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Meeting the future demands for grassland production

Edited by

P. Virkajärvi
K. Hakala
M. Hakojärvi
J. Helin
I. Herzon
V. Jokela
S. Peltonen
M. Rinne
M. Seppänen
J. Uusi-Kämppä

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W. Kessler, Federation Secretary
c/o Agroscope
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Foreword

The 28th General Meeting of the European Grassland Federation is hosted by Finland. The previous EGF meeting held in Finland took place in Lahti in 1992. During the 28 years between these two meetings the challenges presented by climate change and many other environmental issues related to grassland production have increased continuously in their importance. Therefore the main theme of the EGF 2020 General Meeting is 'Meeting the future demands for grassland production'. The conference continues the sustainability themes raised in previous EGF Symposia and General Meetings to answer the challenges that society and environmental constrains have continued to bring forward, such as mitigation of greenhouse gas emissions, improving carbon sequestration and nutrient use efficiency, and finding measures to improve biodiversity in grassland-based systems. In addition, our aim is to strengthen the links between grassland agronomy and ruminant production, including farm economy, which in recent times has attracted much less focus than the other themes. The possibilities offered by new technologies are emphasized because the prospects that a break-through technology may offer are very promising. Obviously, knowledge transfer between scientists, farmers and other stakeholders is crucial for the success with which the European grassland community can meet the challenges that lie ahead of us.

The Covid-19 pandemia has presented an additional challenge for the 2020 EGF General Meeting. At the time of writing this foreword we had postponed the meeting from its original scheduled dates in late June to late October, with the hope that we will be able to meet the European grassland family at that later time.

The meeting has five themes: (1) Crop physiology, plant breeding and nutrient utilisation; (2) Grasses in animal nutrition; (3) Grasslands and environment; (4) Novel technologies in farm management and economy; and (5) Knowledge transfer and consumer perceptions. The largest number of papers were offered for themes 2 and 3. On the other hand, there were surprisingly few papers that included the subject of farm economy, raising a question concerning the general status of farm economics among those involved in grassland science.

We would like to thank all authors for their contributions, numerous referees for their valuable remarks and all delegates attending the conference. We express our sincerest gratitude members of the scientific and organizing committees, the Executive Committee and Secretary of EGF. We also gratefully thank our sponsors for their generous support.

Despite the difficulties caused by the Covid-19 pandemic, we wish that the 28th General Meeting of EGF will provide novel insights for grassland science and stimulate fruitful discussions and networking.

Mervi Seppänen	Perttu Virkajärvi	Marketta Rinne
President *European Grassland Federation*	*Chair* *Scientific Committee*	*Honorary Secretary of* *Organizing Committee*

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Theme 1.
Crop physiology, plant breeding and nutrient utilisation

What European grassland farming will need from grass and legume breeding in the near future

Rognli O.A.[1], Pecetti L.[2], Kovi M.R.[1] and Annicchiarico P.[2]
[1]*Department of Plant Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, 1432 Ås, Norway;* [2]*Council for Agricultural Research and Economics (CREA), Research Centre for Animal Production and Aquaculture, 26900 Lodi, Italy*

Abstract

Plant breeding can be pivotal for the production of new grassland varieties with better adaptation to changes in seasonal patterns of temperature, precipitation and length of the growing seasons, and to increased climate variability caused by climate change. Cultivation of legume species is expected to increase in order to make crop-livestock systems more sustainable and self-sufficient in feed proteins. In northern Europe the higher temperatures and longer growth season will create opportunities for increasing yield, while in southern Europe greater drought tolerance and cool-season growth will be indispensable to maintain crop yields. Greater intra- and interspecific diversity is recommended for grasslands in both regions. In the north, broader genetic diversity is also needed, particularly in terms of adaptation pattern (response diversity), requiring pre-breeding to enlarge the gene pool by introgression of exotic, less-adapted genetic resources as a first step. Genetic gain in forage crop breeding has been modest over time, and new breeding methods need to be implemented to speed up the development of new cultivars with good adaptation to the future climate conditions. Novel technologies, such as genome-enabled selection and high-throughput phenotyping will play a major role in this context. They are evolving rapidly and are increasingly adopted, albeit with technical challenges and a need for optimisation of breeding schemes for optimal exploitation.

Keywords: adaptation, drought tolerance, genomic selection, pre-breeding, phenomics, species mixtures

Introduction

Global climate change is the most important challenge for breeding of forage crops in the near future. The main climate change effects are higher average temperatures, greater climate variability with extreme events, changing precipitation patterns, drought and temperature stress, more wind, less snow cover in continental regions giving more direct frost and ice-encasement, and increased CO_2 content in the atmosphere (Ergon *et al.*, 2018; Olesen *et al.*, 2011). These effects will create new types of abiotic and biotic stresses, requiring increased efforts in breeding new cultivars with improved adaptation to the new climatic conditions. Warming is more rapid in northern regions than the global average, with the largest increases during late autumn, winter and spring. These climate changes will most likely improve the conditions for forage production in these regions, owing to the longer (1-3 months) growing seasons with milder and rainier autumns and winters (Olesen *et al.*, 2011). However, it will be more challenging to harvest the increased biomass in late autumn due to the higher precipitation.

The anticipated greater incidence of drought and higher frequency of extreme climatic events in southern Europe (Alessandri *et al.*, 2014) will affect negatively the productivity of crop-livestock systems. Drought is the main environmental factor influencing forage crop yield, persistence and germplasm × environment (GE) interaction in the Mediterranean basin (Annicchiarico *et al.*, 2011a,b). The increasing costs and/or decreasing availability of irrigation water can exacerbate the negative direct effects of the changing climate. Therefore, there is a need for forage crops with greater tolerance to conditions of increased climate stress. In general, breeding varieties more tolerant to drought is expected to play a crucial role in climate change adaptation and mitigation strategies (Ceccarelli *et al.*, 2010).

Other trends and policies affecting the priorities and breeding goals in the near future are the UN Sustainable Development Goals (SDG). To meet these goals, grassland systems need to be more resilient, and sustainable intensification should provide more output with less input, lower environmental footprints including GHG emissions, and more ecosystem services (biodiversity, pollinators, soil and water quality). A key issue is represented by greater self-sufficiency of livestock feed that is produced locally, especially for feed protein, which today is based largely on imported resources. This context places greater emphasis on the breeding and cultivation of legume species and the selection of varieties with more resilience and higher nutritive quality and digestibility.

How to adapt new cultivars of forage crops to the future climate?

Requirements of plant materials and associated breeding goals for adaptation to and mitigation of climate change effects are indicated in Table 1. Greater intra- and interspecific diversity is recommended for all growing seasons in the north and south of Europe. Broader genetic diversity with more response diversity is needed for northern Europe. Increased growth during cooler seasons and stronger summer dormancy and/or active recovery after drought for perennials, maintenance of growth under moderate drought for annuals, and more persistent seed banks for self-reseeding annuals, are in sharp focus in breeding for the Mediterranean region (Table 1).

Grassland species in northern Europe – characteristics and adaptation to future climate and management

In contrast to southern Europe, grasslands in northern Europe consist exclusively of perennial species, the most important are the grass species perennial ryegrass (*Lolium perenne* L.), timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* Huds.), *Festulolium* and tall fescue (*Festuca arundinacea* Schreb.), and the forage legume species red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.). Lucerne (*Medicago sativa* L.) is currently used very little but has a potential for increased cultivation following climate change. Mäkinen *et al.* (2018) studied diversity in response to agroclimatic variables and the adaptive capacity of forage crop cultivars under climate change. This investigation was based on records of dry matter yields (DMY) obtained on multi-location official variety trials in Finland in the period 2000 to 2012. They found that timothy and meadow fescue cultivars had a low adaptive capacity; thus increased genetic variation is needed, particularly for tolerance to the expected higher temperatures during the growth season. Red clover cultivars were insufficiently adapted to both warm and cold winters, while tall fescue and *Festulolium* were susceptible to drought in autumn. However, *Festulolium* hybrids showed good capacity to adapt to climate change due to high response diversity, which might

Table 1. Requirements of plant materials and associated breeding goals for adaptation to and mitigation of climate change effects (modified from Ergon *et al.*, 2018).

Region in Europe	Growing season[1]	Unfavourable season[2]
Northern	• More intra- and interspecific diversity, broader genetic material with more response diversity • Higher regrowth capacity • Maintenance of growth in water-saturated soils and during dry spells	• More intra- and interspecific diversity, broader genetic material with more response diversity • Utilize earlier spring and later autumn without losing ability to survive winters
Southern	• More intra- and interspecific diversity • Greater ability to grow in cool months (e.g. alfalfa without autumn dormancy) • Maintenance of growth under moderate drought	• More intra- and interspecific diversity • Stronger summer dormancy and active recovery after drought in perennials • More persistent seed bank of annuals

[1] Northern Europe: April – early June to mid-September – early November; Southern Europe: October – November to June.

[2] Northern Europe: Mid-September – early November to April – early June; Southern Europe: July to September – October.

be explained by the larger diversity in the hybrids. Although these results were obtained by studying cultivar responses to the climate of Finland, which is rather continental, they are valid for most Nordic and Baltic regions. An example of how diversity can be increased is the intra-specific diversification by introgressing exotic germplasm into the adapted gene pool to improve northern adaptation of perennial ryegrass (*Lolium perenne* L.), which is the main objective of the Nordic pre-breeding project described below (Rognli *et al.*, 2018).

The future climate will become more maritime also in the continental regions in the north. Under these conditions, active photosynthesis during warmer autumns before snowfall and freezing may be critical for overwintering (Østrem *et al.*, 2018b). Cultivars of adapted species, e.g. timothy and meadow fescue, which cease growth early due to shorter photoperiods and reduced photosynthetic activity in the autumn, will be less winter hardy (Dalmannsdottir *et al.*, 2017); this may be related to increased respiration rates and depletion of reserves during the expected warmer autumns and winters. Growth cessation of plant materials of non-adapted plant species, e.g. perennial ryegrass and *Festulolium*, are less controlled by photoperiod. Thus, in the future longer and warmer autumns, these plant types will maintain growth into the autumn under low light intensities, and this has been shown to delay cold acclimation and reduce winter survival (Østrem *et al.*, 2015).

Grassland species in southern Europe – characteristics and adaptation to future climate and management

Livestock systems of southern Europe have largely relied upon natural grasslands. However, cultivated annual legumes such as berseem clover (*Trifolium alexandrinum* L.) and Persian clover (*Trifolium resupinatum* L.) in pure stand or in mixture with grasses have gained importance as short-term rain-fed forage crops. Likewise, common vetch (*Vicia sativa* L.) and pea (*Pisum sativum* L.) can be valuable for forage production, particularly in moderately favourable environments and in association with cereals (Annicchiarico *et al.,* 2017). Although the growth of these species occurs largely during the cool and moist season, increased tolerance to spring drought will be increasingly important under the changing climate. Some level of cold tolerance is needed as well for adaptation to areas prone to low-temperature winters, such as inland areas of Italy (Piano and Pecetti, 2010). Recently, there has also been increased agronomic interest in southern Europe in annual self-reseeding legumes. Adapted cultivars of subterranean clover (*Trifolium subterraneum* L.) have been selected from local germplasm to exploit pasture productivity and durability thanks to this plant's peculiar morpho-physiology and tolerance to grazing (Nichols *et al.*, 2013). The main selection criteria included suitable maturity for the target environments, high forage and seed yield, good seed-burying capacity and high grazing tolerance. Selection goals for key adaptive traits were derived from ecological studies assessing the relationships between trait variability and climatic conditions of germplasm at collection sites (Piano *et al.*, 1996).

Compared to annuals, perennial species allow the feeding season to be extended and regularized because of prompt regrowth in response to autumn rain and better exploitation of the residual moisture in late spring. A reduction of water consumption by adopting rain-fed cropping or limited irrigation can be pursued by selecting adapted germplasm. While high water-use efficiency provided by extensive growth during the cool rainy season is a desirable trait for annuals, perennials should possess drought tolerance/avoidance mechanisms enabling them to survive through repeated dry summers (Annicchiarico *et al.*, 2011b, 2013a). Different plant species and types, relying on different adaptation strategies, can be useful to cope with different drought stress levels and patterns. Multi-site evaluation across a range of increasingly stressful conditions, together with subsequent modelling of cultivar yield responses, can allow the prediction of possible shifts in top-performing species and cultivars and of useful adaptive traits as a consequence of climate change (Annicchiarico *et al.*, 2013b).

Lucerne is the main forage crop in southern Europe, where it is usually grown under irrigation in low-rainfall areas. Reducing or withholding lucerne irrigation in summer (when the crop water-use efficiency is lowest and water may be precious for alternative uses), or adopting rain-fed cropping, can produce remarkable water savings when using tolerant germplasm, as reported in Annicchiarico *et al.* (2011a) for the western Mediterranean basin. Remarkable crossover GE interaction between top-yielding cultivars occurred across moisture-contrasting environments as a consequence of different and partly incompatible adaptive traits (Annicchiarico *et al.*, 2013a), emphasizing the importance of selecting distinct varieties for relatively favourable and severely drought-stressed conditions. Breeding lucerne for specific adaptation to irrigated or rain-fed cropping exhibited distinctly greater genetic gains than breeding for wide adaptation even for a relatively favourable region such as northern Italy (Annicchiarico, 2007a), also because farm landraces (which may still represent a core genetic base for forage crop breeding) displayed specific adaptation to conditions under which they evolved (Annicchiarico and Piano, 2005).

Cocksfoot (*Dactylis glomerata* L.) and tall fescue are the most important perennial grass species in southern Europe. For long, these species have only been bred for temperate-climate regions, displaying mis-adaptation to environments of southern Europe (Annicchiarico *et al.*, 2011b). Summer dormancy (i.e. the complete aerial senescence at the end of spring irrespective of the water availability: Volaire *et al.*, 2009) is especially useful for cocksfoot plant survival under severe drought (Annicchiarico *et al.*, 2011b), whereas incomplete dormancy (Volaire *et al.*, 2009) and dehydration tolerance (by which sufficient moisture is maintained in leaf basal tissues even at low soil water potential: Volaire, 2008) favour plant adaptation to moderate drought and can be useful in breeding drought-tolerant material that is responsive to possible water availability in summer (Piano *et al.*, 2004). Mediterranean germplasm of tall fescue relies on dehydration delay related to water uptake from deep roots (Volaire and Lelièvre, 2001), although mechanisms of dehydration tolerance can be present (Norton *et al.*, 2006). Selection of tall fescue germplasm with wide adaptation across diversified Mediterranean drought stress levels seems feasible (Pecetti *et al.*, 2011). Mediterranean germplasm of tall fescue has tended to be better yielding than that of cocksfoot (Annicchiarico *et al.*, 2013b), likely because of greater cool-season growth with subsequent higher water-use efficiency and deeper and larger root system (Lelièvre *et al.*, 2011), thereby displaying an optimal combination of endurance to summer drought and high growth under favourable moisture availability.

Pre-breeding

In general, increased intra- and interspecific diversity in needed in order to adapt to and mitigate the effects of climate change (Table 1). Therefore, more emphasis should be devoted to pre-breeding activities. Pre-breeding is defined as 'all activities designed to identify desirable characteristics and/or genes from unadapted materials that cannot be used directly in breeding populations and to transfer these traits to an intermediate set of materials that breeders can use further in producing new varieties for farmers' (The Global Partnership Initiative for Plant Breeding Capacity Building (GIPB)).

Northern Europe

With respect to breeding for climate change in northern Europe, focus is justified on increasing genetic diversity by introgressing exotic material in species that lack sufficient variation for key traits conferring adaptability, e.g. winter hardiness (freezing and ice-encasement tolerance), timing of growth cessation and utilisation of longer growth seasons, and fungal diseases which either cause more damage in the new climate or are moving further north as the climate changes. One example is the Nordic Public-Private Partnership (PPP) on pre-breeding (Nilsson *et al.*, 2016), running since 2012 with one of its projects on perennial ryegrass. This species has superior feed quality and productivity, and it is especially well adapted to the coastal climate of Western Europe (Humphreys *et al.*, 2010). With climate change, we expect its cultivation area to expand further north and east, which implies crop improvement for tolerance to snow-mould, better control of growth cessation in the autumn in order to improve winter survival and persistence, and increased resistance to crown rust (Østrem *et al.,* 2015, 2018a). Ryegrass genetic diversity is restricted, since the species is not native to the northern and continental regions (Rognli *et al.*, 2013). Field testing of Nordic and Central European cultivars across five locations, spanning from continental Estonia to maritime Iceland, found that cultivars developed in the north in general are best adapted, albeit showing large GE interactions (Helgadottir *et al.*, 2018). Thus, a major objective of the pre-breeding project in perennial ryegrass is to introgress and recombine exotic materials with existing breeding populations, to serve as new genetic resources to breed cultivars for the future climate of these regions.

Southern Europe

Current knowledge on useful plant types of forage crops for European Mediterranean regions is likely to be modified by the requirements imposed by the predicted greater incidence of drought. Breeders ought to intercept information on novel germplasm or unexploited adaptive mechanisms and transfer it into their crop improvement programmes, as shown by the following examples. Results on cocksfoot have suggested different genetic resources, plant types and adaptation patterns for North Africa and southern Europe. The completely summer-dormant germplasm of *D. glomerata* subsp. *hispanica* currently has prevalent interest for North Africa but will gain adaptive potential for Mediterranean-climate regions of Europe to ensure summer survival under increasing drought severity (Annicchiarico *et al.*, 2011b). Piano *et al.* (2005) reported a coevolutionary specificity between the native Mediterranean tall fescue germplasm and its associated *Neotyphodium* endophyte, which could enable the selection of novel endophyte strains for targeted infection aimed at increasing the drought tolerance of the host crop (Malinowski *et al.*, 2005). Irrespective of their endophyte status, however, Mediterranean native populations proved more drought-tolerant than 'continental' varieties (Pecetti *et al.*, 2007), and their introgression into the latter could be envisaged to enhance the crop's drought tolerance in southern Europe. In lucerne, single traits or combinations of traits (plant architectures) associated with specific adaptation to drought stress were identified that can be exploited for selection (Annicchiarico, 2007b, 2013a). The integration of adaptive trait-based and yield-based selection may increase the selection efficiency and enable the detection of the best-performing material at early selection stages. In some cases, the relevance of adaptive traits for selection is modulated through the soil type (Annicchiarico, 2007b). The use of managed environments with an accurate control of drought stress levels can be an asset to anticipate the needs that would arise due to climate change and minimise the effect on selection of year-to-year rainfall variation (Annicchiarico, 2007). Prior knowledge borrowed from the relationships between the features of native populations and their environments of origin can provide valuable guidelines for selecting adapted varieties, as in Piano *et al.* (1996), who reported an adjustment of flowering time, seed size and hardseededness for subterranean clover populations that can help define distinct ideotypes for contrasting environments of Sardinia.

Breeding for mixtures

Forage crops are frequently grown in mixed stand (MS), for instance annual legumes with cereals, and perennial legumes with forage grasses, either in binary or complex mixtures. In general, grass-legume mixtures produce higher yields than the pure stands (PS) of each species, and better nutritive quality than the grass PS (Annicchiarico *et al.*, 2019), while contributing to more sustainable cropping systems in various respects (Lüscher *et al.*, 2014). In addition, legumes benefit more from higher atmospheric CO_2 concentration than non-N-fixing species, and this could compensate for the decline in protein content that is expected to occur in grasses in a future scenario with elevated CO_2 and higher temperatures (Thivierge *et al.,* 2016).

Forage species are usually selected in PS, with seed for mixtures pooled later by the seed companies. However, some cultivar combinations are more compatible than others, and direct selection under target

MS conditions can be preferable in various cases. In particular, a recent review of research on breeding for mixed cropping (Annicchiarico *et al.*, 2019) indicated that: (1) averaged across several studies, selection in PS exhibited about 40% lower predicted yield gains than selection in MS; (2) selection under MS tends to be particularly important for species undergoing severe competitive stress, because the genetic correlation for genotype yield response across PS and MS tends to increase as a function of the biotic environment occupied by the associated species in MS – as showed for forage crops by the study of Annicchiarico and Piano (1994); (3) breeding for compatibility with a wide range of plant companions is encouraged by the larger size of general compatibility (or general mixing ability) effects relative to specific compatibility effects. A selection scheme designed to improve the general compatibility of populations was proposed by Hill (1990). Recently, Sampoux *et al.* (2020) estimated the expected performance in MS of different types of forage crop selection (pure stand, reciprocal mixture and general mixture ability).

Breeding for increased compatibility can be highly successful, as shown for a poorly competing species such as white clover through direct selection in MS performed simultaneously with two highly competing grass varieties (Annicchiarico and Proietti, 2010). However, selection under MS may imply greater evaluation costs than in PS, and its convenience ought to be weighted against the expected increase of breeding progress. There is a need for exploring the efficiency of novel and relatively low-cost breeding strategies, among which genomic selection and evolutionary breeding emerge as the most promising ones (Annicchiarico *et al.*, 2019).

The prospects of implementing high-throughput phenotyping in breeding forage crops

Breeding forage crops is probably the most complex and costly type of breeding. Phenotyping a range of traits over several seasons, with biomass yield measurements taken over several cuts each season, is laborious, time consuming and costly. In addition, heterogeneous populations are tested over several locations often with large GE interactions, making precise phenotyping very important in order to select superior genetic materials.

Genomic resources with high-throughput molecular markers are fast developing for forage crops (Byrne *et al.*, 2015; De Vega *et al.*, 2015). High-throughput phenotyping (HTP) of important traits can be important to capitalize timely on the genomic information, to develop genome-enabled breeding in forage crops. NIRS (Near Infrared Reflectance Spectroscopy) has long been used to rapidly phenotype forage quality traits, but in-field measurements are difficult to develop with this technology (Walter *et al.*, 2012). What is needed in a plant breeding context is real-time, non-destructive and in-field high-throughput measurement of yield and other key traits. Recent years have seen a rapid development and application of various sensors (multispectral, hyperspectral, thermal and RGB), and laser scanning (LiDAR) as part of various UAS (Unmanned Aerial Systems) plant phenotyping platforms (see reviews by Gebremedhin *et al.*, 2019a and Yang *et al.*, 2020).

Reliable HTP would be highly valuable also to reduce the phenotypic selection costs, thereby allowing for evaluating more material and applying greater selection intensities. For perennial forages, which typically feature much greater within-population than among-population variation for agronomic traits of landraces or varieties (Annicchiarico *et al.*, 2015a), HTP would provide greater opportunities to exploit within-population diversity of genetic resources.

The question is whether precise phenotyping of complex traits with crucial importance for adaptation can be established using HTP technologies. The most important and costly trait to phenotype is biomass yield. At different stages during the breeding scheme, breeders need to phenotype both single plants, row plots, simulated swards and sward plots. Visual scoring is used to phenotype many traits, and also yield, especially on single plants and row plots early in the breeding process. DMY is best estimated by weighing families tested in sward plots. A challenge for phenotyping yield is the low correlation between yield estimated on single plants and row plots and sward plots (Annicchiarico *et al.*, 2015a). Nevertheless, Ghamkhar *et al.* (2019) used LiDAR to estimate yield traits in single and paired-row plots of perennial ryegrass with high level of accuracy. Significant but highly variable correlations (r=0.12-0.93) were reported between visual scores of biomass yield and normalised difference vegetation index (NDVI) values on spaced plants, and between biomass yield and NDVI in row plot and sward trials in perennial ryegrass (Wang *et al.*, 2019). NDVI obtained from multispectral sensors and UAS can replace visual scoring in spaced plant trials. Gebremedhin *et al.* (2019b) developed a promising method for high-throughput phenotyping of herbage yield of spaced plants by combining NDVI and ultrasonic sonar estimates of plant height. Viljanen *et al.* (2018) used machine learning to integrate various multispectral remote sensing features to accurately estimate biomass of grass swards; this could be relevant both for practical farming and breeding. Østrem *et al.* (2018b) compared chlorophyll fluorescence (Chl-*a*) with traditional whole-plant freezing tests in selection for freezing tolerance in a *Festulolium* population. They found that both methods were effective but gave selections with rather different adaptations. Selection by Chl-*a* gave populations with improved winter survival by maintaining active photosynthesis during the expected warmer autumns, especially in coastal climates, while selection by traditional freezing tests create populations with early growth cessation and reduced photosynthetic activity. These populations show a typical northern continental adaptation but will be less winter-hardy, possibly due to increased respiration and depletion of reserves during the warmer autumns and winters. NDVI measurements on sward plots in the field confirmed the differential adaptation patterns created by the two selection methods. Higher temperatures and associated droughts are expected to increase in prevalence in Europe, especially in the south. Root phenotyping relevant for field conditions is exceedingly difficult, and systems for such high-throughput root phenotyping do not exist. Yates *et al.* (2019) established a non-destructive and largely automated phenotyping platform to collect leaf growth data under various water limiting conditions. They were able to quantify precisely the genotypic response of water deficit on leaf growth.

Genome-enabled breeding in forage crops

The rate of breeding progress for forage crop yields has been low. For perennial ryegrass, Sampoux *et al.* (2011) reported an annual rate of 0.32% (with hardly any improvement for spring growth), whereas McDonagh *et al.* (2016) reported rates of 0.52% under conservation and 0.35% under simulated grazing. For perennial legumes, annual genetic gains ranged between nil and 0.26% for lucerne, while ranging between 0.53 and 0.60% for red or white clover (Annicchiarico *et al.*, 2015a). Hence, new breeding technologies are needed to raise the breeding progress of these crops. Most perennial forage species are outbreeding, frequently with a polyploid, large and highly heterozygous genome. Lack of reference genomes makes it challenging to develop informative and good molecular marker systems. The value of genotyping single plants/parents is much less than in selfing species since the unit of selection are half-sib or full-sib families. Currently it may take 15-20 years to put a new forage crop cultivar on the market. Part of this is progeny testing of families in sward plots, which may take up to 7-8 years (including progeny development and recombination of selected parents). Shortening the breeding cycle is also of utmost importance to cope with the various needs presented by the changing climate.

Genomic selection (GS) has potential for substantially easing these challenges. It was developed to raise the genetic gain for complex quantitative traits by shortening the selection cycles and reducing expensive, time-consuming phenotyping. In GS, a training population is phenotyped and genotyped to train a model, and subsequently used to predict genomically estimated breeding values (GEBVs) of another set of closely related individuals that has only been genotyped but not phenotyped (Meuwissen *et al.*, 2001). Precise phenotypic data and appropriate modelling are crucial requirements for accurate genomic predictions (Bernal-Vasquez *et al.*, 2014). Simple sequence repeat (SSRs) markers, which played a role in traditional marker-assisted selection, are not appropriate for GS because of their small number. Genotyping-by-sequencing (GBS) (Elshire *et al.*, 2011), by which thousands of genome-wide single nucleotide polymorphic (SNP) markers are produced, is a promising cost-effective approach. GBS can also be used on population and family basis for estimation of allele frequencies of SNPs at genome-wide positions (Byrne *et al.*, 2013).

A range of statistical (linear and non-linear) regression models is being applied in GS to predict GEBV (Table 2). Recently machine-learning techniques like deep multi-layered neural network (DNN) have been developed to improve genomic predictions (Montesinos-López *et al.*, 2019), showing an advantage over traditional GS models (González-Camacho *et al.*, 2018).

Genomic selection in forage crops was first outlined by Hayes *et al.* (2013). Annicchiarico *et al.* (2015a) recommended the evaluation of phenotypes of genotyped plant material based on sward plots of their half-sib or selfed progenies rather than as spaced plants (as usually adopted for cloned genotypes), in order to model the additive genetic variation (the only relevant method for synthetic variety improvement), to represent more faithfully actual production environments, and ease the preservation of mapping/reference populations and the implementation of multi-environment experiments. An example of this

Table 2. Overview of genomic prediction studies in forage crops.

Species	Material	# SNP markers[1]	Statistical models[3]	Traitsd[4]	Accuracy[5]	Reference
Perennial ryegrass	Breeding populations (365)	3K SNP chip	GBLUP, KNN, RF, GBM	Biomass yield	0.01-0.22[a]	Grinberg *et al.*, 2016
				DM digestibility	0.07-0.41[a]	
				WSC	0.27-0.59[a]	
	F_2 families (1,515)	137,191[2]		Biomass yield	0.34[b]	Guo *et al.*, 2018
		185,297		Heading date	0.77[b]	
		188,832		Crown rust resistance	0.55[b]	
	Half-sib families (517)	1,023,011	GBLUP, KGD-BLUP, RR, RF	Heading date	0.40-0.52[a]	Faville *et al.*, 2018
	F_2 families (1,757)	144,7122	GBLUP	Heading date	0.9[a]	Fe *et al.*, 2015
	Single plants (1,800)	217,563	rrBLUP, Bayes B, Bayesian Lasso, RF	Crown rust	0.49-0.52[a]	Arojju *et al.*, 2018
Lucerne	Genotypes (322)	44,757	BayesA, B and Cπ	ADF	0.18[c]	Jia *et al.*, 2018
				Crude protein	0.05[c]	
				Biomass yield	0.12[c]	
				Plant regrowth	0.50-0.51[c]	
	Genotypes (154)	11,450	rrBLUP, Bayes B, Bayesian Lasso	Leaf protein content	0.40[a]	Biazzi *et al.*, 2017
				NDF digestibility at 24 hours	0.30[a]	
	Genotypes – subcontinental climate (124)	7,000	rrBLUP, RR, Bayes A, B, Bayesian Lasso	Biomass yield	0.32[a]	Annicchiarico *et al.*, 2015b
	Genotypes -Mediterranean climate (154)	11,000	rrBLUP, RR, Bayes A, B, Bayesian Lasso	Biomass yield	0.35[a]	Annicchiarico *et al.*, 2015b
	Cloned genotypes	10,000	rrBLUP	Biomass yield	0.22-0.40[a,d]	Li *et al.*, 2015

[1] SNP = single nucleotide polymorphism.

[2] Marker type in all studies are GBS: SNP markers derived by genotyping-by-sequencing.

[3] GBLUP = genomic best linear unbiased prediction; KNN = k-nearest neighbour; RFF = random forest fitness; GBM = gradient boosting machine; KGD-BLUP = kinship using GBS with depth adjustment – best linear unbiased prediction; RR = random regression; RF = random forest; rrBLUP = ridge-regression best linear unbiased production.

[4] DMY = dry matter yield; WSC = water soluble carbohydrates; ADF = acid detergent fibre; NDF = neutral detergent fibre.

[5] [a] = Pearson's correlation coefficients between genomic estimated breeding values (GEBvs) and observed phenotypes; [b] = Pearson's correlation coefficients between GEBV and phenotypes by squared root of heritability; [c] = Predictive accuracy measured as the correlation between the EBVs and GEBVs; [d] = Training in one generation for prediction in the following generation.

strategy was reported for lucerne biomass yield in Annicchiarico *et al.* (2015b), where predicted yield gains by GS clearly exceeded predicted gains by phenotypic selection because of the low narrow-sense heritability of the yield trait. This study also showed some scope for applying GS defined for a given genetic base to a completely different genetic base, given a loss of predictive accuracy of 25-30% passing from intra-population predictions to cross-population predictions for sub-continental and Mediterranean germplasm of lucerne.

There is a paucity of results on GS of perennial forages, all relative to perennial ryegrass and lucerne, which are summarised in Table 2. Prediction accuracies ranged from low to good (considering the reproductive systems) for DMY (0.01-0.40) and forage quality traits (0.05-0.41), confirming in most studies the potential interest of GS. They highlight another potential advantage of GS relative to phenotypic selection, namely, easier and more cost-efficient simultaneous selection for several traits (e.g. yield and one or more forage quality traits), as GS implies the same genotyping cost when targeting one or several traits, whereas phenotypic selection implies raising costs as a function of the number of evaluated traits.

Preliminary GS results for timothy yield and forage quality traits, relative to a training population of 720 full-sib (FS) families tested in 3-year field trial sown twice at 2-3 locations of Norway, are quite promising (Rognli *et al.*, unpublished data). GEBV predictions for DMY exhibited outstanding predictive accuracies (>0.90) detected by both gBLUP and machine-learning models (RKHS). Validation of the prediction models in unrelated FS-families (213 FS-families, tested three years at two locations) showed good prediction accuracies for a couple of biomass yield recordings (0.45 for DMY first cut second year; 0.29 for total DMY second year). However, predictions of other yield recordings and traits gave very low correlations, in the same range as that detected by Grinberg *et al.* (2016) in perennial ryegrass.

GS models for cultivars adapted to future climates could be developed by combining historical data from multi-location yield trials with climate, soil and genomic data using advanced machine learning models. Prediction of the effects of changing environments on performance will help breeders to compare the results over multiple years, to gain information about how experimental varieties will likely perform in a target environment.

Conclusions

The combination of new HTP and GS methods may require some changes to future breeding schemes for optimisation. For example, HTP could be applied to the evaluation of a very large number of genotypes that are grown as relatively spaced plants in a non-replicated fashion according to a stratified mass selection lay-out (Annicchiarico, 2004). This evaluation stage would be used for preliminary phenotypic selection of fairly high numbers of genotypes for subsequent GS, on the basis of biomass production and other traits that could meaningfully be recorded on individual plants (e.g. tolerance to diseases, seasonal growth pattern and dormancy, etc.). The selected material would undergo GS for biomass production and a few other key traits (e.g. one or two forage quality traits) by exploiting GS models that were defined at an earlier stage for the same genetic base, selecting genomically a set of plants used as parents of a new synthetic variety. This candidate variety would finally be assessed under field conditions against top-performing commercial varieties before registration. As anticipated, GS could be particularly useful to decrease the cost of breeding for mixed cropping, thereby reversing whenever convenient the current practice of selection in pure stand. Cross-population predictive ability results will be essential to understand the size, genetic diversity and number of genetic bases (ranging from only one, large and highly diversified, to several relatively narrow-based) used by a breeding programme for variety selection.

References

Alessandri A., De Felice M., Zeng N., Mariotti A., Pan Y., Cherchi A., Lee J.Y., Wang B., Ha K.J., Ruti P. and Artale V. (2014) Robust assessment of the expansion and retreat of the Mediterranean climate in the 21st century. *Scientific Reports* 4, 7211.

Annicchiarico P. (2004) A low-cost procedure for multi-purpose, large-scale field evaluation of forage crop genetic resources. *Euphytica* 140, 223-229.

Annicchiarico P. (2007a. Wide- versus specific-adaptation strategy for lucerne breeding in northern Italy. *Theoretical and Applied Genetics* 114, 647-657.

Annicchiarico P. (2007b) Lucerne shoot and root traits associated with adaptation to favourable and drought-stress environments and to contrasting soil types. *Field Crops Research* 102, 51-59.

Annicchiarico P. and Piano E. (1994) Interference effects in white clover genotypes grown as pure stands and binary mixtures with different grass species and varieties. *Theoretical and Applied Genetics* 88, 153-158.

Annicchiarico P. and Piano E. (2005) Use of artificial environments to reproduce and exploit genotype × location interaction for lucerne in northern Italy. *Theoretical and Applied Genetics* 110, 219-227.

Annicchiarico P. and Proietti S. (2010) White clover selected for competitive ability widens the compatibility with grasses and favours the optimization of legume content and forage yield in mown clover-grass mixtures. *Grass and Forage Science* 65, 318-324.

Annicchiarico P., Pecetti L., Abdelguerfi A., Bouizgaren A., Carroni A.M., Hayek T., M'Hammadi Bouzina M. and Mezni M. (2011a) Adaptation of landrace and variety germplasm and selection strategies for lucerne in the Mediterranean basin. *Field Crops Research* 120, 283-291.

Annicchiarico P., Pecetti L., Bouzerzour H., Kallida R., Khedim A., Porqueddu C., Simões N.M., Volaire F. and Lelièvre F. (2011b) Adaptation of contrasting cocksfoot plant types to agricultural environments across the Mediterranean basin. *Environmental and Experimental Botany* 74, 82-89.

Annicchiarico P., Pecetti L. and Tava A. (2013a) Physiological and morphological traits associated with adaptation of lucerne (*Medicago sativa*) to severely drought-stressed and to irrigated environments. *Annals of Applied Biology* 162, 27-40.

Annicchiarico P., Pecetti L., Abdelguerfi A., Bouzerzour H., Kallida R., Porqueddu C,, Simões N.M. and Volaire F. (2013b) Optimal forage grass germplasm for drought-prone Mediterranean environments. *Field Crops Research* 148, 9-14.

Annicchiarico P., Barrett B., Brummer E.C., Julier B. and Marshall A.H. (2015a) Achievements and challenges in improving temperate perennial forage legumes. *Critical Reviews of Plant Science* 34, 327-380.

Annicchiarico P., Nazzicari N., Li X., Wei Y., Pecetti L. and Brummer E. (2015b) Accuracy of genomic selection for alfalfa biomass yield in different reference populations. *BMC Genomics* 16, 1020.

Annicchiarico P., Thami Alami I., Abbas K., Pecetti L., Melis R.A.M. and Porqueddu C. (2017) Performance of legume-based annual forage crops in three semi-arid Mediterranean environments. *Crop and Pasture Science* 68, 932-941.

Annicchiarico P., Collins R.P., De Ron A.M., Firmat C., Litrico I. and Hauggaard-Nielsen H. (2019) Do we need specific breeding for legume-based mixtures? *Advances in Agronomy* 147, 141-215.

Arojju S.K., Conaghan P., Barth S., Milbourne D., Casler M.D., Hodkinson T.R., Michel T. and Stephen B. (2018) Genomic prediction of crown rust resistance in *Lolium perenne*. *BMC Genetics* 19, 35.

Bernal-Vasquez A.M., Möhring J., Schmidt M., Schönleben M., Schön C.C. and Piepho H.P. (2014) The importance of phenotypic data analysis for genomic prediction-a case study comparing different spatial models in rye. *BMC Genomics* 15, 646.

Biazzi E., Nazzicari N., Pecetti L., Brummer E.C., Palmonari A., Tava A. and Annicchiarico P. (2017) Genome-wide association mapping and genomic selection for alfalfa (*Medicago sativa*) forage quality traits. *PLoS ONE* 12, e0169234.

Byrne S., Czaban A., Studer B., Panitz F., Bendixen C. and Asp T. (2013) Genome Wide Allele Frequency Fingerprints (GWAFFs) of populations via genotyping by sequencing. *PLoS ONE* 8, e57438.

Byrne S.L., Nagy I., Pfeifer M., Armstead I., Swain, S., Studer B., Mayer K., Campbell J.D., Czaban A., Hentrup S., Panitz F., Bendixen C., Hedegaard J., Caccamo M. and Asp T. (2015). A synteny-based draft genome sequence of the forage grass *Lolium perenne*. *Plant Journal* 84, 816-826.

Ceccarelli S., Grando S., Maatougui M., Michael M., Slash M., Haghparast R., Rahmanian M., Taheri A., Al-Yassin A., Benbelkacem A., Labdi M., Mimoun H. and Nachit M. (2010) Plant breeding and climate changes. *Journal of Agricultural Science* 148, 627-637.

Dalmannsdottir S., Jørgensen M., Rapacz M., Østrem L., Larsen A., Rødven R. and Rognli O.A. (2017). Cold acclimation in warmer extended autumns impairs freezing tolerance of perennial ryegrass (*Lolium perenne*) and timothy (*Phleum pratense*). *Physiologia Plantarum,* 160, 266-281.

De Vega J.J., Ayling S., Hegarty M., Kudrna D., Goicoechea J.L., Ergon Å., Rognli O.A., Jones C., Swain M., Geurts R., Lang C., Mayer K.F., Rössner S., Yates S., Webb K.J., Donnison I.S., Oldroyd G.E., Wind R.A., Caccamo M., Powell W., Abberton M.T. and Skøt L. (2015) Red clover (*Trifolium pratense* L.) draft genome provides a platform for trait improvement. *Scientific Reports* 5, 17394.

Elshire R.J., Glaubitz J.C., Sun Q., Poland J.A., Kawamoto K., Buckler E.S. and Mitchell S.E. (2011) A robust, simple Genotyping-by-Sequencing (GBS) approach for high diversity species. *PLoS ONE* 6, e19379.

Ergon Å., Seddaiu G., Korhonen P., Virkajärvi P., Bellocchi G., Jørgensen M., Østrem L., Reheul D. and Volaire F. (2018). How can forage production in Nordic and Mediterranean Europe adapt to the challenges and opportunities arising from climate change. *European Journal of Agronomy* 92, 97-106.

Faville M.J., Ganesh S., Cao M., Jahufer M.Z.Z., Bilton T.P., Easton H.S., Ryan D.L., Trethewey J.A.K., Rolston M.P., Griffiths A.G., Moraga R., Flay C., Schimdt J., Tan R. and Barrett B.A. (2018) Predictive ability of genomic selection models in a multi-population perennial ryegrass training set using genotyping-by-sequencing. *Theoretical and Applied Genetics* 131, 703-720.

Fè D., Cericola F., Byrne S., Lenk I., Ashraf B.H., Pedersen M.G., Roulund N., Asp T., Janss L., Jensen C.S. and Jensen J. (2015) Genomic dissection and prediction of heading date in perennial ryegrass. *BMC Genomics* 16, 921.

Gebremedhin A., Badenhorst P.E., Wang J., Spangenberg G.C. and Smith K.F. (2019) Prospects for measurement of dry matter yield in forage breeding programs using sensor technologies. *Agronomy 9*, 65.

Gebremedhin A., Badenhorst P.E., Wang J., Giri K., Spangenberg G.C. and Smith K.F. (2019) Development and validation of a model to combine NDVI and plant height for high-throughput phenotyping of herbage yield in a perennial ryegrass breeding program. *Remote Sensing* 11, 2494.

Ghamkhar K., Irie K., Hagedorn M., Hsiao J., Fourie J., Gebbie S., Hoyos-Villegas V., George R., Stewart A., Inch C., Werner A. and Barrett, B. (2019) Real-time, non-destructive and in-field foliage yield and growth rate measurement in perennial ryegrass (*Lolium perenne* L.). *Plant Methods* 15, 72.

González-Camacho J.M., Ornella L., Pérez-Rodríguez P., Gianola D., Dreisigacker S. and Crossa J. (2018) Applications of machine learning methods to genomic selection in breeding wheat for rust resistance. *Plant Genome* 11, 170104.

Grinberg N.F., Lovatt A., Hegarty M., Lovatt A., Skøt K.P., Kelly R., Blackmore T., Thorogood D., King R.D., Armstead I., Powell W. and Skøt L. (2016) Implementation of genomic prediction in *Lolium perenne* breeding populations. *Frontiers in Plant Science* 7, 133.

Guo X., Cericola F., Fè D., Pedersen M.G., Lenk I., Jensen C.S., Jensen J. and Janss L.L. (2018) Genomic prediction in tetraploid ryegrass using allele frequencies based on genotyping by sequencing. *Frontiers in Plant Sciences* 9, 1165.

Hayes B.J., Cogan N.O.I., Pembleton L.W., Goddard M.E., Wang J., Spangenberg G.C., and Forster J.W. (2013). Prospects for genomic selection in forage plant species. *Plant Breeding* 132, 133-143.

Helgadóttir Á., Aavola R., Isolahti M., Marum P., Persson C., Aleliūnas A., Brazauskas G., Kristjánsdóttir T.A., Asp T. and Rognli O.A. (2018) Adaptability and phenotypic stability of *Lolium perenne* L. cultivars of diverse origin grown at the margin of the species distribution. *Journal of Agronomy and Crop Science* 204, 493-504.

Hess M., Druet T., Hess A. and Garrick D. (2017) Fixed-length haplotypes can improve genomic prediction accuracy in an admixed dairy cattle population. *Genetics Selection Evolution* 49, 54.

Hill J. (1990) The three C's – competition, coexistence and coevolution – and their impact on the breeding of forage crop mixtures. *Theoretical and Applied Genetics* 79, 168-176.

Humphreys M., Feuerstein U., Vandewalle M. and Baert J. (2010) Ryegrasses. In: Boller B., Posselt U.K. and Veronesi F. (eds.) *Handbook of Plant Breeding Vol. 5. Fodder Crops and Amenity Grasses*, Springer: New York, pp. 211-260.

Jia C., Zhao F., Wang X., Han J., Zhao H., Liu G. and Wang Z. (2018) Genomic prediction for 25 agronomic and quality traits in alfalfa (*Medicago sativa*). *Frontiers in Plant Sciences* 9, 1220.

Lelièvre F., Seddaiu G., Ledda L., Porqueddu C. and Volaire F. (2011) Water use efficiency and drought survival in Mediterranean perennial forage grasses. *Field Crops Research* 121, 333-342.

Li X., Wei Y., Acharya A., Hansen J.L., Crawford J.L., Viands D.R., Michaud R., Claessens A. and Brummer E.C. (2015) Genomic prediction of biomass yield in two selection cycles of a tetraploid alfalfa breeding population. *Plant Genome* 8, 2.

Lüscher A., Mueller-Harvey I., Soussana J.F., Rees R.M. and Peyraud J.L. (2014). Potential of legume-based grassland-livestock systems in Europe: a review. *Grass and Forage Science* 69, 206-228

Mäkinen H., Kaseva J., Virkajärvi P. and Kahiluoto H. (2018) Gaps in the capacity of modern forage crops to adapt to the changing climate in northern Europe. *Mitigation and Adaptation Strategies for Global Change, 23*, 81-100.

Malinowski D.P., Belesky D.P. and Lewis G.C. (2005). Abiotic stresses in endophytic grasses. In: Roberts C.A., West CP. and Spiers DE. (eds), *Neotyphodium in cool-season grasses*. Blackwell Publishing, Carlton, Victoria, Australia, pp.187-199.

McDonagh J., O'Donovan M., McEvoy M. and Gilliland T.J. (2016) Genetic gain in perennial ryegrass (*Lolium perenne*) varieties 1973 to 2013. *Euphytica* 212, 187-199.

Meuwissen T.H., Hayes B.J. and Goddard M.E. (2001) Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157, 1819-1829.

Montesinos-López O.A., Martín-Vallejo J., Crossa J., Gianola D., Hernández-Suárez C.M., Montesinos-López A., Juliana P. and Singh R. (2019) New deep learning genomic-based prediction model for multiple traits with binary, ordinal, and continuous phenotypes. *G3: Genes|Genomes|Genetics* 9, 1545-1556.

Nilsson A., von Bothmer R., Johanesson T., Nybom H., Bendevis M.A., Bengtsson T. and Rognli O.A. (2016) Promoting Nordic plant breeding for the future. In: Hägnefelt A. (ed) PPP public private partnership for pre-breeding. Nordic Council of Ministers, Copenhagen, ANP, 730, p 27.

Nichols P.G.H., Foster K.J., Piano E., Pecetti L., Kaur P., Ghamkhar K. and Collins W.J. (2013). Genetic improvement of subterranean clover (*Trifolium subterraneum* L.). 1. Germplasm, traits and future prospects. *Crop & Pasture Science* 64, 312-346.

Norton M.R., Volaire F. and Lelièvre F. (2006) Summer dormancy in *Festuca arundinacea* Schreb.: the influence of season of sowing and a simulated mid-summer storm on two contrasting cultivars. *Australian Journal of Agricultural Research* 57, 1267-1277.

Olesen J.E., Trnka M., Kersebaum K.C., Skjelvåg A.O., Seguin B., Peltonen-Sainio P., Rossi F., Kozyra J. and Micale F. (2011) Impacts and adaptation of European crop production systems to climate change. *European Journal of Agronomy* 34, 96-112.

Østrem L., Rapacz M., Larsen A., Dalmannsdottir S. and Jørgensen M. (2015) Influences of growth cessation and photoacclimation on winter survival of non-native *Lolium-Festuca* grasses in high-latitude regions. *Environmental and Experimental Botany* 111, 21-31.

Østrem L., Asp T., Ghesquière M., Sanada Y. and Rognli O.A. (2018a) Low crown rust resistance in Norwegian material of *Lolium perenne* and ×*Festulolium*. In: Brazauskas G. *et al.* (eds.) *Breeding grasses and protein crops in the era of genomics*. Springer International Publishing AG, pp. 145-149.

Østrem L., Rapacz M., Larsen A., Marum P. and Rognli O.A. (2018b) Chlorophyll a fluorescence and freezing tests as selection methods for growth cessation and increased winter survival in ×Festulolium. *Frontiers in Plant Science* 9, 1200.

Pecetti L., Romani M., Carroni A.M., Annicchiarico P. and Piano E. (2007) The effect of endophyte infection on persistence of tall fescue (*Festuca arundinacea* Schreb.) populations in two climatically contrasting Italian environments. *Australian Journal of Agricultural Research* 58, 893-899.

Pecetti L., Annicchiarico P., Abdelguerfi A., Kallida R., Mefti M., Porqueddu C., Simões N.M., Volaire F. and Lelièvre F. (2011) Response of Mediterranean tall fescue cultivars to contrasting agricultural environments and implications for selection. *Journal of Agronomy and Crop Science* 197, 12-20.

Piano E., Pecetti L. and Carroni AM. (1996) Climatic adaptation in subterranean clover populations. *Euphytica* 92, 39-44.

Piano E., Pecetti L., Annicchiarico P., Carroni A.M., Fornasier F. and Romani M. (2004) Combining drought tolerance and responsiveness to summer moisture availability in cocksfoot (*Dactylis glomerata* L.) germplasm grown in Mediterranean environments. *Australian Journal of Agricultural Research* 55, 1197-1204.

Piano E., Bertoli F.B., Romani M., Tava A., Riccioni L., Valvassori M., Carroni A.M. and Pecetti L. (2005) Specificity of host-endophyte association in tall fescue populations from Sardinia, Italy. *Crop Science* 45, 1456-1463.

Piano E. and Pecetti L. (2010) Minor legume species. In: Boller B., Posselt UK. and Veronesi F. (eds) *Handbook of Plant Breeding. Vol. 5. Fodder Crops and Amenity Grasses*, Springer: New York, pp. 477-500.

Rognli O.A., Aavola R., Aleliūnas A., Asp T., Brazauskas G., Gylstrøm K.H., Helgadottir A., Isolahti M., Kovi M.R., Kristjánsdóttir T.A., Larsen A.S., Marum P., Paina C., Persson C. and Rancāne S. (2018) Utilization of genebank accessions to improve northern adaptation of perennial ryegrass (*Lolium perenne* L.). In: Brazauskas G., Statkeviciute G. and Jonaviciene K. (eds.): *Breeding Grasses and Protein Crops in the Era of Genomics*, Springer International Publishing AG, pp. 3-8.

Sampoux J.P., Baudouin P., Bayle B., Béguier V., Bourdon P., Chosson, J.F., Deneufbourg F., Galbrun C., Ghesquiere M., Noel D., Pietraszek W., Tharel B. and Viguie A. (2011). Breeding perennial grasses for forage usage: an experimental assessment of trait changes in diploid perennial ryegrass (*Lolium perenne* L.) cultivars released in the last four decades. *Field Crops Research* 123, 117-129.

Sampoux J.-P., Giraud H. and Litrico I. (2020) Which recurrent selection scheme to improve mixtures of crop species? Theoretical expectations. *G3: Genes|Genomes|Genetics* 10, 89-107.

Thivierge, M.-N., G. Jégo, G. Bélanger, A. Bertrand, G. F. Tremblay, C. A. Rotz and B. Qian (2016). Predicted yield and nutritive value of an alfalfa-timothy mixture under climate change and elevated atmospheric carbon dioxide. *Agronomy Journal* 108, 585-603.

Viljanen N., Honkavaara E., Näsi R., Hakala T., Niemeläinen O. and Kaivosoja J. (2018) A novel machine learning method for estimating biomass of grass swards using a photogrammetric canopy height model, images and vegetation indices captured by a drone. *Agronomy* 8, 70.

Volaire F. (2008) Plant traits and functional types to characterise drought survival of pluri-specific perennial herbaceous swards in Mediterranean areas. *European Journal of Agronomy* 29, 116-124.

Volaire F. and Lelièvre F. (2001) Drought survival in *Dactylis glomerata* and *Festuca arundinacea* under similar rooting conditions in tubes. *Plant Soil* 229, 225-234.

Volaire F., Norton M.R. and Lelièvre F. (2009) Summer drought survival strategies and sustainability of perennial temperate forage grasses in Mediterranean areas. *Crop Science* 49, 2386-2392.

Walter A., Studer B. and Kölliker R. (2012). Advanced phenotyping offers opportunities for improved breeding of forage and turf species. *Annals of Botany* 110, 1271-1279.

Wang J., Badenhorst P., Phelan A., Pembleton L., Shi F., Cogan N., Spangenberg G. and Smith K. (2019) Using sensors and unmanned aircraft systems for high-throughput phenotyping of biomass in perennial ryegrass breeding trials. *Frontiers in Plant Science* 10, 1381.

Yang W., Feng H., Zhang X., Zhang J., Doonan J.H., Batchelor W.D., Xiong L. and Yan, J. (2020) Crop phenomics and high-throughput phenotyping: past decades, current challenges, and future perspectives. *Molecular Plant* 13, 187-214.

Yates S., Jaškūnė K., Liebisch F., Nagelmüller S., Kirchgessner N., Kölliker R., Walter A., Brazauskas G. and Studer B. (2019). Phenotyping a dynamic trait: leaf growth of perennial ryegrass under water limiting conditions. *Frontiers in Plant Science* 10, 344.

Building high herbage masses in autumn for the extension of the grazing season

Looney C.[1,2], Wingler A.[2] and Egan M.[1]

[1] *Teagasc Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland;* [2] *School of Biological, Earth & Environmental Sciences, University College Cork, Distillery Fields, North Mall, Cork, Ireland*

Abstract

Targeting a high pre-grazing herbage mass in early autumn facilitates the extension of the grazing season and maintains grass within the diet of a lactating dairy cow; however, ceiling yield can be reached at higher herbage masses, resulting in reduced grass growth. The objectives of the current experiment were: (1) to determine the effect of defoliation date (DD) on the actual herbage mass (AHM) in autumn and (2) the effect of these different target herbage mass (THM) and DD on the spring grass availability. An experiment was established with three DD (15 Oct, 7 Nov and 21 Nov) and four THM (low, medium, high, very high) on the 15 Oct. The AHM (>3.5 cm kg DM ha^{-1}) was measured on each DD in autumn and again in spring. Pre-grazing sward heights were taken prior to defoliation. Data were analysed using PROC mixed in SAS. There was a significant (P<0.001) effect of autumn DD on AHM in spring. The very high THM decreased in AHM between DD2 and DD3 (3,666 and 3,177±160.5 kg DM ha^{-1}, respectively). This indicates leaving very high HM swards undefoliated for longer leads to a reduction in AHM. Targeting high herbage mass early in autumn allows for the extension of the grazing season.

Keywords: grazed grass, herbage mass, autumn management, defoliation date

Introduction

Higher utilisation of grazed grass and extension of the grazing season are key objectives for the future sustainability of the pasture-based dairy production system (Donnellan *et al.*, 2018). Grazed grass is the cheapest feed source available for ruminant production systems (Finneran *et al.*, 2010) and it is beneficial to increase the proportion of grazed grass in the diet for autumn as the milk protein concentration decreases as grazed grass is replaced by silage in late lactation (Claffey *et al.*, 2019). Extending the autumn grazing season requires specific grazing management targets, as growth rates are reduced in autumn due to environmental conditions (Hennessy *et al.*, 2006). As a result of reduced grass growth in late autumn, large quantities of herbage are built up, prior to the grass growth decline in autumn, to facilitate the extension of the grazing season (Claffey *et al.*, 2019). Herbage continues to accumulate over this period albeit at a slower rate and tissue turnover still occurs even when swards have reached a ceiling yield (no further accumulation of herbage) (Brereton *et al.*, 1985). The actual herbage mass (AHM; >3.5 cm kg DM ha^{-1}) available to graze could have a variable herbage mass (HM) containing different proportions of green leaf, stem and dead material; this is dependent on when the target herbage mass (THM) is reached and when the defoliation date (DD) occurs. Consideration must also be given in autumn to future spring grass availability, as the value of spring grass in terms of animal production is well documented (Claffey *et al.*, 2019). Closing swards earlier in autumn results in a greater HM available the following spring (Hennessy *et al.*, 2006; Lawrence *et al.*, 2017; Ryan *et al.*, 2010). The objectives of the current experiment were (1) to determine the effect of DD on the actual AHM in autumn and (2) the effect of these different THM and DD on the AHM in spring.

Materials and methods

The current experiment was carried out in autumn 2018 at the Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Ireland (50°07'N, 08°16'W). The soil type at the experimental site was a free-draining acid brown earth of sandy to loam texture. Soils had a pH of 6.4, phosphorus (P) Index of 4 and potassium (K) Index of 3. The experiment was a 3×4 factorial design with three DD (15 Oct, 7 Nov and 21 Nov) and four THM (500 – low; 1,500 –medium; 2,000 – high; and 3,000 – very high; kg DM ha^{-1}) by 15 Oct. Forty-eight plots (1.5×6 m) were used and a split-plot design applied. The four THM were randomly assigned to three plots and within each of the 3 DD. Target HM was built from August and cutting was used to attain the THM for 15 Oct (DD1). Actual HM was measured on DD1, DD2 and DD3 (Measurement period; MP1), and again on all plots on 21 Feb in spring (MP2). A rising plate meter was used to measure the pre-grazing sward height (cm) of the plots prior to defoliation. The entire plot was harvested using the Etesia mower (Etesia UK Ltd., Warwick, UK) and the mown herbage from each strip was collected and weighed. A sample of 100 g of herbage was collected from each cut strip and dried for 16 h at 90 °C to determine DM content. From this the AHM was calculated. Data were analysed using a PROC mixed model in SAS 9.4 (SAS Institute Inc., Cary, NC, USA, 2002), where DD, THM and MP were included in the model, with plot the experimental unit.

Results and discussion

There was a tendency (P=0.08) for an interaction between DD and THM on AHM in MP1 (Figure 1). Actual herbage mass increased for the low and high THM treatments from DD1 to DD2 and DD3. Whereas AHM for the medium HM increased from DD1 to DD2 (1,241 to 2,148±160.5 kg DM ha^{-1}, respectively), AHM did not increase from DD2 to DD3 (2,182±160.5 kg DM ha^{-1}, respectively). The lack of increase in overall herbage mass over two weeks of growth could reflect the low growth rate (Hennessy *et al.*, 2006). The very high THM increased AHM from DD1 to DD2 (2,587 and 3,666±160.5 kg DM ha^{-1}, respectively); however, there was a reduction in AHM between DD2 and DD3 (3,666 and 3,177±160.5 kg DM ha^{-1}, respectively) as the ceiling yield was attained (Brereton *et al.*, 1985). Actual HM significantly (P<0.001) increased from DD1 to DD2 and DD3 (1,602, 2,398 and 2,516±69.2 kg DM ha^{-1}, respectively) across all THM. This shows that the targeting of the HM used in the current study allows for the extension of the grazing season in autumn. Pre-grazing sward height was significantly (P<0.01) affected by the interaction between DD and THM (Figure 1). For the low and medium THM the pre-grazing sward height increased between all three DD. However, for the high and very high THM, the pre-grazing sward height decreased from DD1 to DD2 ((high; 11.65 to 11.45±0.357 cm, respectively) (very high; 13.82 to 12.63±0.357 cm, respectively)) and increased again between DD2 and DD3 ((high; 11.45 to 11.75±0.357 cm, respectively) (very high; 12.63 to

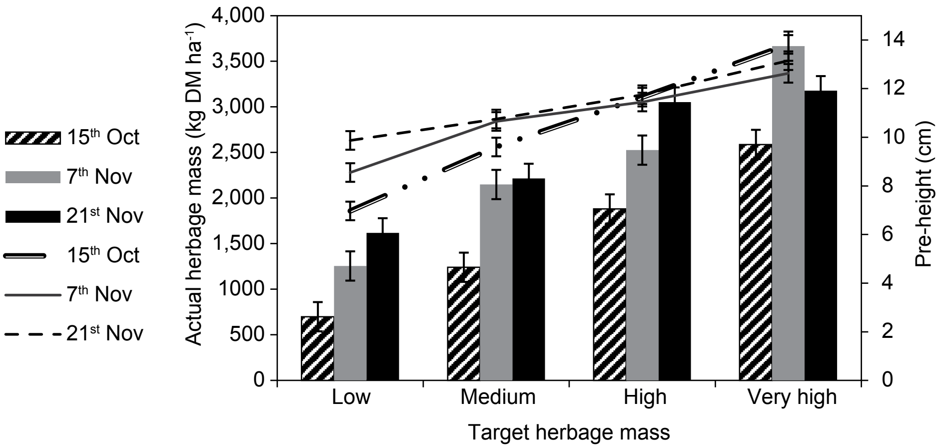

Figure 1. The effect of target herbage mass and defoliation date on the actual herbage mass (primary vertical axis and bar chart; kg DM ha^{-1}) and the pre-height (secondary axis and the line graph; cm).

13.15±0.357 cm, respectively)). There was a significant (P<0.001) effect of autumn DD on AHM in spring. Defoliation date 1 (1,310±36.5 kg DM ha^{-1}) had a greater AHM compared with both DD2 and DD3 (1,058 and 872±36.5 kg DM ha^{-1}, respectively), similar to Lawrence *et al.* (2017), Ryan *et al.* (2010) and Hennessy *et al.* (2006). It is evident, from the above results, that targeting to build high levels of herbage mass in autumn for the extension of the grazing season can be achieved. The very high HM should be defoliated earlier in autumn to ensure grass is utilised and there is no reduction in AHM. Low HM continued to increase herbage mass across the autumn period, and as a result it can be grazed later.

Conclusions

From the current experiment it is concluded that targeting high levels of herbage mass (>1,500 kg DM ha^{-1}) for 15 Oct in autumn in order to extend the grazing season can be achieved successfully. However, leaving these swards undefoliated later into autumn does not increase the AHM, and on very high swards the AHM can reduce. Earlier defoliation of high herbage mass swards will allow for increased utilisation and increase the herbage available for spring.

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References

Brereton A., Carton O. and O'Keeffe W. (1985) Tissue turnover in perennial ryegrass (*Lolium perenne* L.) during winter. *Irish Journal of Agricultural Research*, 24, 49-62.

Claffey A., Delaby L., Boland T. and Egan M. (2019) Implications of adapting autumn grazing management on spring herbage production – the effect on late lactation milk production and the subsequent response in early lactation animal performance. *Livestock Science*, 231, 103870.

Donnellan T., Hanrahan K. and Lanigan G. (2018) Future scenarios for Irish agriculture: Implications for Greenhouse Gas and Ammonia Emissions. *Teagasc, Athenry. June 2018.*

Finneran E., Crosson P., O'Kiely P., Shalloo L., Forristal D. and Wallace M. (2010) Simulation modelling of the cost of producing and utilising feeds for ruminants on Irish farms. *Journal of Farm Management,* 14(2), 95-116.

Hennessy D., O'Donovan M., French P. and Laidlaw A. (2006) Effects of date of autumn closing and timing of winter grazing on herbage production in winter and spring. *Grass and Forage Science,* 61(4), 363-374.

Lawrence D.C., O'Donovan M., Boland T.M. and Kennedy E. (2017) Effects of autumn and spring defoliation management on the dry-matter yield and herbage quality of perennial ryegrass swards throughout the year. *Grass and Forage Science,* 72(1), 38-49.

Ryan W., Hennessy D., Murphy J. and Boland T. (2010) The effects of autumn closing date on sward leaf area index and herbage mass during the winter period. *Grass and Forage Science,* 65(2), 200-211.

Response of grass yield to soil acid-extractable potassium

Kurki P.[1], Kykkänen S.[2], Termonen M.[2], Mustonen A.[2], Korhonen P.[2] and Virkajärvi P.[2]
[1]*Natural Resources Institute Finland (Luke), Mikkeli, Finland;* [2]*Natural Resources Institute Finland (Luke), Maaninka, Finland*

Abstract

In Finland, forage grass yields respond more to soil acid-extractable potassium concentration (K_{HCl}) than to the traditionally analysed soil acid ammonium acetate-extractable potassium concentration (K_{AAc}). A set of four-year grass experiments was established in 2011 and treated with K mineral fertiliser and cattle slurry. To confirm the long-term effects, the same plots were followed at two of the sites for another four-year period with the same treatment. The effect of the cattle slurry and mineral K-fertilisation (0, 50, 100, 150, 200 kg ha^{-1} yr^{-1}) on grass dry matter (DM) production and K concentration and soil K_{AAc} and K_{HCl} was measured. The study was carried out as a split-plot experiment. At Site1, soil K_{HCl} was high and, under dry conditions in 2018, K fertilisation (50 kg K ha^{-1} yr^{-1}) increased grass DM yield by 470 kg ha^{-1} with no-slurry (S0) and 390 kg ha^{-1} with slurry (S). At Site2, in 2017 K fertilisation with rates up to 200 kg ha^{-1} yr^{-1} increased the grass DM yield and also the K concentration, despite medium to high soil K_{HCl}. After eight years of no K-fertilisation, soil K_{AAc} had declined to a poor level at Site2.

Keywords: dry matter production, grass, K fertilisation, soil acid-extractable K

Introduction

In Finland, the recommendation of K fertilisation for cultivated short-term grass leys has traditionally been based on concentration of ammonium acetate-extractable K (K_{AAc}) in the soil (Vuorinen and Mäkitie, 1955). Based on a meta-analysis of 17 Finnish experiments with 36 different K-fertilisation levels (Virkajärvi *et al.,* 2014) soil acid-extractable potassium (K_{HCl}) was found to be a better predictor of yield response than K_{AAc}. The meta-analysis included only studies with mineral K-fertilisation, and only four of these included more than one grass rotation on the same plots.

To ensure the effects of K-fertilisation including cattle slurry application, three experiments under different levels of soil K_{HCl} were established in 2011. Contrary to Virkajärvi *et al.* (2014), the effect of K-fertilisation on the grass DM yield was observed occasionally during the first rotation (2011-2014) (Kykkänen *et al.*, 2015). The aim of this study was to continue treatments on a second grass rotation (2015-2018) at two of the previous sites to confirm the long-term effects of K-fertilisation on grass DM yield and K concentration.

Materials and methods

Field experiments, described in detail in Kykkänen *et al.* (2015), were carried out at Site1 (Maaninka, 63°08' N, 27°19' E, sandy loam) and Site2 (Mikkeli, 61°40' N, 27°13' E, loamy sand) in Finland. In spring 2015, the average concentration of K_{HCl} in topsoil (0-25 cm) was high (3,000 mg l^{-1}) and medium (1,300 mg l^{-1}) at Site1 and Site2, respectively (the Finnish Soil Fertility Testing Scheme). At both sites, K_{HCl} in subsoil (25-50 cm) was high (>2,000 mg l^{-1}). The study was carried out as a split-plot experiment with four replicates. The effect of cattle slurry was investigated as a main plot (MP) (slurry, S; no slurry, S0) and mineral K-fertilisation as a sub-plot (SP) (0, 50, 100, 150, 200 kg ha^{-1} yr^{-1}; split in half to the first and the second cut). Both main plots received the same mineral K fertiliser levels. Cattle slurry was injected directly into the soil after the first cut at a mean rate of 30 Mg ha^{-1} and complemented with mineral N to correspond to the amount of soluble N of each cut on the main plot S0 (100+100+40 kg soluble N ha^{-1}, respectively). Plots were established with a mixture of timothy (*Phleum pratense* L., cv. Tenho) and meadow fescue (*Festuca pratensis* Huds., cv. Inkeri) in 2015 using whole crop barley (*Hordeum vulgare* L.) as a cover crop. The cover crop in S plots received 40 and 30 Mg ha^{-1} of cattle slurry at Site1 and Site2, respectively. Mineral K-fertilisation (0, 25, 50, 75, 100 kg ha^{-1}) was applied to all cover crop plots. The grass plots were harvested three times per year over three years. The DM yield and K concentration (NIRS) of yield were measured from each cut. The soil K_{HCl} was analysed with 2 M HCl-solute extraction in boiling water bath (Kaila, 1967) and K_{AAc} with acid ammonium acetate-extraction according to the method described by Vuorinen and Mäkitie (1955). Statistical analyses were performed using ANOVA (Mixed Procedure of SAS 9.4). Due to interaction with treatments and site, experimental sites were analysed separately.

Results and discussion

The effect of K-fertilisation on the grass DM yield differed significantly ($P<0.05$) between sites and years (Figure 1). At Site1, both the topsoil and subsoil K_{HCl} were high and the effect of K fertilisation was less than at Site2. At Site1, in 2018 under dry growth conditions, K fertilisation with 50 kg K ha^{-1} $year^{-1}$ increased grass DM yield by 470 kg DM ha^{-1} on S0 plots and by 390 kg ha^{-1} on S plots (Figure 1). The K concentration of grass averaged 26.9 and 28.0 g kg^{-1} DM^{-1} in plots with 0 and 200 kg^{-1} mineral K ha^{-1} yr^{-1}, respectively. The soil K_{HCl} seemed to be less usable for plants under dry periods, which was in accordance with previous results (Kuchenbuch *et al.*, 1986).

At Site2, the topsoil and subsoil K_{HCl} concentrations were medium and high, respectively. The highest DM yield (7,000 kg DM ha^{-1}) was observed for the S0 plot at the level K200. The least significant ($P>0.03$) difference was between levels K100 (6,060 kg DM ha^{-1}) and K200 (7,000 kg DM ha^{-1}) (Figure 1). Over the years, the yield response to K fertilisation was variable (Figure 1) and the K concentration of grass averaged 19.3 and 32.0 g kg^{-1} DM^{-1} in plots with 0 kg^{-1} ha^{-1} and 200 kg^{-1} mineral K ha^{-1} yr^{-1}, respectively. In the plots without K-fertilisation during 2011-2018, soil K_{AAc} had declined to a poor level and a clear K deficiency was seen, indicating that grass could not use efficiently soil K_{HCl}.

The soil at Site1 was a sandy loam, and Site2 a loamy sand. At Site1 slurry or 50 kg mineral K ha^{-1} yr^{-1} was enough to keep DM yield stable, which is similar to the results of the meta-analysis (Virkajärvi *et*

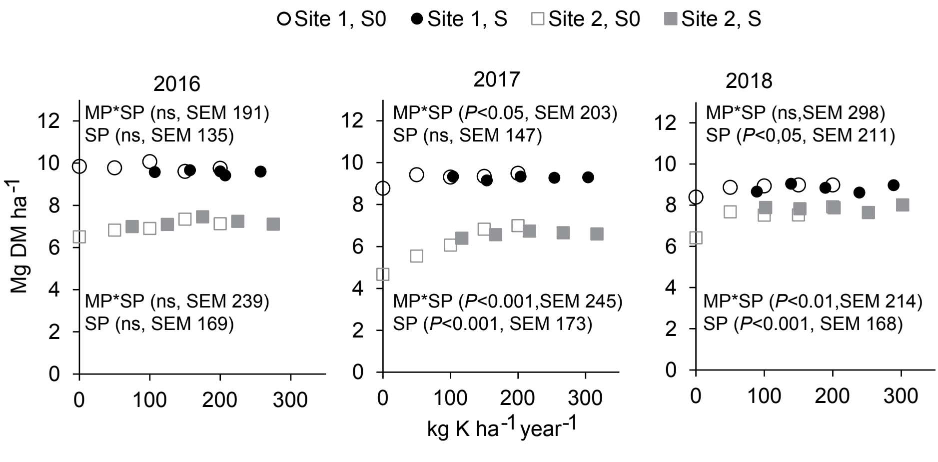

Figure1. Effect of K fertilisation on the grass DM yield (kg ha^{-1} yr^{-1}) at Site1 and Site2 in Finland. Main plot (MP) S0 = No slurry, and S=30 Mg^{-1} ha^{-1} yr^{-1} cattle slurry after the first cut. S plots received both slurry K and mineral K according to the sub plot (SP) K rate of 0, 50, 100, 150, 200 kg ha^{-1} yr^{-1}, respectively. The experimental sites and years were analysed separately. *, **, *** indicate the treatment (MP*SP and SP) effect is significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively, SEM = standard error of the mean, non-significant effect is denoted by ns ($P>0.05$)

al., 2014). In contrast, the results at Site2 differed to some extent from that of the meta-analysis because medium K_{HCl} was not sufficient for grass DM production without K fertilisation. The soil mineralogy, especially the proportions of feldspars and micas in the soil, can be an important determinant of how well different acid K extraction methods predict plant K uptake because, e.g. the K in feldspars is bound covalently to the crystal structures and is harder to extract with acid solutions than K from the micas where it is bound by electrostatic forces between the sheet structure. Extraction of K with cold (20 °C) 2 M HCl has been proposed as a better alternative for predicting K uptake than the more commonly used extraction with boiling 2 M HCl (Saarela and Mäntylahti, 2002), and should thus be considered, together with other potentially better methods, as an option for developing better K analytics.

Conclusions

The response of K fertilisation to DM yield was partly contradictory between the sites despite relatively equal levels of soil K_{HCl}. At Site1, the slurry or 50 kg mineral K ha^{-1} yr^{-1} was enough to keep the DM yield stable. In site2, the K-fertilisation strongly increased the grass DM yield despite a medium level of soil K_{HCl}. The interaction with year and K fertilisation was clear. The extraction methods of soil acid-extractable K have to be studied further.

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References

Kaila, A. (1967) Release of nonexchangeable potassium from Finnish mineral soils. *Journal of the Scientific Agricultural Society of Finland* 39, 107-118.

Kuchenbuch R., Claassen N. and Jungk A. (1986) Potassium availability in relation to soil moisture. *Plant and Soil* 95, 221-243.

Kykkänen S., Virkajärvi P., Hyrkäs M., Järvenranta K., Kurki P. and Suomela R. (2015) The effect of potassium on dry matter production and nutritive value of grass on three different soil types. *Grassland Science in Europe,* 20, 245-247.

Saarela I. and Mäntylahti V. (2002) Release of potassium to plants from micaceous sandy soils. In: Pietola L. and Esala M. (eds.). *Maa, josta elämme. II. Maaperätieteiden päivät*, Helsinki 19-20.11.2002. Finnish Society of Soil Sciences. *Pro Terra* 15, 140-142.

Virkajärvi P., Kykkänen S., Räty M., Hyrkäs M., Järvenranta K., Isolahti M. and Kauppila R. (2014) Potassium fertilization of grass in Finland. Use of acid-soluble potassium as a basis for potassium fertilization. *MTT Report* 165. 54 p.

Vuorinen J. and Mäkitie O. (1955) The method of soil testing in use in Finland. *Agrogeological Publications* 63, 1-44.

The effect of kurzrasen and strip-grazing on grassland performance and load bearing capacity of a peat meadow

Hoekstra N.J.[1], Holshof G.[2], Schils R.L.M.[2], Philipsen B.[2] and Van Eekeren N.[1]
[1]*Louis Bolk Institute, Kosterijland 3-5, 3981 AJ Bunnik, the Netherlands;* [2]*Wageningen University and Research, P.O. Box 9101, 6700 HB Wageningen, the Netherlands*

Abstract

Herd size per farm is increasing in the Netherlands. Therefore, there is need for assessing the performance of different grazing systems at high stocking densities. The objective of the current experiment was to assess the effect of two contrasting grazing systems, kurzrasen (KR, continuous grazing at 3-5 cm sward height) and strip-grazing (SG) at a high stocking rate, on load bearing capacity, sward density, grass production and morphology on peat soil. To this end, a two-year grazing trial with four herds of 15 cows on 2 ha each was conducted. Sward density was higher for KR compared with SG, which had a positive impact on load bearing capacity. This is an important feature on peat soils, where load-bearing capacity is often limited. KR showed on average 18% lower herbage dry matter production than SG. The yield penalty of using a shorter regrowth period under KR was limited due to the strong response in grass morphology, resulting in a dense and lamina-rich sward.

Keywords: grazing systems, swards, load bearing capacity, root density, nutritional value

Introduction

Due to the increased herd size in the Netherlands, there is need for assessing the performance of different grazing systems at high stocking densities. Strip-grazing (SG) is a system in which cows are allowed a fresh strip of pasture herbage (with back-fence) each day, followed by a regrowth period. This system generally results in the highest grass and milk production. Recently, there is increasing interest in kurzrasen (KR), a continuous grazing system, in which sward height is always kept between 3 and 5 cm. Herbage production is generally lower; however, the high quality of the ingested herbage results in a good conversion into milk. A major potential benefit of this system for the Dutch peat meadow region is its positive effect on sward density, which may increase the load bearing capacity.

The objectives of the current experiment were to assess the effect of these two contrasting grazing systems, SG and KR at a high stocking rate, on load-bearing capacity, sward density, grass production and morphology on peat meadows.

Materials and methods

The experiment was conducted on permanent grassland at KTC Zegveld on drained peat soil in the western peat region in the Netherlands (see Hoekstra *et al.*, 2019 for more details). The experimental treatments consisted of two grazing systems, SG and KR, in two replications. In 2016 and 2017, four independent farmlets were formed, each consisting of 15 cows (9 Holstein Friesian, 6 Jersey) on 2 ha, representing a stocking rate of 7.5 LU ha^{-1} on the grazing platform. For KR, each farmlet consisted of one single grazing block, with the aim to maintain a constant sward height between 3 and 5 cm. These fields had already been subjected to a KR grazing regime in 2015, in order to allow the sward to adapt. For SG, cows had access to a new strip each day, with strip-size depending on the herbage supply. Herbage in excess of animal requirements was harvested for silage production. Yearly N application rate was approximately 140 kg N ha^{-1}.

Soil parameters were measured 2-5 times per year on 5×5 m observation plots (two per farmlet) and included load-bearing capacity (analogue penetrometer), soil moisture content (SMC; gravimetric) and sward density (point quadrat method). Herbage measurements included weekly sward height measurements (rising plate meter) and weekly herbage growth rate (for KR under growth cages) based on sward height difference, using a calibration method for conversion into DM yield. Additionally, herbage morphology (tiller length, free-leaf lamina length, number of leaves tiller^{-1}) was measured for 25 tillers per observation plot, 3 times per year.

The effect of grazing system on the measured parameters were analysed with GLM, taking into account the random structure of repeated measures over time and sub-plots within each farmlet, using the LMER package in R 3.4.4.

Results and discussion

Load-bearing capacity was on average 10% higher for KR than for SG (Figure 1A), but the difference was only statistically significant during July 2016, November 2016 and March 2017. Load-bearing capacity is an important parameter that has a large impact on grassland utilisation, as it affects the number of available grazing days. Load-bearing capacity showed a strong negative correlation with SMC, both within and between measurement periods (data not shown). Also, load-bearing capacity was positively correlated with sward density (Figure 1B). The sward density was 39% higher for KR compared with SG (Table 1).

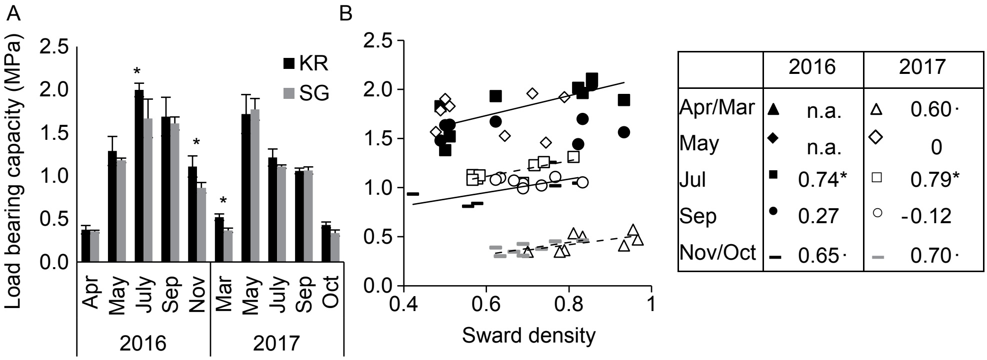

Figure 1. (A) The load-bearing capacity (LBC) for the KR and SG systems during 2016 and 2017. Error bar = 2SE, n=4. (B) Correlations between LBC and sward density (prop. cover) during 2016 and 2017. Regression lines indicate significant correlations or trends as represented in the Table (• = P<0.1; * = P<0.05).

Table 1. Mean sward density, sward height and herbage morphology for KR and pre and post-grazing SG swards.

Parameter[1]	KR		SG-pre		SG-post		P-value[2]
Sward density (prop)	0.80	b	0.60	a			**
Sward height (cm)	4.5	a	11.3	c	6.0	b	*
No. of ungrazed leaves per tiller	1.2	b	2.0	c	0.1	a	**
Total FLL length (cm tiller^{-1})	9.4	a	30.2	b	5.9	a	***
Proportion FLL	0.76	b	0.75	b	0.47	a	***

[1] FLL = free leaf lamina.

[2] * P<0.05; ** P<0.01; *** P<0.001; means followed by different letters in same row are significantly different at P<0.05.

In 2016, the cumulative herbage DM production on the grazed area was 7.4 and 8.8 Mg DM ha^{-1} for kurzrasen and strip-grazing, respectively. In 2017, the cumulative herbage dry matter production was 23% higher for strip-grazing than kurzrasen (12.8 and 10.4 Mg DM ha^{-1}, respectively). Generally, highest herbage yields are achieved by grazing at the two to three leaf stage, to a residual grazing height of approximately 5 cm. Just after grazing, there is relatively little photosynthetically active plant material (i.e. laminae), and growth is mainly dependent on carbohydrate reserves in the stubble and roots. As soon as sufficient lamina material has developed, further growth is based on photosynthesis, and at this stage the sugar reserves can be replenished. If swards are grazed too often (before the sugar reserves have been restored), this can result in decreased growth capacity. In the current experiment, on average, the leaf stage at time of grazing was lower for KR than for SG, and the residual grazing height was also lower for KR (Table 1).

However, the yield penalty for KR was smaller than expected, as a result of the morphological changes in the perennial ryegrass plants in response to these different systems.

For KR, the amount of lamina material (expressed as free-leaf lamina length per tiller) was smaller than the pre-grazing free-leaf lamina length of SG. However, just after grazing, the free-leaf lamina length was greater for KR than for SG. Additionally, free leaf lamina, as a proportion of total tiller length, was always greater for KR (Table 1). This might imply that the amount of photosynthetically active material just after defoliation is less limiting for the regrowth of the KR sward (but is even higher for KR compared with SG) and therefore the depletion of sugar reserves just after defoliation is smaller. Additionally, the KR system resulted in a much higher sward density, and the lower productivity per tiller was partly compensated by an increase in the number of tillers.

Conclusions

Sward density was higher for KR than for SG, and this had a positive effect on load-bearing capacity. Therefore, KR may be employed as a strategy to increase the load-bearing capacity and therefore to increase the number of days available for grazing and to reduce sward damage caused by treading. KR showed a lower amount of herbage dry matter production compared with SG. The yield penalty of using a shorter regrowth period under KR was limited due to the strong response in grass morphology, resulting in a dense and lamina-rich sward, even at very low stubble heights.

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References

Hoekstra,N.J., Holshof G., Schils R., Philipsen B., van Reenen K., van Houwelingen K. and van Eekeren N. (2019) The effect of kurzrasen and strip-grazing on grassland performance and soil quality of a peat meadow. *Sustainability,* 11, 6283.

Effect of the regrowth on the prediction of forage quality based on growing degree days

Peratoner G., Figl U., Mittermair P., Soini E. and Matteazzi A.
Laimburg Research Centre, Vadena/Pfatten, 39040 Ora/Auer (BZ), Italy

Abstract

The web application webGRAS (https://webgras.civis.bz.it) provides an on-line estimation of the potential forage quality of permanent meadows at the first cut by means of statistical predictive models. As these models rely on a sequential sampling of forage at a large number of environments, the extension of this application to the regrowths implies time-consuming sampling activities. The information whether the first regrowth can be used as a representative for all other subsequent regrowths represents, therefore, pivotal information for the preparation of an efficient investigation plan. At nine environments a sequential sampling at the first cut and of the following two regrowths was performed in a randomized complete block design, and the forage quality of the samples was determined in the laboratory. The statistical analysis, including also growing degree days (GDD) as a covariate, showed a differentiation between all the regrowths. This shows that the models based on GDD alone are not sufficient to describe the forage quality regardless of the regrowth and that a sampling campaign of each regrowth or the use of correction factors would be necessary.

Keywords: permanent meadows, potential forage quality, growing degree days, regrowth effect

Introduction

The web application webGRAS (https://webgras.civis.bz.it) provides an on-line estimation of the potential forage quality of permanent meadows at the first cut by means of statistical predictive models which are built on the basis of a dataset describing the changes of forage quality along the phenological development of grassland and relying on a weekly sequential sampling at each environment (site × year) at seven sampling events starting from the stage of stem elongation (Peratoner *et al.*, 2016). An extension of the application to regrowths following the first cut would be a desirable complement, in order to provide the users with a comprehensive description of the potential forage quality during the whole growing season. However, the intensive and time-consuming sampling activities are major issues for achieving this aim. If performed for each regrowth, they require an effort being a multiple of those performed for the first cut. In order to optimise the effort for sampling activities, this study explores the possibility to use only one of the regrowths to develop statistical models based on the sampling of just one regrowth following the first one.

Materials and methods

A three-year field experiment was conducted at three sites in South Tyrol, NE Italy (Salern/Vahrn: 46°44'11" N; 11°37'59" E, 725 m a.s.l, aspect W, 12% slope; Dietenheim: 46°48'05" N; 11°57'36" E, 920 m a.s.l., aspect SW, 8% slope; Aldein: 46°21'49" N; 11°22'60" E, 1415 m a.s.l., aspect SE, 20% slope). The effect of the factor regrowth ($1^{st}/2^{nd}/3^{rd}$) was investigated. Each plot was assigned to one of the regrowth treatments and small quadrats (four replicates of 0.5×0.5 m) were randomly sampled within the same plot for each sampling date during the respective regrowth. For the first cut, sampling starting time (SST) was at a sward height of 15 cm and sampling was performed weekly for a total of seven events. For the regrowths, SST was three weeks after mowing the previous regrowth. The mowing of the whole plot area was synchronized with the harvesting dates of the surrounding meadows of the farm at which the experiment took place. Also, for the regrowths, seven sampling events were performed. For each sample, the plant stand type was visually assessed based on the yield proportion of grasses, forbs and legumes and classified into four categories: rich in grasses, balanced, rich in forbs, or rich in legumes (Peratoner *et al.*, 2018). The plots were arranged according to a randomized complete block design with four replicates. The field was randomised each year. Details about sampling and forage analysis are given in Peratoner *et al.* (2020). At each site, air temperature at 2 m above ground was recorded during the whole growing season. Growing degree days (GDD) were computed within the time span between one week before SST and the harvest date. A base temperature according to Romano *et al.* (2014) was used.

The data were analysed by means of mixed models accounting for the design effects (site, year, site × year, block × site and block × site × year) as random terms, as well as for the plant stand type and the regrowth as fixed effects. The repeated measurements due to sampling over time within the same plot were taken into account with a spatial power covariance structure based on the time in days since SST. The effect of GDD, included into the model as a covariate, was modelled by means of polynomial regression, using the Akaike Information Criterion for determining the degree of the polynomial. Normal distribution of the residuals and homoscedasticity were visually checked, and appropriate data transformation of the dependent variable was carried out if necessary. Post hoc comparisons between regrowths were made by Least Significant Difference.

Results and discussion

The relationship between GDD and the forage quality parameters was found to be best described in most cases by a second-order polynomial (Table 1 and 2). Only for Ca, Mg and Na was first order polynomial already sufficient. As expected, based on the predictive models implemented in webGRAS, the fibre components (neutral detergent fibre (NDF), acid detergent fibre (ADF)) and Ca increased by increasing GDD, whilst the opposite was true for all other parameters. The plant stand type also affected about half of the investigated parameters (crude ash (CA), crude protein (CP), NDF, net energy for lactation (NEL), Ca and Mg).

The regrowth was found to affect all parameters but ADF, with an increase of the value of the dependent variable from the first to the third regrowth. The lowest value of the 2nd regrowth was only for Na and Fe. In general, there was not only a clear differentiation between the first and the following regrowths,

Table 1. *P*-values of the investigated factors (growing degree days (GDD), plant stand type and regrowth) on crude ash (CA), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and net energy for lactation (NEL). Regression parameters as well as estimated marginal means (EMMEANS) ± standard errors for each regrowth are shown.[1]

	CA (g kg^{-1})	CP (g kg^{-1})	NDF (g kg^{-1})	ADF (g kg^{-1})	NEL (MJ kg^{-1})
P-values of effects					
Regrowth	<0.001	<0.001	<0.001	0.2016	<0.001
Plant stand type	<0.001	<0.001	<0.001	0.0735	<0.001
GDD	<0.001	<0.001	<0.001	<0.001	<0.001
GDD^2	<0.001	<0.001	<0.001	<0.001	<0.001
Parameter estimates for GDD					
Intercept	106.99	257.56	326.99	242.1	6.2453
GDD	-0.03664	-0.227	0.2229	0.2142	-0.00257
GDD^2	0.000027	0.000139	-0.00012	-0.00012	1.296E-6
EMMEANS for regrowth					
1st	82.4±2.1^a	153.0±7.1^a	428.7±5.5^a	293.1±10.6^a	5.62±0.11^a
2nd	91.0±2.1^b	174.4±7.1^b	433.1±5.6^a	296.7±10.6^a	5.73±0.11^b
3rd	96.9±2.1^c	186.5±7.1^c	401.7±5.2^b	289.6±10.5^a	5.91±0.11^c

[1] Means without superscript letters in common significantly differ at *P*=0.05.

Table 2. *P*-values of the investigated factors (growing degree days (GDD), plant stand type and regrowth) on concentrations of mineral elements.[1]

	Ca (g kg^{-1})	P (g kg^{-1})	Mg (g kg^{-1})	Na (g kg^{-1})[2]	Zn (mg kg^{-1})	Fe (mg kg^{-1})[1]
P-values of effects						
Regrowth	<0.001	<0.001	<0.001	0.0012	<0.001	<0.001
Plant stand type	<0.001	0.0989	0.0032	0.4660	0.7415	0.0924
GDD	<0.001	<0.001	0.0152	0.0409	0.0002	<0.001
GDD^2		0.0103			0.0071	<0.001
Parameter estimates for GDD						
Intercept	12.4517	4.3122	4.1099	0.5635	47.5848	162.89
GDD	0.002475	-0.0282	0.000506	-0.00008	-0.02286	-0.2835
GDD^2		$1.518E^{-6}$			0.000015	0.0002
EMMEANS for regrowth						
1st	8.7 ± 0.9^a	2.9 ± 0.2^a	2.8 ± 0.2^a	0.428^{ab}	29.2 ± 2.0^a	97.4^b
2nd	10.1 ± 0.9^b	3.2 ± 0.2^b	3.5 ± 0.2^b	0.388^a	34.7 ± 2.0^b	76.6^a
3rd	12.2 ± 0.9^c	3.6 ± 0.6^c	4.2 ± 0.2^c	0.466^b	41.4 ± 2.0^c	95.3^b

[1] Means without superscript letters in common significantly differ at P=0.05.

[2] Analysis with log-transformed (base 10) data. Back-transformed functions and means are shown.

which was expected because of the high proportion of grasses entering the generative phase in the first regrowth, but also between the following regrowths. In most cases, the difference between the first and the second regrowth and that between the second and the third regrowths were similar. This suggests that factors other than GDD (representing the phenological advance) cause these differences. We tentatively suggest that this may be caused by a shift in the botanical composition concerning species less affected than grasses by GDD, i.e. legumes or forbs.

Conclusions

The differences found between the forage quality parameters of the 2nd and of the 3rd regrowth show that the model based on GDD alone is not sufficient to describe the forage quality regardless of the regrowth to be estimated. This suggests that a sampling campaign of each regrowth or the use of correction factors based on the results presented here will be necessary.

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References

Peratoner G., Kasal A., Florian C. and Pramsohler M. (2018) Accuracy of visual assessment of the plant stand type for the estimation of forage quality. *Grassland Science in Europe* 23, 950-952.

Peratoner G., Niedrist G., Figl U., Della Chiesa S., Vitalone L. and Matteazzi A. (2020) Effect of growing degree days and soil moisture on forage quality. *Grassland Science in Europe*, this volume.

Peratoner G., Romano G., Schaumberger A., Piepho H.-P., Resch R. and Bodner A. (2016) webGRAS: a web application to estimate the potential forage quality of mountain permanent meadows. *Grassland Science in Europe* 21, 203-205.

Romano G., Schaumberger A., Piepho H.-P., Bodner A. and Peratoner, G. (2014) Optimal base temperature for computing growing degree-day sums to predict forage quality of mountain permanent meadow in South Tyrol. *Grassland Science in Europe* 19, 655-657.

Sensitivity of soil quality indicators in agricultural land

Baizán S., Vicente F. and Martínez-Fernández A.
Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), P.O. Box 13, 33300 Villaviciosa (Asturias), Spain

Abstract

Preserving quality and health of agricultural soils is one of the main aims of sustainable agriculture. Simple, fast and reliable soil quality indicators are necessary to evaluate the agro-ecosystem sustainability. The aim of this study was to evaluate the changes produced in chemical and biological parameters with different managements of fodder crops to determine their capacity as indicators of soil quality. Three adjacent plots were used, each with different managements: chemical fertilisation and Italian ryegrass (IR); organic fertilisation and faba bean crop (FB); and organic fertilisation and faba bean-Italian ryegrass intercrop (FBIR). In each plot the rotation was completed with maize. Soil samples were taken randomly at nine points in each plot from the upper layer, after each winter and summer harvest, during two consecutive years, and several chemical and biological parameters were analysed. Nitrogen was the chemical parameter that presented less balance, and potassium showed a tendency of separation among treatments. Earthworm abundance and soil mesofauna were the main biological parameters, whereas the respiratory quotient presented a lower balance. The biological indicators were more sensitive and able to differentiate faster among treatments than chemical indicators; these need a greater response time.

Keywords: agricultural management, sustainability, fertilisation, legumes

Introduction

Physicochemical parameters are the main indicators used to assess soil quality. Nevertheless, several years are required to detect significant changes as a result of disturbances in the soil (Costa *et al.*, 2014; Trasar-Cepeda *et al.*, 2008). Biological and biochemical properties, however, may respond more quickly to disturbances caused by management activities than physical and/or chemical properties (Paz-Ferreiro *et al.*, 2007) and so they are more suitable for estimating changes in soil over a shorter time scale. The aim of this study was to assess the changes produced by different management practices on soil physicochemical and biological parameters in order to evaluate their capacity as sensitive soil quality indicators.

Materials and methods

The study was undertaken at the SERIDA experimental farm (43°28'20" N, 5°26'10" W; 10 m a.s.l.) which has a climate with warm template according to Papadakis climate classification. The experiment was established on Mollisol Order, Udol Suborder, Halpludol Group and Fluventic Subgroup (Soil Survey Staff, 2014) with a sandy-loam texture (11% clay, 13% silt and 76% sand). There were three adjacent one ha plots with different managements, each was used during two consecutive years: chemical fertilisation and Italian ryegrass (*Lolium multiflorum* Lam.) crop (IR); organic fertilisation and faba bean (*Vicia faba* L.) crop (FB); and organic fertilisation and faba bean-Italian ryegrass intercrop (FBIR). The rotation was completed with maize (*Zea mais* L.) in all plots. The sowing and fertiliser rates were carried out according to Baizán *et al.* (2018). Soil samples were taken randomly in nine points on each plot from the 0-20 cm layer depth. Chemical parameters were determined at University of Oviedo following the methodology described by Afif-Khouri *et al.* (2011). Biological parameters were determined at Department of Ecology and Natural Resources of Neiker-Tecnalia (Spain) using the protocols described by Soilmontana (2012). Results were contrasted with R (R Core Team, 2017) using a two-way ANOVA, considering management (crop and fertilisation) as main factor and the year as random effect. Means were compared using Duncan's Multiple Range Test.

Results and discussion

Soil chemical parameters and main edaphic services of plots are shown in Table 1. The IR management treatment presented a slightly more acidic pH value than FB and FBIR, although no change was made in the pH range, considered suitable for most crops and the maximum nutrient availability (5.6-7.3; Soil Survey Staff, 2014). The nitrogen (N) content was higher in IR than FB and FBIR, but they remained in the adequate range (Junta de Extremadura, 1992). FB and FBIR showed higher concentrations of potassium (K) than IR; however, they were low considering that these plots were fertilised with cattle slurry. The organic matter (OM) did not show significant differences among treatments, suggesting that this parameter may be a poor indicator for agricultural land. Some edaphic services were affected by IR management, with noted decreases for the FB and FBIR managements. Earthworm abundance was higher in FB and FBIR and, consequently, soil infiltration was improved due to earthworm activity (Huggins and Reganold, 2008).

Chemical and biological indicators are shown in Figure 1. K and N had the lowest balance, although some small separation between management treatments was observed for K. The biological indicator

Table 1. Soil chemical properties and main edaphic services according to the Pastoral Agrosystem Health Cards (PAHC) of plots under study.[1]

	IR	FB	FBIR	rse	*P* (M)	*P* (Y)	*P* (M×Y)
Soil chemical properties							
pH	6.18^b	7.16^a	7.07^a	0.562	<0.001	0.574	0.267
Electrical conductivity (dS m^{-1})	0.06	0.09	0.07	0.034	0.130	<0.001	0.050
Organic matter (g kg^{-1})	51.71	52.50	53.80	5.667	0.599	<0.001	0.158
C (g kg^{-1})	30.00	30.45	31.21	3.282	0.597	<0.001	0.155
N (g kg^{-1})	2.08^a	1.80^b	1.79^b	0.328	0.031	0.294	0.044
C/N ratio	14.89^b	17.48^{ab}	18.29^a	3.566	0.036	<0.001	0.131
Ca (cmol$^{(+)}$ kg^{-1})	11.42^b	12.04^{ab}	12.74^a	1.111	0.011	<0.001	0.024
Mg (cmol$^{(+)}$ kg^{-1})	0.61^b	0.69^{ab}	0.76^a	0.132	0.015	<0.001	0.004
K (cmol$^{(+)}$ kg^{-1})	0.24^c	0.42^a	0.30^b	0.060	<0.001	<0.001	<0.001
Na (cmol$^{(+)}$ kg^{-1})	0.91	0.94	0.89	0.252	0.890	0.094	0.981
Al (cmol$^{(+)}$ kg^{-1})	0.01	0.01	0.02	0.022	0.246	<0.001	0.210
Cation exchange capacity (cmol$^{(+)}$ kg^{-1})	13.19^b	14.10^{ab}	14.72^a	1.213	0.006	<0.001	0.010
Al exchangeable (%)	0.06	0.04	0.10	0.115	0.336	<0.001	0.372
P (mg kg^{-1})	17.86^b	20.94^{ab}	23.84^a	4.084	0.002	<0.001	0.147
Edaphic services							
Macrofauna (n°)	3.28	3.72	4.02	0.682	0.213	<0.001	0.467
Mesofauna (BQ)	3.87^c	4.52^b	5.22^a	0.361	<0.001	0.001	0.019
Bacteria (H′)	3.93^b	5.05^a	5.78^a	0.660	0.001	<0.001	0.104
Earthworms (n° m^{-2})	1.47^b	5.03^a	5.25^a	1.068	<0.001	<0.001	0.099
Infiltration (min)	6.52^b	8.40^a	8.73^a	0.887	0.002	0.001	0.003
Basal respiration mg (C $kg^{-1}h^{-1}$)	6.82^b	8.62^a	8.78^a	0.514	<0.001	0.002	0.128
Induced respiration mg (C $kg^{-1}h^{-1}$)	2.58^b	3.43^a	3.37^a	0.258	<0.001	<0.001	0.158
Respiratory quotient (qCO_2)	1.00	1.00	1.00	<0.001	0.397	0.337	0.397
Compaction (MPa)	5.75^b	6.68^a	6.73^a	0.698	0.053	0.058	0.004
Soil colour (colour)	6.17	6.67	6.58	0.425	0.134	0.422	0.797
CO_2 emissions (g CO_2 m^{-2} h^{-1})	7.98^a	7.13^b	6.52^b	0.493	<0.001	0.358	0.130

[1] IR = Italian ryegrass crop and chemical fertilisation; FB = faba bean and organic fertilisation; FBIR = faba bean-Italian ryegrass intercrop and organic fertilisation; rse = residual standard error; M = management (crop and fertilisation); Y = year. [a,b,c] Different letters indicate significant differences among managements (*P*>0.05).

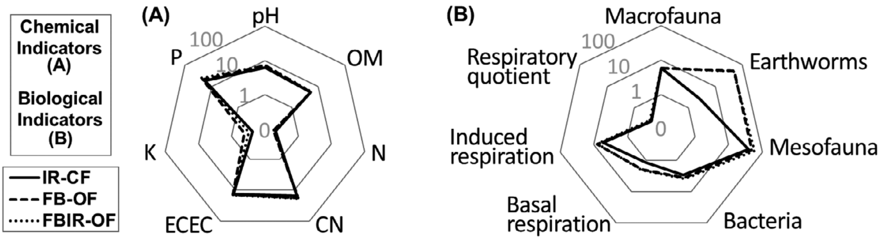

Figure 1. Soil chemical and biological indicators of the plots under study. IR = Italian ryegrass crop and chemical fertilisation; FB = faba bean and organic fertilisation; FBIR = faba bean-Italian ryegrass intercrop and organic fertilisation; OM = organic matter; CN = carbon/nitrogen ratio; ECEC = effective cation exchange capacity. Indicators units were balanced using a logarithmic scale.

of least balance was the respiratory quotient, while the abundance of earthworms and the mesofauna showed a clear separation among managements.

Conclusions

The biological indicators were more sensitive and able to show faster differentiation among the different treatments than chemical indicators due to these needing a greater response time.

Acknowledgements

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References

Afif-Khouri E., Álvarez-Álvarez P., Fernández-López M.J., Oliveira-Prendes J.A. and Cámara-Obregón A. (2011) Influence of climate, edaphic factors and tree nutrition on site index of chestnut coppice stands in north-west Spain. *Forestry* 84, 385-396.

Baizán S., Vicente F. and Martínez-Fernández A. (2018) Haba forrajera en monocultivo o asociada con raigrás italiano: rendimiento y calidad de sus ensilados. In: Roig S. and Barrantes O. (eds.) *Pastoralismo y Vías Pecuarias. Conectando Tradición e Innovación*. 57ª Reunión Científica de la SEP, Teruel, Spain, pp. 478-486.

Costa J.L., Aparicio V.C. and Cerda A. (2014) Soil physical quality changes under different management systems after 10 years in Argentinian Humid Pampa. *Solid Earth Discuss* 6, 2615-2644.

Huggins D.R. and Reganold J.P. (2008) Agricultura sin labranza. *Investigación y Ciencia* 384, 66-73.

Junta de Extremadura. (1992) *Interpretación de análisis de suelos, foliar y agua de riego*. *Consejo de abonado (normas básicas)*, Mundi-Prensa, Madrid, Spain. 280 pp.

Paz-Ferreiro J., Trasar-Cepeda C., Leirós M.C., Seoane S. and Gil-Sotres F. (2007) Biochemical properties of acid soils under native grassland in a temperate humid zone. *NZ Journal of Agricultural Research* 50, 537-548.

R Core Team (2017) R: A Language and Environment for Statistical Computing. Foundation for Statistical Computing, Vienna, Austria. Available from: http://www.R-project.org/.

Soilmontana (2012) Informe de creación de las TSA. Tarjetas de Salud de los Agroecosistemas. Available from: http://www.soilmontana.com/src/uploads/2011/12/Proceso-de-creaci%C3%B3n-de-las-TSA.pdf.

Soil Survey Staff (2014) *Keys to Soil Taxonomy,* 12th ed., USDA-Natural Resources Conservation Service, Washington, DC, USA, 372 pp.

Trasar-Cepeda C., Leiros M.C., Seoane S. and Gil-Sotres F. (2008) Biochemical properties of soils under crop rotation. *Applied Soil Ecology* 39, 133-143.

Estimation of the botanical composition of leys using field spectroscopy and chemometric models

Morel J.[1], Zhou Z.[1,2], Gustavsson A.M.[1] and Parsons D.[1]
[1]*Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umeå, Sweden;* [2]*College of Biosystems Engineering & Food Science, Zhejiang University, 310058 Hangzhou, China P.R.*

Abstract

Mixed leys are among the most important crops in Northern Europe for livestock production. Farming practices and productivity of leys rely to a large extent on their botanical composition, as the nitrogen fertilisation rate will be adjusted based on the clover content. Developing tools that would enable a near-real time estimation of the clover content should lead to increased profitability for the farmer and reduced impacts on the environment through optimised fertilisation. We used a commercially available field spectrometer (Yara-N sensor) to estimate the botanical composition of experimental plots of various mixed leys located at four sites across northern Sweden. Canopy spectral measurements were performed concurrently to harvests, and the estimation of the botanical composition was performed by collecting and hand separating samples. Various chemometric models were built to link the spectral measurements with botanical compositions. Results showed large variations in performances across the models: while partial least square regression only yielded an R^2 of 0.49, support vector regression showed a better accuracy with a coefficient of determination R^2=0.70. As the sensor used in this study is already available commercially, these results, if confirmed, could easily be converted into a practical application for farmers.

Keywords: field spectroscopy, botanical composition, chemometric models, leys, clover

Introduction

Forage crops, predominantly leys, contribute to a large level of total feed for ruminants in Northern latitudes. As a consequence, the productivity and quality of leys have a large effect on the economic efficiency of meat and dairy production. The botanical composition of leys is particularly important for optimising farming practices, as the recommended rate of fertilisation depends directly on the amount of legumes within the sward. At present, a botanical composition estimation is either performed in the field, with a high risk of bias due to the subjective reading and inherent difficulty of the task, or in the lab (only for research purposes), making the result potentially more accurate but also much more time-consuming. Consequently, a tool that performs near-real time estimation of botanical composition would also lead to a better management of fertilisers, and eventually help to improve the economic and environmental performances of livestock farms.

In recent years, field spectrometers have become increasingly used both in research and industry. Indeed, these sensors capture the information carried by vegetation-reflected light that can ultimately be used to evaluate traits of the vegetation. Recent solutions have been developed using field sensors to evaluate the vigour of crops (Zhang *et al.*, 2014), their biomass production (Biewer *et al.*, 2009) or nitrogen uptake (Zhou *et al.*, 2019).

Among other sensors, the Yara-N sensor (YNS) is an already a commercially produced tool used mainly to assess nitrogen needs of cereal crops. As a consequence, a YNS-based tool for adjusting fertilisation rates from sensor-estimated botanical composition could easily be implemented at an industrial level, as the sensor is already widely used by farmers in Nordic countries.

Therefore, the main objective of this work is to develop mathematical models that would link the spectral information acquired with a YNS to a lab-determined botanical composition. A Partial Least Square Regression (PLSR) and a Support Vector Regression (SVR) models were adjusted and their respective performances to estimate the botanical composition were assessed using statistical indicators.

Materials and methods

A total of 103 samples were taken at Öjebyn, Röbäcksdalen, Ås and Lännäs (northern Sweden) during the field season of 2017 on experimental plots and production fields with mixtures of grass (timothy, *Phleum pratense* L.) and legume (red clover, *Trifolium pratense* L.). Sample spots included various nitrogen fertilisation rates and botanical compositions. For each sample spot, a round hoop 50 cm of diameter was used to delineate the sample area. The average heights of grass and legume were measured with a ruler. Canopy spectral measurements were performed close to solar noon on clear sky days, with a zenithal viewing angle of 45° using a YNS spectrometer (Yara International ASA, Oslo, Norway) before harvesting the sample. The sensor was held at a constant height of 0.86 m above the canopy and 0.55 m away from the edge of the hoop so that the area measured by the sensor would approximately match with the sampling area. Acquired spectra contained 60 bands ranging from 400 to 1000 nm, with a spectral resolution of 10 nm and a field of view of 25°. The solar irradiance was measured simultaneously using an external sensor and used to convert the spectral raw measurements to reflectance. Samples were cut at 7 cm above the ground level and hand separated into grass and legume fractions. Sub-samples were then oven-dried at 70 °C for 48 h until they reached a constant weight, enabling estimation of botanical composition on a dry weight basis.

Two chemometric models were calibrated to estimate the botanical composition of the samples using the canopy spectral reflectance (CSR), namely a Partial Least Square Regression (PLSR) and a Support Vector Regression (SVR). All analyses were performed using R 3.6.1 (R Core Team, 2019), and SVR and PLSR models were built using the e1071 and pls packages, respectively. For both models, a leave-one-out cross validation was used, as no validation dataset was available for a regular calibration-validation procedure. A radial-basis kernel was used with SVR to account for the potential non-linear relationship between the botanical composition and the spectral data. Models were evaluated using the root mean squared error (*RMSE*) and the coefficient of determination (R^2).

Results and discussion

PLSR and SVR models showed various performances for the estimation of the botanical composition. Although the regression slopes of both models' predictions are relatively close to the 1:1 line (Figure 1), the PLSR results indicated a poor correlation between the values simulated by the model and the reference ones, with a R^2 of 0.49 and a *RMSE* of 11.0. On the other hand, SVR showed better predictive performances, with a R^2 of 0.70 and a *RMSE* of 8.4. The PLSR model showed a large scattering of values around the 1:1 line, whereas for SVR the scattering tended to increase with the amount of legumes in the sample (Figure 1).

The differences in performance between PLSR and SVR could probably be explained by the inherent differences of both models, the latter being able to deal with non-linear relationships thanks to a non-linear kernel.

Interestingly, the grass to clover height difference did not affect the accuracy of the results (Figure 1). Indeed, it could have been expected that samples where the grass height largely exceeded the legume height would have a poor prediction of botanical composition, as the effects of legumes on reflected light would be masked by those of grasses. Nevertheless, both PLSR and SVR indicate that there is no influence of the grass to clover height difference.

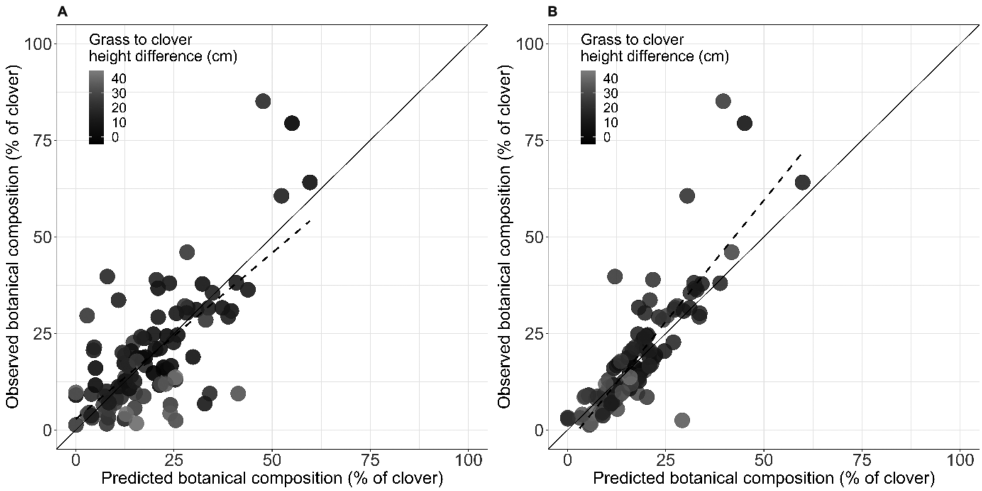

Figure 1. Predicted vs observed botanical compositions for (A) Partial Least Square Regression (PLSR) and (B) Support Vector Regression (SVR).

Conclusions

This work proved the potential of spectral devices associated with SVR for performing estimations of botanical composition. The results, if confirmed, could be developed rapidly as a tool that would help farmers improve their profitability and reduce their environmental footprint.

Acknowledgements

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References

Biewer S., Fricke T. and Wachendorf M. (2009) Determination of dry matter yield from legume–grass swards by field spectroscopy. *Crop Science*, 49, 1927-1936. https://doi.org/10.2135/cropsci2008.10.0608

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Zhang F., John R., Zhou G., Shao C. and Chen J. (2014). Estimating canopy characteristics of inner Mongolia's grasslands from field spectrometry. *Remote Sensing,* 6, 2239-2254. https://doi.org/10.3390/rs6032239

Zhou Z., Morel J., Parsons D., Kucheryavskiy S.V. and Gustavsson A.-M. (2019) Estimation of yield and quality of legume and grass mixtures using partial least squares and support vector machine analysis of spectral data. *Computers and Electronics in Agriculture,* 162, 246-253. https://doi.org/10.1016/j.compag.2019.03.038

Morphological plasticity of white clover grown in pure and mixed stands

Nölke I.[1,2], Tonn B.[1,2] and Isselstein J.[1,2]

[1]*Division of Grassland Science/Department of Crop Sciences, University of Goettingen, Germany;* [2]*Centre of Biodiversity and Sustainable Land Use (CBL), University of Goettingen, Germany*

Abstract

Environmental factors, including competition between plants, are able to alter functional plant traits. Among them are morphological characteristics linked to light capture. We wanted to provide insight into their plasticity and determinant factors for different *Trifolium repens* populations grown in pure or mixed stands with *Lolium perenne* or *Cichorium intybus*. Therefore, we measured the leaf area, petiole length and internode length over four regrowths. Leaf area and petiole length were related linearly and varied among populations. Across populations, leaf and petiole were largest in *T. repens* pure stands and smallest in mixed stands with *L. perenne*, yet without any population × stand effects. In contrast, internode length varied either between populations or stands, depending on regrowth. *T. repens* morphological characteristics thus reacted plastically and were largely dependent on the competitor identity, but the direction and extent of plasticity was similar for all populations.

Keywords: white clover, morphological characteristics, competition, plasticity

Introduction

Trifolium repens L. (white clover) is characterised by great intraspecific variation in morphological traits related to light capture, such as leaf area (LA), petiole length (PL) and internode length (IL) (Annicchiarico, 2003). These traits affect its competitive ability in mixtures with other forage species and may show a plastic response to changes in the competition environment. For example, *T. repens* PL increased in mixed stands with grasses compared with pure stands as a shade-avoidance reaction (Annicchiarico, 2003), and IL was greater in more heterogeneous competition environments to allow better exploitation of favourable microsites (Bittebiere *et al.*, 2012). This ability to respond to characteristics of mixture partners with morphological changes may be linked with *T. repens* competitive ability. We expected that *T. repens* populations do not only differ in the mean values of LA, PL and IL, but also in the degree of plasticity these traits show in reaction to different partner species. To test this expectation, we grew different *T. repens* populations in pure swards and in mixture with *Lolium perenne* (perennial ryegrass) or *Cichorium intybus* (chicory) and measured their LA, PL and IL over four regrowths.

Materials and methods

The underlying field experiment was set up in late 2017 at a fertile site in north Germany. Eight novel *T. repens* populations from DSV (Deutsche Saatveredelung) with a considerable variability in morphological traits were each grown in unfertilised pure (1000 seeds m^{-2}) or mixed stands with *L. perenne* or *C. intybus* (400:600 seeds m^{-2}). The plots (4.5×5 m) were arranged randomly and replicated in three blocks. In 2019, we harvested all plots four times. Prior to each harvest, we collected fifteen randomly chosen plants per plot (sampling of mixed stands with *L. perenne* started from regrowth two). We selected the youngest fully expanded leaf and the related petiole and internode of each plant to determine LA, PL and IL. Linear models implemented in the package 'nlme' (Pinheiro *et al.*, 2018) of the software R (R Foundation for Statistical Computing, Vienna) were used to model LA, PL and IL as a function of population, stand and block for each regrowth. Further, we modelled PL as a function of LA, with stand, regrowth, their pairwise interactions with LA, and block as fixed effects and plot as random effect. We checked the models for homoscedasticity and normality of residuals and included variance structure functions where necessary. Model reduction was performed based on second order Akaike Information Criterion. As significance tests, we used Wald tests and Tukey tests.

Results and discussion

Both population and stand significantly affected LA and PL in all regrowths (R), while IL was either dependent on population (R1, R4) or stand (R2, R3) (Table 1). However, since no population × stand effects were found, the direction and extent of plasticity in reaction to the competitive environment did not vary between populations. This contrasts with previous studies that found that *T. repens* genotypes differed in their IL response to a grass partner (Annicchiarico, 2003) or their LA response to shading (Weijschedé *et al.*, 2006).

Across populations, LA and PL were greatest in pure stands, and smallest in mixed stands with *L. perenne* (Figure 1). This result contradicts earlier findings of increased PL in mixed compared with pure stands

Table 1. *P*-values for effects on leaf area (LA), petiole length (PL) and internode length (IL) per regrowth (R). Dashes signify effects not retained in the final model.

	Effect	R1	R2	R3	R4
LA	Population	<0.001	<0.001	<0.001	<0.001
	Stand	<0.001	<0.001	<0.001	<0.001
PL	Population	0.002	<0.001	<0.001	<0.001
	Stand	0.026	<0.001	<0.001	<0.001
IL	Population	<0.001	–	–	<0.001
	Stand	–	<0.001	<0.001	–

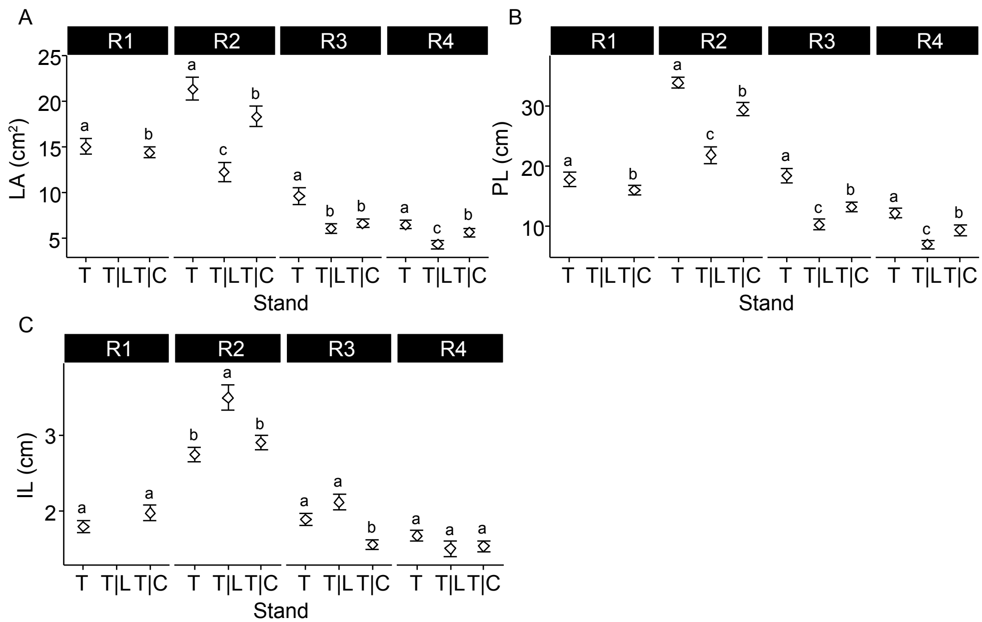

Figure 1. Means and standard error of (A) leaf area (LA), (B) petiole length (PL) and (C) internode length (IL) for *T. repens* pure (T) or mixed stands with *L. perenne* (T|L) or *C. intybus* (T|C). Different letters signify significant differences (P<0.05) between means per regrowth (R).

(Annicchiarico, 2003). The response of IL was also not consistent with previous results. While the caespitose growth form of *C. intybus* meant that it provided the most heterogeneous competition environment as a partner species, mixed stands with *L. perenne* (R2) and mixed stands with *L. perenne* together with pure stands (R3) had the longest IL (Figure 1). We further detected that PL was related linearly to LA in each regrowth, but was additionally dependent on stand (Figure 2), pointing out that PL and LA responded to stand to a different degree.

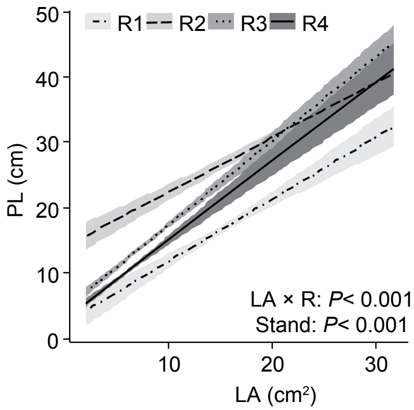

Figure 2. Model estimates and 95%CI for the relation between petiole length (PL) and leaf area (LA) per regrowth (R) and *P*-values for effects on petiole length (PL). Pseudo-R^2=0.94.

Conclusions

Our results indicate that the competitor identity, along with other factors, determines the morphological characteristics linked to light capture in *T. repens*, but that the direction and extent of morphological plasticity did not differ between *T. repens* populations.

Acknowledgements

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References

Annicchiarico P. (2003) Breeding white clover for increased ability to compete with associated grasses. *Journal of Agricultural Science* 140, 255-266.

Bittebiere A.-K., Renaud N., Clément B. and Mony C. (2012) Morphological response to competition for light in the clonal *Trifolium repens* (Fabaceae). *American Journal of Botany* 99, 646-654.

Pinheiro J., Bates D., DebRoy S., Sarkar D. and R Core Team (2018) nlme: Linear and Nonlinear Mixed Effects Models, version 3.1-137, https://CRAN.R-project.org/package=nlme.

Weijschedé J., Martínková J., De Kroon H. and Huber H. (2006) Shade avoidance in *Trifolium repens*: costs and benefits of plasticity in petiole length and leaf size. *New Phytologist* 172, 655-666.

Prediction of herbage mass in pure stands of lucerne and red clover using a plate meter

Paczkowski A.[1,2], Isselstein J.[2] and Hartmann S.[1]

[1]*Bavarian State Research Center for Agriculture (LfL), Institute for Crop Science and Plant Breeding, Germany;* [2]*Department of Crop Science, Division of Grassland Science, University of Göttingen, Germany*

Abstract

The plate meter method is a simple and widely used method to estimate herbage mass in pasture management since there is a strong relation between herbage height and mass within the sward. In our experiment, we evaluated three plate meter calibration models in pure stands of red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*) for prediction of herbage mass. The experiment was conducted during the growing season 2018 in Freising, Germany. Plant stand height was measured by lowering the acrylic plate along the ruler. The best-fitted prediction model, based on measured plant height and sampling date, explained 94 and 96% of the variation in predicted herbage mass of lucerne and red clover, respectively.

Keywords: herbage mass prediction, plate meter calibration

Introduction

Plate meters (PM), e.g. rising or falling plate meters, are commonly used to estimate herbage mass and are useful for pasture management. They are seldomly used in cutting management due to decreasing accuracy with increasing stand height. However, the application of PM in cutting management could help breeders estimate the yield over the entire growing period. Plant breeders cope with extremely high numbers of plots and almost always determine only the final biomass yield when registering the herbage mass. PM could show the change in crop growth rate (CGR), in particular, since CGR responds sensitively to short-term fluctuating environmental conditions (such as drought-stress phases). Moreover, CGR determination across a range of varieties is time consuming and PM could accelerate the CGR determination process. Despite this, few researchers have described the use of the PM method to predict herbage mass in pure stands of red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*) (Griggs and Stringer, 1988), or for red clover and lucerne in mixed stands (Harmoney *et al.*, 1997).

Materials and methods

The experiment was carried out in 2018 at the Bavarian Research State Centre for Agriculture in Pulling near Freising, Germany. Long-term annual average temperature for the location is 8.8 °C and long-term mean annual rainfall 820 mm. The experimental year 2018 was the hottest and driest since the start of weather recording for the location with annual average temperature of 10.2 °C and mean annual rainfall 745 mm.

Pure stands of six lucerne and five tetraploid red clover varieties were sown in a completely randomized design in the spring of 2017. The selected varieties differed in: flowering time, herbage yield, crude protein concentration, plant height and form. Lucerne and red clover stands were harvested four times in 2018. Between regular cuts, additional samples were taken to improve the calibration accuracy (Table 1).

The PM was built from a 30 cm diameter acrylic circular disc (plate) with a 2 cm hole in the centre. The plate was 7 mm thick, weighed 500.6 g and was hung from a string attached to four regularly placed points on the plate. Readings were taken by carefully lowering the plate along the ruler until the uppermost leaves of the canopy made contact with the plate. All measurements were taken on subplots of 0.25 m^2. The height was measured four times per subplot. Afterwards, the herbage was clipped 5 cm above the ground and the dry matter content was determined (2 days, 60 °C).

Table 1. Red clover and lucerne measured plant height and clipped herbage mass.[1]

	Cut no.	Sampling date	Regrowth days	No. of observations	Measured plate height [cm] range	Measured plate height [cm] mean	Clipped herbage mass (g DM $(0.25\ m^2)^{-1}$) range	Clipped herbage mass (g DM $(0.25\ m^2)^{-1}$) mean
Red clover	1	9 May	39	15	30.3-72.5	48.1	74.9-136.2	96.7
		20 June	42	15	16.0-30.5	23.6	30.3-72.5	48.1
		29 June	51	15	18.5-35.8	25.8	36.4-78.3	55.3
	2	8 July	60	15	17.5-42.0	29.5	27.6-102.2	60.4
		3 Aug	26	15	12.0-17.5	14.9	10.9-36.4	20.1
	3	13 Aug	36	15	10.0-18.3	13.3	9.0-29.5	18.4
		5 Sep	23	15	7.5-12.8	9.4	6.6-13.4	9.1
		17 Sep	35	15	10.3-15.3	12.7	5.5-18.0	13.2
	4	27 Sep	45	15	7.5-17.0	12.7	6.9-21.5	14.4
Lucerne	1	9 May	39	17	59.5-70.5	66.5	118.7-153.5	134.6
		20 June	42	18	32.5-49.5	41.2	42.2-84.5	61.7
		29 June	51	18	35.5-59.0	49.1	52.2-103.6	78.8
	2	8 July	60	17	39.5-69.8	56.6	48.8-119.0	87.9
		3 Aug	26	18	27.8-47.0	38.5	33.6-67.4	49.8
	3	13 Aug	36	17	30.3-56.5	46.5	26.8-81.2	51.7
		27 Aug	14	17	14.0-24.3	18.2	4.9-24.1	15.4
		6 Sep	24	18	21.3-34.8	28.2	15.7-43.3	28.3
	4	17 Sep	35	18	23.0-39.0	32.0	20.8-39.2	32.2

[1] DM = dry matter herbage mass.

All statistical analyses were conducted in R (version 3.6.1) using MASS, caret, and e1071 packages. Measured plate height (PH) was set as a continuous variable and sampling date (SD) as a categorical variable. Three regression models were evaluated: (1) simple linear regression with PH as an explanatory variable, and two multiple regression models, where dry matter of clipped herbage mass (DM) was fitted to (2) PH and SD or to (3) PH, SD and PH × SD interaction. All models were evaluated in terms of best fitting by Akaike information criterion (AIC). The validation was carried out by 5-fold cross-validation for best-fitted regression model.

Results and discussion

For red clover, PM height and DM varied widely (Table 1). Until the end of July, DM and PH increased proportionately. Further regrowth after July was considerably less; none or only a small change in PH was observed combined with a slow DM change. Plants under drought stress (August and beginning of September) had a lower turgor pressure, which may have resulted in a higher dry matter content and/or senescent material and thus lower PH readings (Ferrero *et al.*, 2012; L`Huillier and Thomson, 1988). Nevertheless, pooled data in one set resulted in a good calibration model with high prediction power. Already a simple linear regression model explained 95% of the variation of DM. This is a considerably higher precision than that found by Harmoney *et al.* (1997) for red clover growing in mixed stands. Adding SD as explanatory variable into the model slightly increased the explanatory power and the fit of the model (Table 2). In the validation, all tested models showed a similar explanatory power. Differences were observed in the prediction power, where SD and PH as explanatory variables resulted in the best model (Figure 1A).

Lucerne stands were not affected by the hot dry period and no depression in PM height or DM was observed. A simple linear regression model explained 84% of the variation of DM. This is a considerably higher precision than those found by Griggs and Stringer (1988) or described by Harmoney *et al.* (1997).

Table 2. Comparison of red clover and lucerne calibration and validation models.[1]

	Model	Calibration				Validation		
		Adj.-R^2	F-statistics	*P*-value	AIC	R^2	RMSE	MAE
Red clover	DM = SD + PH	0.96	338	<0.001	506	0.96	6.4	4.86
	DM = SD + PH + (SD × PH)	0.96	190	<0.001	505	0.95	6.6	4.86
	DM = PH	0.95	2,430	<0.001	912	0.95	6.9	5.27
Lucerne	DM =SD + PH	0.94	276	<0.001	678	0.94	9.2	7.12
	DM = SD + PH + (SD × PH)	0.94	153	<0.001	679	0.94	9.4	7.39
	DM = PH	0.86	963	<0.001	1,241	0.94	9.2	7.14

[1] DM = dry matter herbage mass; PH = measured plate height; SD = sampling date; AIC = Akaike information criterion; RMSE = root mean square error; MAE = mean absolute error.

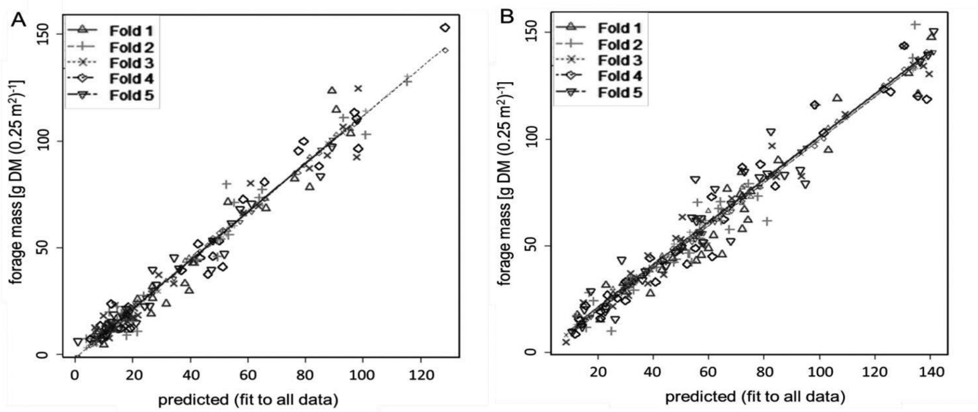

Figure 1. Five-fold cross-validation of best-fitted model of (A) red clover and (B) lucerne herbage mass to measured plant height and sampling date. Small symbols show cross-validation predicted values.

Adding SD as explanatory variable into the model increased the explanatory power and fit of the model (Table 2). In the validation, all tested models showed the same explanatory power. Differences were observed in the prediction power, where SD and PH as explanatory variables resulted in the best model (Figure 1B).

Conclusions

The herbage mass of red clover was well predicted with plate meter measurements in all three models. Herbage mass of lucerne was also well predicted when SD was included in the calibration model; the addition of PH × SD did not improve the model further. The calibration accuracy should be further improved by including more growing stages and strongly varying or extreme weather conditions. Also, a variety effect, suggested by Griggs and Stringer (1988) on herbage mass prediction, should be considered.

References

Ferrero F.P., Nave R.L.G., Sulc R.M. and Barker D.J. (2012) Seasonal variation in the rising plate meter calibration for forage mass. *Agronomy Journal* 104, 1-6.

Griggs T.C. and Stringer W.C. (1988) Prediction of alfalfa herbage mass using sward height, ground cover, and disk technique. *Agronomy Journal* 80, 204-208.

Harmoney K.R., Moore K.J., George J.R., Brummer E.C. and Russel J.R. (1997) Determination of pasture biomass using four indirect methods. *Agronomy Journal* 89, 665-672.

L'Huillier P.J. and Thomson N.A. (1988) Estimation of herbage mass in ryegrass/white clover dairy pastures. *Proceedings of the New Zealand Grassland Association* 49, 117-122.

Morphogenesis and functional traits of contrasting growth strategies of forage grass species growing in pure stands or intercropped

Duchini P.G.[1], Guzatti G.C.[2], Americo L.F.[1], Echeverria J.R.[1] and Sbrissia A.F.[1]
[1]Santa Catarina State University (UDESC/CAV), Avenida Camões, 2090, 88520-000, Lages, SC, Brazil; [2]Santa Catarina Federal Institute (IFSC), April 22nd street, 2440, 89900-000, São Miguel do Oeste, SC, Brazil

Abstract

Forage plants can adjust their morphophysiological and functional traits when subjected to stress and/or disturbance, and subsequently change their productivity and ecological adaptation. The aim of this work was to test the hypothesis that contrasting growth strategies of forage grasses do not change their morphogenesis and functional traits when cultivated in mixtures in nutrient-rich soil with lenient defoliations. Morphogenic, structural and functional characteristics of three grass species with contrasting growth strategies (*Arrhenatherum elatius*, *Dactylis glomerata* and *Festuca arundinacea*) were evaluated in mixed or pure stands for 24 uninterrupted months. *F. arundinacea* was more associated with characteristics of resource-conservative species, with high values for leaf lifespan and phyllochron, while *A. elatius* and *D. glomerata* presented higher values for specific leaf area, nitrogen tissue concentration and leaf elongation rates. *D. glomerata* tillers showed higher photosynthetic apparatus, while *A. elatius* tillers presented higher relative growth rate. No change in functional traits was observed in the evaluated species whether cultivated alone or as a mixture. Our results indicate that fertile environment and lenient defoliation could allow the expression of species intrinsic traits what could be used to conceive stable agronomic mixtures composed by contrasting growth strategy grasses.

Keywords: conservative, exploitative, lenient defoliation, nutrient-rich soil, plant development

Introduction

Scientific literature reports interspecific variations in morphogenic traits which have been related with plant's growth strategies (Pontes *et al.*, 2012). In this way, exploitative species present greater phyllochron and leaf elongation rates, while greater leaf lifespan is normally observed in more conservative species. These characteristics, associated with tiller population density, define relevant ecological features of the ecosystem, such as the residence time of carbon and nutrients in the aboveground biomass (Kazakou *et al.*, 2007; Schleip *et al.*, 2012) and its ecological adaptation and survival capacity (Briske, 1996). Although morphogenic traits and growth strategies are genetic attributes, adaptive responses can occur when plants are submitted to stress and/or disturbance (Al Haj Khaled *et al.*, 2005; Baruch and Guenni, 2007). However, depending on the duration and intensity with which a plant community is submitted to stressful and disturbing conditions, these adaptive responses may not be effective, and some species can become dominant (Grime, 1974), decreasing the biodiversity of grassland ecosystems. Therefore, we hypothesised that minimising stresses and disturbance over mixed populations of grasses allow the expression of species intrinsic morphogenic and functional traits that help to maintain stability of agronomic grass mixtures over time.

Materials and methods

Monocultures of *Arrhenatherum elatius* 'SCS314 Santa Vitória' (exploitative species), *Festuca arundinacea* 'Quantum II' (conservative species) and *Dactylis glomerata* 'Ambar' (moderately exploitative species) and a mixture of these three species in the same proportions (weight basis) were sown by broadcasting on 13 June 2013, at a density of 18 kg ha^{-1} of pure viable seeds, in Lages, Brazil (27°47' S, 50°18' W, altitude: 960 m above sea level). Plots of 9×5 m, with three replicates of each treatment, were established in a completely randomised design. From May 2014 to June 2016, cuts at 10 cm above ground level were performed when sward heights had reached 20 cm. The pre-cutting height corresponded to an average canopy condition that intercepts around 95% of the incident radiation during a full vegetative development stage of plants. After soil analyses, phosphorus and potassium were applied each autumn to maintain a high soil fertility. Nitrogen fertilisation was applied every 40 days as urea, with 30 kg of N ha^{-1} (270 kg ha^{-1} yr^{-1}) during the first experimental year (2014/2015), and 50 kg of N ha^{-1} (450 kg ha^{-1} yr^{-1}) during the second experimental year (2015/2016). The N input was increased to maintain the nitrogen nutrition index closer to 1.0. Tissue flow was evaluated continuously from May 2014 to June 2016 in 14 (mixture) or 20 (monocultures) tillers per species per plot at about 7-day intervals. From this evaluation, leaf elongation rate per tiller (LER_T) and per leaf (LER_L), phyllochron (Ph), number of leaves growing simultaneously on the same tiller (α), final leaf length (FLL), and leaf lifespan (LLS) were calculated according to Lemaire and Agnusdei (2000). Moreover, specific leaf area (SLA) and leaf area per tiller (LA_T) were measured in 50 randomly collected tillers per plot, and nitrogen concentration in the upper canopy layer (N_{up}) was determined according to Farrugia *et al.* (2004). Relative growth efficiency (RGE) was calculated as LER/LA_T. Data were analysed by principal components using the software R, version 3.4.2.

Results and discussion

Approximately 65% of the total data variation was explained by the first two principal components (Figure 1). The first principal component (PC1) explained 45.29% of data variation and contrasts *F. arundinacea* with *D. glomerata* and *A. elatius* along N acquisition/conservation trade-off axis (Figure 1A). In this way, stronger correlations were observed between Ph and LLS with *F. arundinacea*, while *D. glomerata* and *A. elatius* were more associated with LER_T, LER_L, SLA, and N_{up} (Figure 1A and 1B), similar to the results found in the literature (Pontes *et al.*, 2012). The second principal component (PC2)

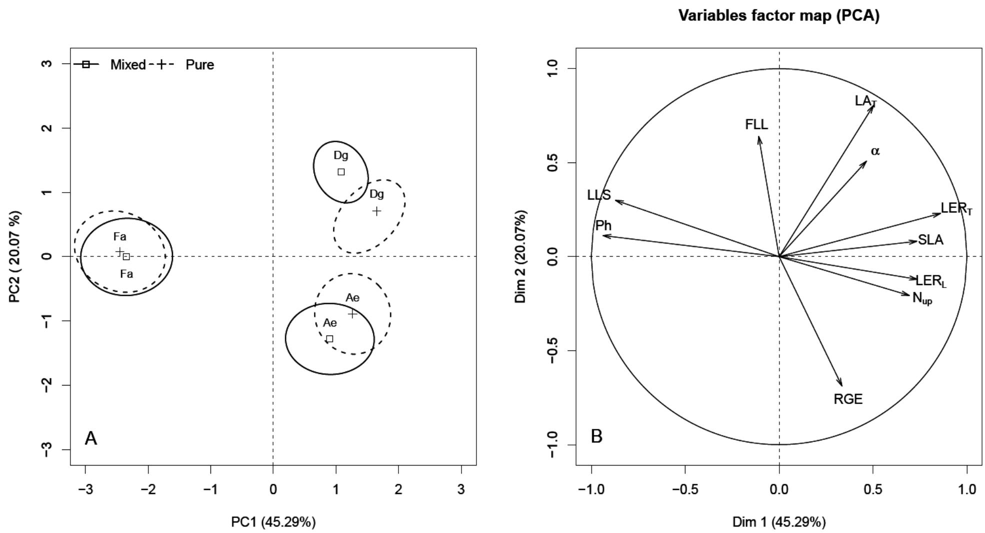

Figure 1. Principal components diagram placing *Festuca arundinacea* (Fa), *Dactylis glomerata* (Dg) and *Arrhenatherum elatius* (Ae) in monocultures (cross) or in a mixture (square) according to their traits. Ellipses (A) indicate the 95% confidence interval. Traits used in this analyses are presented in (B). FLL = final leaf length; LA_T = leaf area per tiller; LER_L = leaf elongation rate per leaf; LER_T = leaf elongation rate per tiller; LLS = leaf life spam; α = number of elongating leaves; N_{up} = N% in the upper canopy layer; Ph = phyllochron; RGE = relative growth efficiency; SLA = specific leaf area.

explained 20.07% of data variation and distinguished *D. glomerata* from *A. elatius*, differentiating their tiller photosynthetic apparatus size to support their tissue production. In this way, *A. elatius* was more related to N_{up} and RGE whilst *D. glomerata* was more associated with FLL, LA_T, and α. Therefore, although these species presented similar N acquisition/conservation (CP1), they had different strategies in using this nutrient: *A. elatius* prioritizes N concentration and *D. glomerata* use N to increase its LA_T. This difference could benefit either species when nutrient or light is the most limiting resource. Finally, principal components showed that, based on the measured traits, the studied species differ functionally, but they presented similar features when cultivated as monoculture or as a mixture under the imposed managements.

Conclusions

Cool season perennial grasses with contrasting growth strategies do not change their morphogenic, structural and functional traits when cultivated as a monoculture or as a mixture in nutrient-rich soil and submitted to low level of defoliation.

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References

Baruch Z. and Guenni O. (2007) Irradiance and defoliation effects in three species of the forage grass *Brachiaria*. *Tropical Grassland* 41, 269-276.

Briske, D.D. (1996) Strategies of plant survival in grazed ecosystems: A functional interpretation. In: Hodgson J. and Illius A.W. (eds.). *The Ecology and Management of Grazing Systems*. Wallingford, UK: CABI, pp. 37-68.

Farrugia A., Gastal F. and Scholefield D. (2004) Assessment of the nitrogen status of grassland. *Grass and Forage Science* 59, 113-120.

Grime J.P. (1974) Vegetation classification by reference to strategies. *Nature* 250(5461), 26-31.

Kazakou E., Garnier E., Navas M-L., Roumet C., Collin C. and Laurent G. (2007) Components of nutrient residence time and the leaf economics spectrum in species from Mediterranean old-fields differing in successional status. *Functional Ecology* 21, 235-245.

Khaled A.H., Duru M., Theau J.P., Plantureux S. and Cruz P. (2005) Variation in leaf traits through seasons and N-availability levels and its consequences for ranking grassland species. *Journal of Vegetation Science* 16, 391-398.

Lemaire G. and Agnusdei M. (2000) Leaf tissue turnover and efficiency of herbage utilization. In: Lemaire G., Hodgson J., De Moraes A., Carvalho P.C.F. and Nabinger C. (eds.) *Grasland Ecophysiology and Grazing Ecology*, Wallingford, UK: CABI, pp. 265-288.

Pontes L.S., Maire V., Louault F., Soussana J-F. and Carrère P. (2012) Impacts of species interactions on grass community productivity under contrasting management regimes. *Oecologia* 168(3), 761-771.

Schleip I., Lattanzi F.A. and Schnyder H. (2012) Common leaf life span of co-dominant species in a continuously grazed temperate pasture. *Basic and Applied Ecology* 14, 54-63.

Fight it or fit it in: *Holcus lanatus* on peat land

Becker T.[1], Isselstein J.[1] and Kayser M.[1,2]

[1] *Georg-August University Göttingen, Department of Crop Sciences, Germany;* [2] *University of Vechta, Geo-Lab, Germany*

Abstract

Holcus lanatus often dominates grass swards on peat land and is unpopular among farmers as it is associated with declining yields and poor forage quality. As a consequence, farmers often react by renewing swards to increase proportions of *Lolium perenne*, however, usually not with lasting effects. Moreover, sward renewal is costly and losses of CO_2 are inevitable; emissions of greenhouse gases from intensive use of peat land are regarded as a main contribution of agricultural production to climate change. On peat land, despite regular renovation measures, *H. lanatus* will make up varying proportions of the sward over time and farmers might adopt management to deal with that. In a three-year experiment, we examined yields and forage quality of simple grass mixtures, with *Festuca arundinacea*, *Lolium perenne* and *Phleum pratense* as main species sown on peat land, under different cutting frequencies. All main species were displaced in different amounts by *H. lanatus* over time. We found that under conditions of frequent defoliations, *H. lanatus*-rich swards had comparatively good yields and a forage quality that would be acceptable for dairy cows. Among the three mixtures, *F. arundinacea* was best suited to resist invasion of *H. lanatus*.

Keywords: *Holcus lanatus*, *Festuca arundinacea, Lolium perenne*, peat land, renovation

Introduction

Increasing proportions of *Holcus lanatus* (HoLa) in grass swards on peat land are often related to declining sward productivity and serve as an indicator of the need for sward renewal. The aim of a renewal is mainly to increase the proportions of *Lolium perenne* (LoPe) in the sward. While sward renewal can be costly and accelerate CO_2 losses from peat land, positive effects on sward composition are often short-lived (Kayser *et al.*, 2018). There have been few studies on the influence of HoLa on yield and feed quality on peat land. We hypothesise that accepting certain amounts of HoLa in the sward, in combination with more competitive species like *Festuca arundinacea* (FeAr), might reduce the frequency of sward renewals. In a three-year field trial on peat land we compared mixtures with *Phleum pratense* (PhPr) and FeAr as the main species with LoPe. We analysed, how the feed quality and yield were influenced by the invading species HoLa.

Materials and methods

The experiment was established in early autumn 2013 on peat soil in northwest Germany (53° 9' N and 8° 5' E; 5 m above sea level). Plant available concentrations of P and K were sufficient, pH was at 4.1.

The experimental set-up followed a split-plot design with the treatment 'management regime' forming three sub-blocks within the three main blocks (replications) and plots of the treatment 'mixtures' randomly allocated to the sub-blocks. In all sown mixtures, the main species LoPe, FeAr, and PhPr (main species: 25 kg ha^{-1}) were accompanied by *Trifolium repens* (3 kg ha^{-1}) and *Poa pratensis* (3 kg ha^{-1}) and subjected to a cutting-only regime, a simulated grazing regime, and a mixed regime with a first cut followed by simulated grazing. All plots received 320 kg N ha^{-1}, 75 kg P ha^{-1}, and 150 kg K ha^{-1} per year; after an initial supply of N (60 kg N ha^{-1}) in March the remaining N was applied after each cut. In 2014, 2015 and 2016 the plots were cut four times in the cutting-only treatments and up to seven times to simulate grazing. All yields were recorded. At each cut, samples of 500 g were taken, oven-dried for 48 h at 60 °C, and ground to 1 mm. Near infrared reflectance spectroscopy (NIRS) was used to determine net energy and crude protein concentrations. Energy content (MJ net energy kg^{-1} DM) was calculated according to the guidelines of the German Society of Nutrition Physiology (GfE, 2009). In the third year, herbage mass proportions of the different species were determined by separation of samples of 500 g fresh matter from each plot.

For the statistical analysis of yield persistence (mass proportions of species in the third year), 'mixture' and 'management' were considered as fixed factors in a mixed-model approach with replications in blocks and sub-blocks as random factors.

Results and discussion

All sown mixtures were to a certain degree displaced by HoLa (Table 1). PhPr was affected the most and proportions of PhPr in the sward were already reduced in the first year. The displacement of LoPe also was substantial, while proportions of FeAr remained high until the third year resulting also in the highest yields. Mixtures with PhPr and LoPe showed a higher energy concentration than mixtures with FeAr as main species. The cutting interval had no influence on the extent of the displacement of main sown species by HoLa.

HoLa is well adapted to poorly drained soils (Hopkins, 1986) and had been one of the main species in the grassland of the experimental field. It started to invade the plots already in the first year, probably from the seed bank in the soil. With grazing, feed intake of HoLa can be reduced: the hair coat, infections with rust and a high proportion of inflorescences, dead leaf and sheath material reduce the palatability. These aspects can be controlled by the grazing management (Cameron, 1979). Generally, negative effects of HoLa in a sward might be overrated. Swards with PhPr as the main sown species had high amounts of HoLa in the third year but still showed high contents of crude protein and an acceptable content of net energy (Table 1), which would still meet the requirements of dairy cows (Spiekers, 2004). Also, Frame (1992) and Watt (1987) found HoLa to be a productive grass.

It might thus be an option to integrate HoLa into the grassland management instead of fighting it by frequent renewal. Actually, in the years before the decision for renewal, HoLa is for some time already an important part of the swards and contributes to the nutrition of dairy cows. The idea would then be to keep the quality of swards containing HoLa at an acceptable level. An option would be to increase the frequency of defoliations. More frequent defoliations generally result in better feed quality and palatability of grasses (Frame and Hunt, 1971; Pontes *et al.*, 2007). In grazing, HoLa is better accepted at a younger growth stadium (Cameron, 1979). This should be accompanied by introducing more competitive grasses to the sward and to suppress further extension of HoLa. For a better energy content

Table 1. Mass proportions (%) of the sown grass species (25 kg ha^{-1}) FeAr, LoPe, and PhPr and of the invading species *Holcus lanatus* on peat soil, dry matter yields (Lsmeans, in kg DM ha^{-1}), content of net energy and crude protein.[1,2,3]

	FeAr	LoPe	PhPr
Mass proportion (%) of main species (FeAr, LoPe, PhPr) in the respective mixture	76[a]	61[a]	20[b]
Mass proportion (%) of *H. lanatus* in the mixture	18[c]	26[b]	49[a]
Dry matter yields (kg DM ha^{-1})	11,484[a]	8,993[b]	8,787[b]
Net energy (MJ kg^{-1} DM)	6.15[b]	6.30[a]	6.17[ab]
Crude protein (g kg^{-1} DM)	194[b]	205[a]	212[a]

[1] Different letters indicate significant differences.

[2] FeAr = *Festuca arundinacea*; LoPe = *Lolium perenne*; PhPr = *Phleum pratense.*

[3] Lsmeans averaged over the three types of managements (sim. grazing, mixed, and cutting-only) in the third year.

of the grass and to improve the sward they might use direct seeding of LoPe and *Lolium multiflorum* (Grund and Bonckholt, 2002) and FeAr.

Some studies have observed a good persistence and yield stability of FeAr on peat soil (Becker *et al.*, 2018; Kalzendorf and Hinrichsen, 2017). This could help to establish swards where the main sown species lasts much longer and reduce the need for renewal.

Conclusions

Instead of spending time and money for regular sward renewal on peat land, it might be an option that farmers accept certain proportions of HoLa. Which proportions of HoLa under given circumstance could be tolerated is currently unknown and should be further analysed. Along with this, the introduction of competitive grasses like FeAr and competitive varieties of LoPe could be a management option.

References

Becker T., Isselstein J., Benke M. and Kayser M. (2018) Persistence of modern varieties of *Festuca arundinacea* L. and *Phleum pratense* L. as an alternative to *Lolium perenne* L. in intensively managed sown grasslands. *Grassland Science in Europe*, 23, 117-119.

Cameron N.E. (1979) A study of the acceptability of *Holcus* spp. to Perendale sheep. M. Agric. Sci. Thesis, Massey University, New Zealand. Available at: http://hdl.handle.net/10179/5905

Frame J. (1992). *Improved grassland management*. Farming Press Books, Ipswich, UK.

Frame J. and Hunt, I.V. (1971) The effects of cutting and grazing systems on herbage production from grass swards. *Grass and Forage Science* 26, 163-172.

GfE (German Society of Nutrition Physiology) (2009) New equations for predicting metabolisable energy of compound feeds for cattle. *Proceedings of the Society of Nutrition Physiology* 18, 143-146.

Grund C. and Bockholt R. (2002) Futterqualität und Siliereigenschaften von *Lolium*. *Annual meeting of the AGGF,* 46, 88-91.

Hopkins A. (1986) Botanical composition of permanent grassland in England and Wales in relation to soil, environment and management factors. *Grass and Forage Science,* 41, 237-246.

Kalzendorf C. and Hinrichsen H.C. (2017). Ertragsleistungen und Futterqualitäten von Rohrschwingel, *Festulolium*, Lieschgras und Deutschem Weidelgras – dreijährige Untersuchungen in Nordwestdeutschland. *Mitteilungen der AG Grünland und Futterbau*, 18, 195-198.

Kayser M., Müller J. and Isselstein J. (2018) Grassland renovation has important consequences for C and N cycling and losses. *Food and Energy Security* 2018, e00146. https://doi.org/10.1002/fes3.146.

Pontes L.S., Carrère P., Andueza D., Louault F. and Soussana J.F. (2007) Seasonal productivity and nutritive value of temperate grasses found in semi-natural pastures in Europe: responses to cutting frequency and N supply. *Grass and Forage Science* 62, 485-496.

Spiekers H. (2004) *Tierphysiologische Anforderungen an die Silagequalität*. Institut für Tierernährung (LfL).

Watt T.A. (1987) A comparison of two cultivars of *Holcus lanatus* with *Lolium perenne*, under cutting. *Grass and Forage Science* 42, 43-48.

Modelling radiation use efficiency of red clover (*Trifolium pratense* L.) under the Nordic climate

Ahmed M.[1,2], Parsons D.[1] and Gustavsson A.[1]

[1]*Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, 90183 Umeå, Sweden;* [2]*Department of Agronomy, PMAS Arid Agriculture University, 46300 Rawalpindi, Pakistan*

Abstract

Radiation-use efficiency (RUE) describes biomass produced per unit of photosynthetically active or total solar radiation intercepted. RUE is used to analyse crop growth based on resource capture and use efficiency. Northern Sweden has a cool climate with short growing season, long day length and high RUE. Thus, to capture the impact of RUE on red clover crop biomass production, APSIM next generation red clover model was calibrated using data from four study sites in the Nordic Field Trial System (NFTS). Biomass accumulation in APSIM is the product of intercepted radiation, RUE and stress factors. This model was tested to see its potential to simulate biomass production for northern Sweden. The approach simulates net photosynthesis rather than giving separate estimates of growth and respiration. Potential photosynthesis is calculated using RUE by considering stress factors, i.e. plant nutrition, air temperature, vapour pressure deficit, water supply and atmospheric CO_2 concentration. The results showed that model was able to simulate the dry matter production with good accuracy but during the establishment year it simulated higher biomass than observed. Thus, the model needs further calibration particularly under stress conditions.

Keywords: radiation-use efficiency, red clover, APSIM next generation red clover model, dry matter, Nordic climate

Introduction

Modelling and experimental climate change impact studies for the agricultural sector have largely focused on a small set of annual, staple food crops, which are most relevant for global calorie supply and land use. Natural grasslands cover 26% of global land area and 70% of the agricultural area, and provide livelihoods for about 1 billion people and one third of global protein intake (FAO, 2019). Biomass crops differ from annual grain crops in physiology, environmental requirements, and management and therefore require dedicated modelling approaches. Red clover is mainly used for cutting in sown grass clover leys of 2-4 years duration. Factors responsible for the low persistence of red clover include weather, particularly less survival during cold winter, cultivar, nutrition, intensity of cutting, pests and diseases or a combination of these. Sweden wishes to increase the proportion of organic farming, which requires nitrogen-fixing crops like red clover. No modelling studies have yet been reported on the impact of radiation use efficiency (RUE) on red clover dry matter (DM) production in high latitude Sweden. RUE describes the amount of DM produced per photosynthetically active photons absorbed by the leaves. This is an important parameter used by the process-based crop growth models to simulate photosynthesis. Improving RUE is a promising avenue for increased crop yield. The steps forward involve firstly to assess the impact of RUE on crop yield across different environments, management, and cultivars with different genetic backgrounds. This is only possible through crop models with a range of possible changes in RUE (e.g. step wise increase to 30%) (Asseng *et al.*, 2019). Secondly, modifications in the traits towards increased RUE need to be explored. Based upon this background it is essential to study the effect of RUE on red clover DM production at high latitudes of Sweden. The objectives of this study include: (1) calibration of Agricultural Production Systems sIMulator (APSIM) next generation red clover model under high latitudes; and (2) simulations of RUE impacts on red clover production under high latitudes.

Materials and methods

Data regarding crop, soil and weather for four study sites: Röbäcksdalen (63°81' N, 20°24' E), Öjebyn (65°34' N, 21°39' E), Lännäs (63°11' N, 17°74' E) and Ås (63°24' N, 14°56' E) were collected from Nordic Field Trial System (NFTS). One red clover cultivar, SW Torun (4n) DM data was considered to calibrate the APSIM next generation red clover model. Plant modelling framework (PMF) (Brown *et al.* 2014) is used to simulate the growth of red clover as forage crop in APSIM next generation model. The focus in this approach is to describe biomass accumulation and regrowth after harvest on monoculture swards. Development of the crop has been simulated through successive developmental phases. The phenological changes in these phases are controlled by thermal time. Monteith and Moss (1977) approach is used to model biomass fixation as the product of intercepted radiation and its conversion efficiency (RUE). This simulates net photosynthesis and afterwards potential photosynthesis is calculated using adjusted RUE based upon different stress factors. The stress factors include plant nutrition (FN), air temperature (FT), vapour pressure deficit (VPD), water supply (FW) and atmospheric carbon dioxide concentration (FCO_2). The equation used to calculate potential photosynthesis or radiation limited biomass accumulation in APSIM is:

$$\textit{Radiation limited biomass accumulation } (\Delta Q_r) = \textit{Intercepted radiation } (I) \times RUE \times f_d(\textit{Diffue factor}) \times f_s(\textit{Stress factor}) \times f_c(\textit{Carbon dioxide factor})$$

General value of RUE reported for red clover is in the range of 2.5-3.0 g DM MJ^{-1} (Riesinger *et al.*, 2009) while standard value of RUE in the model is 2.95 g MJ^{-1}. Sowing, establishment and harvesting (H) of red clover used to run the model during 2015-18 are in Table 1 for all four sites. Calibration with standard RUE was conducted initially; first year was considered as establishment year, in the next three years, defoliation command was initiated to have three cuts per year. After simulation the simulated DM was plotted against observed NFTS data set to evaluate the performance of model using built in red clover coefficients.

Results and discussion

DM accumulation by APSIM next generation showed variable trend with observed data during the three harvest years (Figure 1). This could be due to crop response to stress factors that might result in the differences in the intercepted photosynthetically active radiation (IPAR), RUE and DM partitioning (Chakwizira *et al.*, 2018). Asseng *et al.* (2019) reported that a potential avenue to increase crop production

Table 1. Sowing and harvesting dates of red clover during 2015-2018 at four sites.[1]

Years	Harvest at Röbäcksdalen (Umea)			Years	Harvest at As		
	H1	H2	H3		H1	H2	H3
EY (2015)	21 June	-	-	EY (2015)	21 June	-	-
2016	21 June	10 Aug	10 Sept	2016	21 June	10 Aug	10 Sept
2017	21 June	10 July	28 Aug	2017	29 June	05 Aug	05 Sept
2018	17 June	26 July	05 Sept	2018	27 June	27 Aug	15 Sept
Years	**Harvest at Öjebyn**			**Years**	**Harvest at Lännäs**		
	H1	**H2**	**H3**		**H1**	**H2**	**H3**
EY (2015)	21 June	-	-	EY (2015)	21 June	-	-
2016	21 June	10 Aug	10 Sept	2016	21 June	10 Aug	10 Sept
2017	10 July	28 Aug	-	2017	21 June	10 July	28 Aug
2018	11 July	04 Sept	-	2018	17 June	26 July	05 Sept

[1] Sowing at all sites: 21/05/2015; EY = establishment year; H1 = first harvest; H2 = second harvest and H3 = third harvest.

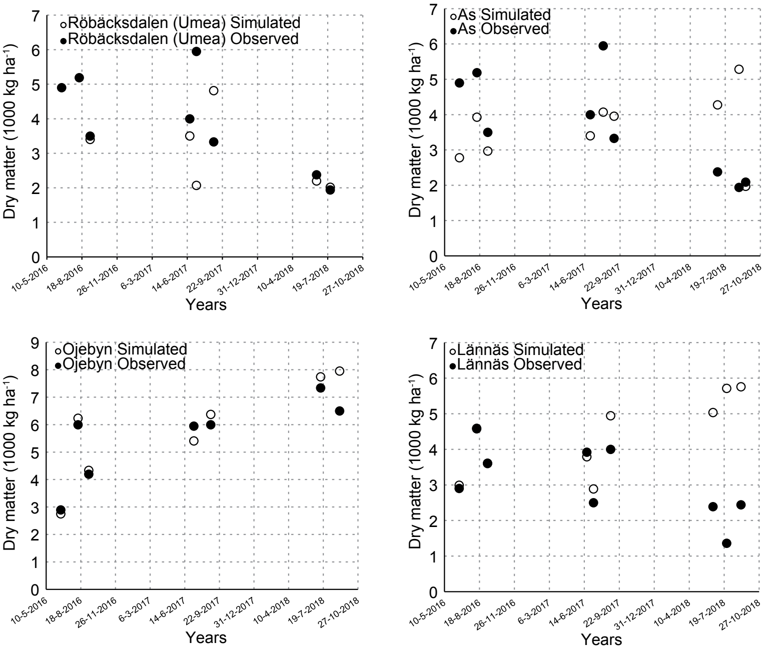

Figure 1. Observed and simulated red clover DM accumulation at four different study sites.

is improving RUE through modifications in crop traits linked with the interception of PAR. Therefore, modification in the leaf RUE parameters could result in increased DM. Furthermore, in order to get accurate estimation from APSIM next generation in northern Sweden, we focus on the parameters that have direct linkage with radiation limited biomass accumulation. These include intercepted radiation, RUE, CO_2 concentration, stress factors and light extinction coefficient. Since proper phenotypic data is important to implement APSIM next generation with true spirit at ground scale, we will run this model with collected phenological field data in the future.

Conclusions

APSIM next generation has good potential to simulate red clover biomass accumulation at high latitudes. However, additional field-based data are required to further calibrate and evaluate the model performance in the Nordic climate. Since improving RUE is a promising avenue to substantially increase crop production, applying the model in latitudes where growing season daylength exceeds 20 h could help to simulate higher RUE impact on crop production.

References

Asseng S., Martre P., Ewert F., Dreccer M.F., Beres B.L., Reynolds M., Braun H.J., Langridge P., Le Gouis J., Salse J. and Baenziger P.S. (2019) Model-driven multidisciplinary global research to meet future needs: the case for improving radiation use efficiency to increase yield. *Crop Science* 59, 843-849.

Chakwizira E., Teixeira E., Meenken E., Michel A.J. and Maley S. (2018) Radiation use efficiency and biomass partitioning to storage roots in fodder beet crops. *European Journal of Agronomy* 92, 63-71.

FAO (2019) FAOSTAT database, Food and Agriculture Organization of the United Nations. Available at http://faostat.fao.org/ (accessed 30 November 2019).

Monteith J.L. and Moss C.J. (1977) Climate and the efficiency of crop production in Britain. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 281 (980), 277-294.

Riesinger P., Eckersten H., Torssell B. and Forkman J. (2009) Radiation-use efficiency in leys: influences of growth period and clover proportion. *Acta Agriculturae Scandinavica, Section B – Soil & Plant Science* 59, 465-474.

A simple model for simulating water-limited ley production potential

Baadshaug O.H. and Skjelvåg A.O.
Department of Plant Sciences, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway

Abstract

This version of the COUP-ENGNOR model simulates ley production from daily records of air temperature, solar radiation, air humidity, wind speed, and precipitation. Light-use efficiency is calibrated to fit experimental data. Absorbed radiation is calculated from measured global radiation, leaf angle, and leaf area. The carbohydrate produced is partitioned between leaves, stems, stubbles and roots according to development stage. To estimate the effect of water limitation, the factor EAEP, the relation between actual (Ea) and potential (Ep) evapotranspiration is included. Ep is estimated by Penman's formula. To estimate Ea, a special routine gives root depth and density. For soil water balance calculation, the maximal root depth (65 cm) is divided into six soil layers, each with its specific plant available soil moisture capacity and budget. The field capacity is divided in a readily available fraction, and a fraction from which the uptake is reduced proportionally to its remaining moisture content, leading to reduced Ea and EAEP. After a situation with all layers at field capacity, the easily available fraction of each soil layer is emptied from the top and down the root zone, before starting the emptying of the less-available fraction; this also from the top layer and downwards. The results, the benefit obtained, and the possible area of application in using the model are briefly presented and discussed.

Keywords: climatic factors, growth model, ley, soil factor, soil moisture routine

Introduction

In Norway, arable soil resources are sparse and scatted and of extremely variable type and quality. Therefore, surveys of this resource for potential food production are highly relevant. A simple reliable plant growth model could be a useful tool for evaluating potential both at a single farm scale and for whole communities. When viewing the effects of climate change on plant production potential, not least as realized in our marginal regions, such a model would be a valuable facility (Baadshaug and Haugen, 2009). The objective of the work is to develop a tool that simultaneously takes both climate and soil physical variation into account.

Materials and methods

The model was derived from the ENGNOR crop growth model (Baadshaug and Lantinga, 2002), and was calibrated to first-year growth of a pure timothy ley. Some main traits are outlined in Figure 1. 'The equation of life' (1) (Figure 1) gives gross photosynthesis (A, GPHOT) as a function of maximum attainable photosynthesis, Amax, (Figure 2), absorbed photosynthetic active radiation (PAR) Ia, and initial light use efficiency ε. This potential gross photosynthesis is reduced by the 'drought term' EAEP and converted from absorbed CO_2 to carbohydrate produced, $(CH_2O)_n$ by the ratio 30/44 to form TOTASS, which is stored, and subsequently mobilized as a function of temperature (Figure 3) and allocated to growth of shoots and roots.

The daily radiation as PAR is distributed per hour according to the sinusoidal solar angle course over of the photoperiod. The hourly light interception for each LAI unit layer downwards is calculated according to Beer's law for light attenuation down the canopy. The resulting TOTASS emerges from summing up during the light period to make the daily output.

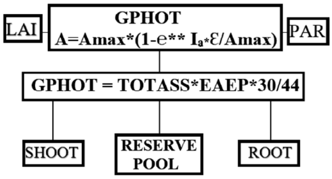

Figure 1. Gross photosynthesis (TOTASS (A) GPHOT) calculated from leaf area (LAI) and photosynthetic active radiation (PAR).

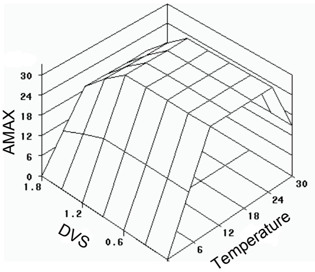

Figure 2. Maximum gross photosynthesis (AMAX) as function of development stage (DVS) and temperature.

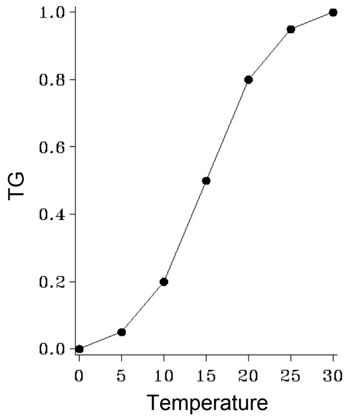

Figure 3. Mobilisation of reserve pool, TG (0.0-1.0) as function of daily temperature (0-30 °C).

The soil water balance routine starts with filling eventual precipitation, if possible, up to saturation, layer by layer from the top and downwards. A soil layer filled above field capacity (-10 kPa tension) is allowed to keep parts of this content above field capacity during a drainage period of three days maximum. Plant available water is considered as readily available in the tension range 0 to -100 kPa and gradually less available from -100 to the wilting point, -1,500 kPa. The water loss by soil surface evaporation, Es, is estimated according to Ritchie's (1972) method for a partly plant-covered land surface. Plant water uptake, Epp, depends on potential evapotranspiration, Ep (Penman, 1956), and soil surface evaporation, Es, which depends on leaf area and radiation reaching the ground surface. The procedure starts with taking Es from the top layer. For meeting plant water demand, the search starts from the uppermost layer for readily available water sufficient to meet this demand. In case that is not met, the next layer downwards is loaded, and so on until the lowermost layer within the current root zone is emptied for its readily available content. In non-precipitation cases the search then starts again from the top layer and downwards for the 'best' one as to less available water content. At this stage the plant water uptake is restricted as compared with potential evapotranspiration (Ep), so the actual evapo-transpiration (Ea) and the relative reduction, EAEP (= Ea/(Ep – Es)) are calculated. This quotient, 'the drought term', is used to calculate assimilation (GPHOT) from potential assimilation (TOTASS) (Figure 1).

Results and discussion

The observations needed for the production routine of the model are daily global radiation, air temperature, air humidity, and wind speed. All except radiation are usually available from most weather stations. In cases where global radiation records are lacking, sunshine hours or cloudiness can be used to give reliable estimations.

The curvilinear shape of the of the light saturation curve (Equation 1) implies a relatively higher efficiency for assimilation of the low light intensities in early morning or late evening and in the lower part of the canopy, than that by the midday hours or in the higher canopy layers (Figure 4A,B). The beneficial effect of long photoperiods can be seen in Figure 4B and 4C. If, in a situation with a daily global radiation of 22.2 MJ m^{-2} over a photoperiod of 18 h, this total is distributed according to a sinus curve over 24 h day,

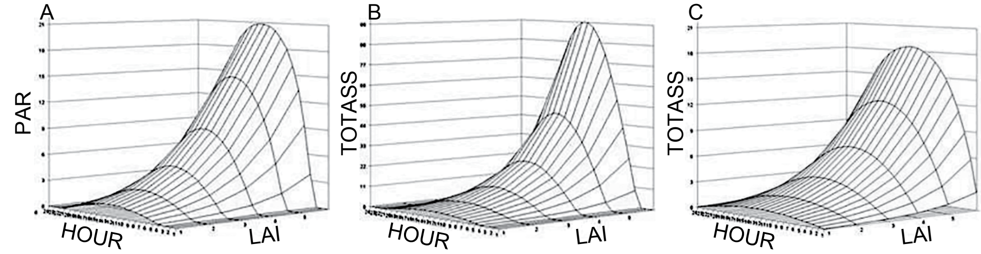

Figure 4. PAR, (0-99 J m^{-2} sec^{-1}), (A) and hourly assimilation, TOTASS (0-21g CO_2 m^{-2} h^{-1}), (B-C), as depending on hour of the light day (24-0 from left) and number of unit leaf layer (1-6 from left) of a stand from a top layer, numbered 6, down a 6 layer canopy.

the total daily TOTASS output will increase by some 9%, and in the uppermost leaf layer by as much as 14%. This is due to the gains during the extra light hours, which more than outweigh the loss during the midday hours at the higher leaf layers.

A great advantage of our soil water balance routine, compared with more sophisticated ones, is the relatively easily available soil characteristics needed to run the model. Soil pF-curves for determining the water holding capacities at the cardinal tension levels (0, -10, -100, and -1,500 kPa) in a multi-layer soil profile used here, are seldom at hand. But estimates of water retention capacities with reasonable precision can be derived from pedotransfer functions of soil organic matter content and texture analysis (Riley, 1996) or conventional texture classification. By interpolations, a convenient layer depth range in practical soil classification can be adjusted to the more universal one suggested above.

The model running procedure further includes allocation of assimilates to shoots (Figure 1) between a fraction for harvestable shoots and one for stubbles, and also calculation of dead leaves in an ageing grass stand. By summing these as litter, all the unharvested plant parts, including non-functioning roots, an input is obtained for estimating soil carbon sequestration, if the model is combined with a proper one for soil humus turnover. In this way, the status of leys, and of natural grasslands as 'green lungs' in the ecosystem, can be fully recognised.

References

Baadshaug O.H. and Lantinga E.A. (2002) *ENGNOR, a grassland crop growth model for high latitudes*. Documentations. Report no 2/2002, Reports from UMB. Dept of Plant and Environmental Sciences, Norwegian University of Life Sciences. 18 pp.

Baadshaug O.H. and Haugen L.E. (2009) Effect of climate change on growth potential in the mountainous region of southeast Norway. *Idojárás* 113, (1-2), 129-133.

Penman H.L. (1956) Evaporation: an introductory survey. *Netherlands Journal of Agricultural Science,* 4, 9-29.

Riley H. (1996) Estimation of physical properties of cultivated soil in southeast Norway from readily available soil information. *Norwegian Journal of Agricultural Sciences*, Supplement No. 25. 51 pp.

Ritchie J.T. (1972) Model for predicting evaporation from a row crop with incomplete cover. *Water Resources Research,* 8, 1204-1213.

Effect of pre-mowing on seed production of white clover

Bender A.
Estonian Crop Research Insitute, J. Aamisepa 1, Jõgeva alevik, 48309, Estonia

Abstract

A field experiment was conducted at the Estonian Crop Research Institute in 2016-2018 to study the effect of pre-mowing in a white clover field, in terms of biomass at harvest, harvest index, seed yield and seed quality. The treatments were: (1) two harvest times (onset of colouration of the first clusters or 7 days later); (2) two mowing heights (3 cm or 6 cm); and (3) two mower types (a rotary mower that crushed and spread the biomass or a sickle bar mower that collected and removed the biomass). Results showed that pre-mowing synchronized the flowering of white clover, reduced biomass at harvest and increased the harvest index, which leads to increased seed yield. All pre-mown treatments had higher seed yields compared with the unmown control. Maximum and significant yield surplus was obtained as a result of later mowing irrespective of the mower type. There were no significant differences between the treatments in seed quality characteristics, although there were differences between the 1000 seed weights, germination ability and the proportion of hard seeds produced in years differing in weather conditions.

Keywords: white clover, pre-mowing, harvest index, seed yield, seed quality

Introduction

White clover (*Trifolium repens* L.) is a species with continuous indeterminate growth during the flowering period. The activity of different plant processes such as flowering, seed setting or vegetative biomass production is influenced by soil moisture content. Under conditions of abundant rainfall, leaves will overgrow inflorescences, in which the seeds will germinate and become unviable. In areas where white clover seed fields are irrigated, the vegetative growth is controlled by reduced watering during the flowering period (De Barro, 2014). An alternative approach is to limit vegetative growth by mowing or grazing on non-irrigated areas (Pederson and Brink, 2000). Different results have been published regarding the time and height of pre-mowing. In Sweden, the highest seed production was reported when pre-mowing was performed at early bloom (Wallenhammar *et al.*, 2007), whereas in New Zealand and the UK, the largest seed production was achieved when the stand was mown two to three weeks after emergence of the first coloured inflorescences (Clifford, 1985; Marshall *et al.*, 1993). The objective of this study was to assess the effects of timing and height of pre-mowing and the type of mower on seed production of white clover, as expressed by harvest index, seed yield and quality.

Materials and methods

In July 2016, experimental plots (1.5×5.0 m) in four replicates were seeded with white clover 'Tooma' at the rate of 4 kg ha^{-1}. The field experiment was located on a calcareous cambisol, sandy loam in texture, with the following agrochemical parameters: pH_{KCl} 6.4, P 90, K 113, Ca 2041, Mg 116 mg kg^{-1}measured by the Mehlich 3 method, C_{org} 17 g kg^{-1}. Before soil preparation, the experimental field was fertilised with P 19 and K 67 kg ha^{-1}. In this study, after the first coloured inflorescences emerged, one of the pre-mowing treatments was applied: cutting with a rotary mower at 3 cm (1a) or 6 cm (1b); using a sickle bar mower at 3 cm (1c) or 6 cm (1d). One week later, the same cutting pattern was repeated on new plots: cutting with a rotary mower at 3 cm (2a) or 6 cm (2b), with sickle bar mower at 3 cm (2c) or 6 cm (2d). Control plots (C) without pre-mowing were also included. In treatments where a rotary mower was used, the grass clippings were removed from plots, whereas in the case of cutting with a sickle bar mower the clippings were left on the plots. Peak of blooming was estimated by the method of Chynoweth and Rolston (2010), where number of coloured flower buds was counted from 0.5×0.5 m plots in four replicates. At maximum bloom the heights of the flower clusters and leaves were measured from the ground surface. During seed harvest, plots were cut at a height of 4 cm, and fresh biomass was weighed and dried in the oven. Seeds were threshed with a Hege 140 combine harvester and cleaned with a laboratory seed cleaner (Kamas Westrup LA-LS). Harvest index was calculated based on the ratio of seed yield to biomass. Seed quality was estimated three months after the harvest. Statistical analyses were carried out by program Agrobase 20™. Yearly seed harvest was considered in relation to weather data for the two years. In 2017, the temperature remained below the long-term average, whereas in 2018 air temperature was above average with very low precipitation. There was only 55 mm of precipitation between 1 May and 31 July 2018, which was only 28% of the long-term average. The mean air temperature exceeded the long-term average by 4 °C in May, 1 °C in June, 3 °C in July and 2.6 °C in August.

Results and discussion

Compared with the control plots, all pre-mown treatments produced more flower clusters, had a lower height of foliage and flower clusters, less biomass during seed harvest, greater harvest index and greater seed yield in the year 2017 (Table 1). Exceptional drought and higher temperatures in 2018 resulted in statistically non-significant difference in seed production between the treatments mown at a one-week interval.

The number of flower clusters was affected by the height of pre-mowing: richer blooms were detected with lower pre-mowing height in both test years, although the results were not significantly different. Low pre-mowing also resulted in greater harvest index and seed yields. The two types of mowers used in this experiment did not cause any differences in the studied parameters. In 2017, all experiments had an excellent seed quality (germination rate on non-scarified seeds was 96-98%, hard seeds constituted 1-3% and the thousand seed weight was 0.663-0.671 g). In contrast, in 2018 the germination rate of non-scarified seeds was only 22-37%, proportion of hard seeds was 55-75%, and the weight of thousand seeds was 0.495-0.593 g. A high proportion of hard seeds in a lot is regarded as an outcome of water deficiency during seed maturation (Australian Government Office of Gene Technology Regulator, 2008).

Table 1. Characteristics of white clover seed stands, seed yields and quality in 2017 and 2018.

		C	1a	1b	1c	1d	2a	2b	2c	2d	LSD 0.05
2017	Flower clusters, m^{-2}	378	463	465	523	473	435	494	503	462	66
	Foliage height, cm	27.2	19.3	21.5	18.8	21.7	13.6	18.1	13.6	16.3	1.2
	Flower buds' height, cm	34.9	27.8	31.2	28.3	30.3	20.9	26.4	20.9	23.6	1.8
	Biomass, Mg ha^{-1}	12.67	10.37	10.83	10.20	10.43	8.53	8.93	7.97	10.37	1.37
	Harvest index, %	3.25	5.11	6.05	5.55	4.72	7.32	6.95	7.69	5.19	
	Seed yield, kg ha^{-1}	412	530	655	566	492	624	521	613	538	76
	1000 seed weight, g	0.669	0.667	0.687	0.671	0.669	0.663	0.667	0.665	0.673	
	Germination, %	96	96	97	98	97	96	96	98	97	
	Hard seeds, %	3	1	1	0	1	2	2	1	2	
2018	Flower clusters, m^{-2}	133	172	162	180	182	183	167	191	172	50
	Foliage height, cm	17.5	8.9	10.9	8.2	10.5	8.6	10.8	7.5	9.0	1.7
	Flower clusters' height, cm	21.3	13.1	15.7	13.4	15.4	12.9	15.3	13.0	13.8	2.2
	Biomass, Mg ha^{-1}	2.67	1.71	2.10	1.84	1.86	1.44	1.99	1.66	1.60	0.43
	Harvest index, %	2.88	5.38	4.14	4.67	4.19	4.72	3.37	5.00	4.44	
	Seed yield, kg ha^{-1}	77	92	87	86	78	68	67	83	71	20
	1000 seed weight, g	0.544	0.593	0.562	0.551	0.549	0.544	0.565	0.555	0.543	
	Germination, %	24	37	27	24	27	22	36	21	30	
	Hard seeds, %	73	55	66	75	68	73	64	63	68	

Conclusions

Pre-mowing of white clover seed field helps to synchronize flowering, reduce biomass during harvest and increase seed yield. Low pre-mowing at one-week after colouring of the first flower clusters resulted in higher seed yield in a rainy season, whereas in drought conditions this should be avoided. The type of mower did not affect seed yield. White clover seed quality mainly depends on the weather conditions after flowering, but not on the time of pre-mowing, mowing height or the type of mower used.

References

Australian Government Office of Gene Technology Regulator (2008) The biology of *Trifolium repens* L. (White clover) 39 p. Available at: http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/clover-3/$FILE/biologywclover2008.pdf.

Chynoweth R.J. and Rolston M.P. (2010) Determination of optimum desiccation in timing of white clover seed crops. *Proceedings of the 7th International Herbage Seed Conference*. Dallas, Texas 11-13 April 2010, pp. 172-176.

Clifford P.T.P. (1985) Effects of cultural practice on potential seed yield components of 'Grasslands Huia' and 'Grassland Pitau' white clover. *New Zealand Journal of Experimental Agriculture*. 13, 301-306.

De Barro J.E. (2014) *Irrigating for white clover seed production*. Rural Industries Research and Development Corporation. Publication No. 14/086. 25 p. https://www.agrifutures.com.au/wp-content/uploads/publications/14-086.pdf.

Marshall A.H., Hollington P.A. and Hides D.H. (1993) Spring defoliation of white clover seed crops. I. Inflorescence production of contrasting white clover cultivars. *Grass and Forage Science*, 48, 301-309.

Pederson G.A. and Brink G. (2000) Seed production of white clover cultivars and naturalized populations when grown in a pasture. *Crop Science* 40, 1109-1114.

Wallenhammar A.-Ch., Stahl P., Cristiansson B. and Andersson L. (2007) Weed regulation by cutting in organic seed crops of *Trifolium pratense* L. and *Trifolium repens* L. In: Aamlid T.S., Havstad L.T. and Boelt B. (eds.) *Seed production in the northern light.* Proceedings of the sixth international herbage seed conference, Gjennestad, Norway 18-20 June 2007. Grimstad, pp. 156-159.

Investigating the effect of nitrogen application level on grass production and quality under simulated grazing

Chesney L.[1,2], Scollan N.[2], Gordon A.[3], McConnell D.A.[1] and Lively F.O.[1]
[1]Agri-Food and Biosciences Institute (AFBI), Hillsborough, Northern Ireland, United Kingdom; [2]School of Biological Sciences, Queens' University Belfast, Northern Ireland, United Kingdom; [3]Agri-Food and Biosciences Institute (AFBI), 23a Newforge Lane, Belfast, Northern Ireland, United Kingdom

Abstract

Current estimate of herbage utilisation on Northern Ireland beef and sheep farms is 4.1 Mg of dry matter (Mg DM) per hectare per year. This experiment involved four inorganic nitrogen (N) application levels (0, 90, 180 and 270 kg N ha^{-1} yr^{-1}), which were applied at an experimental plot level in 2018. Fresh herbage yields, growth, quality, monthly trace mineral and botanical composition were recorded. Annual herbage production increased by 30% from 0 to 90 kg N ha^{-1} yr^{-1} and from 90 to 180 kg N ha^{-1} yr^{-1}, and by 18% between 180 and 270 kg N ha^{-1} yr^{-1}. For herbage quality, no significant effects (P>0.05) were seen in acid degradable fibre (ADF) and metabolizable energy (ME). However, crude protein (CP), water soluble carbohydrates (WSC) and dry matter (DM) were significantly affected (P<0.001) by the inorganic N fertilisation rate.

Keywords: N application rate, simulated grazing, grassland

Introduction

In Ireland over 90% of the agricultural land is devoted to grassland, with grass being the main feed for the ruminants. It is estimated that only 4.1 Mg of dry matter (DM) ha^{-1} yr^{-1} of herbage is utilised on beef and sheep farms in Northern Ireland, which is substantially lower than what is utilised on dairy farms (7.5 Mg DM ha^{-1} yr^{-1}) (DAERA, 2016). The average amount of nitrogen (N) fertiliser used on UK beef and sheep farms is 94 kg N ha^{-1} yr^{-1} (DEFRA, 2016) which is lower than that on dairy farms (146 kg N ha^{-1} yr^{-1}), which may account for the difference in utilisation. The objective of this study was to investigate the effect of inorganic N application level on herbage DM production and quality (crude protein (CP), acid degradable fibre (ADF), water soluble carbohydrates (WSC), metabolizable energy (ME) and trace element content), when managed under simulated grazing (herbage harvested every 21 days).

Materials and methods

The experiment was set up at AFBI Hillsborough with four different N application rates and was replicated across two sites. These four N fertiliser application rates were: 0, 90, 180 and 270 kg N ha^{-1} yr^{-1}. Plots measured 2×6 m, with each plot further sub divided into three 2×2 m plots. The plots were defoliated on a 21-day cycle to simulate the optimum grazing interval. Each fertiliser level was randomly assigned to each plot with three replicates per treatment at each site. Fertiliser was applied by hand to each plot after each defoliation until the total amount of N (90, 180 and 270 kg N ha^{-1} yr^{-1}) had been applied by mid-September. The plots were defoliated using an Agria-mower to a height of 4-5 cm to simulate grazing. The total herbage yield (Mg DM ha^{-1}) was calculated from a strip measuring 1.1×2 m cut from each 2×2 m plot, of which a fresh weight was recorded. The herbage recorded from each cutting was used to calculate a total yield (Mg DM ha^{-1}) for each treatment level. Any remaining grass on the plot was trimmed off and discarded. From this strip a sub-sample was taken and dried at 60 °C for 48 h for DM determination. Each dry sample was then bulked together for treatment (0, 90, 180 or 270 kg N/ha) within field site and then milled. This milled sample was then analysed for N, WSC, ADF, gross energy (GE) and ash by wet chemistry. A fresh sample was taken and was analysed for DM, CP, ADF, WSC and ME by near-infrared spectroscopy (NIRS). Monthly fresh herbage samples (250 g) per treatment from each site were taken and analysed for trace mineral content (phosphorus, magnesium, calcium, sodium, potassium, chloride, manganese, copper, zinc, selenium, cobalt, iodine, iron, aluminium, molybdenum, sulphur and lead). Sward botanical composition was determined at three separate time points during the year (April, June and August) from each plot. A 150 g sample of herbage was split into perennial ryegrass (*Lolium perenne* L.), weed grass species and broad leaf weeds. These different fractions were dried at 85 °C for 24 h to calculate the percentage for each species on a DM basis. Plot heights (cm) were measured weekly with a rising platemeter (Jenquip, Fielding, New Zealand) to give weekly grass growth (kg DM ha^{-1} day^{-1}). Cutting began on the 13 April 2018 and finished 30 Oct 2018. All data were analysed with GenStat. Herbage DM, CP, ADF, WSC and ME, botanical composition, yield and growth was analysed by a REML mixed model. Data on mineral content in the fresh herbage were analysed by ANOVA.

Results and discussion

Total herbage production increased significantly (P<0.001) for each increase in N application rate; with herbage yields increasing by 30% from 0 to 90 and from 90 to 180, and by 18% from 180 and 270 kg N ha^{-1} yr^{-1}. The total lowest average yield across all treatments was in October (0.36 Mg DM ha^{-1}) while, unusually, July had the second lowest average yield at 0.57 Mg DM ha^{-1} compared to 1.39 Mg DM ha^{-1} for May and June and 1.32 Mg DM ha^{-1} for August across all yields. This can be explained by the unusual drought conditions experienced during 2018 in which only 61.4 mm of rain fell between June and August. The average growth for each month showed highly significant (P<0.001) differences with 0 kg N ha^{-1} yr^{-1} and 90 kg N ha^{-1} yr^{-1} having significantly lower growth rates than 180 and 270 kg N ha^{-1} yr^{-1} (Figure 1).

N application rate had no significant effect on ADF and ME. Increasing N application rate significantly (P<0.001) increased the CP content of the herbage and significantly (P<0.001) decreased the WSC and DM of the herbage. There were significant effects seen (P<0.001) for DM, CP, WSC, ADF and ME per month; which were in line with expectation due to weather. Interactions between treatment and month were seen for ADF, ME, DM (P<0.001) and WSC (P=0.012). Increasing N application rate increased magnesium (Mg) (P=0.002) and zinc (Zn) (P<0.001) content and decreased chloride (P=0.005) and molybdenum (Mo) (P<0.001) content. No other significant effects were observed for trace element content.

There was no significant difference seen for botanical analysis for the sward species in April or June. However, in August there were significant differences (P=0.009) in the perennial ryegrass and non-sown grass species proportions of the sward between 0, 180 and 270 kg N ha^{-1} yr^{-1} but not between 0 and 90 kg N ha^{-1} yr^{-1} or between 90, 180 and 270 kg N ha^{-1} yr^{-1}. The lowest percentage of ryegrass was seen in

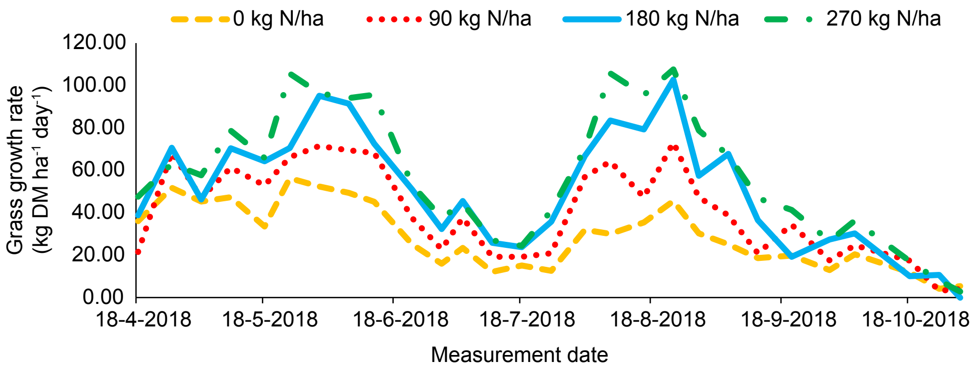

Figure 1. Herbage growth (kg DM ha^{-1} day^{-1}) curve for the four application rates in 2018.

the sward in June, with it higher in both April and August. This higher amount of perennial ryegrass in August may have been due to the drought conditions earlier in the season having a more severe effect on the shallower rooting non-sown weed grass species during this period.

Conclusions

Increasing N application rate improved average herbage growth and resulted in a higher total herbage yield. Increasing N application rate improved herbage quality, although there was a substitution for an increase in CP for a decrease for WSC. Plots receiving the higher rates of N application had a higher proportion of perennial ryegrass in the sward relative to plots receiving no added N. Trace element content was affected by increasing the N application rate. Further research is needed into looking at the response of N between the two highest N application rates as although the yields were significantly different between 180 kg N ha^{-1} yr^{-1} and 270 kg N ha^{-1} yr^{-1} the proportion of herbage produced from each kg of N applied decreased between 180 kg N ha^{-1} yr^{-1} and 270 kg N ha^{-1} yr^{-1} compared to the lower levels. Further research should also look into grazing animals on swards that have received these N levels.

Acknowledgements

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References

DAERA (2016) Delivering Our Future, Valuing Our Soils: A Sustainable Agricultural Land Management Strategy for Northern Ireland. (SALMS). 132 pp. Available at: https://tinyurl.com/y822fzly.

DEFRA (2016) The British Survey of Fertiliser Practice: fertiliser use on farm crops for crop year 2016, 114 pp. Available at: https://tinyurl.com/w7hpgdg.

Perennial ryegrass versus tall fescue on winter flooded grasslands

Cougnon M., Wyckaert A. and Reheul D.
Department of Plant and Crops, Ghent University, Proefhoevestraat 22, 9090 Melle, Belgium

Abstract

On winter flooded pastures, perennial ryegrass (*Lolium perenne* L.) is outcompeted by species with poor agronomic value. The potential of tall fescue (*Festuca arundinacea* Schreb.) on winter flooded grassland was tested both in a lysimeter study and in a field trial. In the lysimeter study, ground water level was set either at 0 cm (high groundwater) or at -30 cm (low groundwater) during 2 months in two consecutive winters. In the following growing seasons, tall fescue yield was significantly higher, both in the high and in the low ground water level. The effect of ground water level on yield was not consistent in the two years. To compare the two species in real farming conditions, degenerated swards, close to a waterway, were resown with either perennial ryegrass or tall fescue during the summer of 2017. Frequent flooding occurred in the winter of 2017-18, whereas the winter of 2018-19 was rather dry. Botanical analysis of the swards revealed that after two growing seasons both swards were still dominated by the sown species, but the perennial ryegrass plots contained a higher proportion of undesirable species at the end of the second growing season.

Keywords: flooding, *Elymus repens*, botanical analysis, lysimeter

Introduction

In the valley of the IJzer river, an important grassland area in the west of Belgium, around 15% of the 30,000 ha of grassland is frequently flooded in winter. Whereas these winter flooded grasslands were highly appreciated as hayland until the 1960s, this in not so today for grassland farming. The grass species typical for these grasslands, including couch grass (*Elymus repens*), tufted hairgrass (*Deschampsia cespitosa*), bent grasses (*Agrostis* sp.), marsh foxtail (*Alopecurus geniculatus*), and floating sweet grass (*Glyceria fluitans*), produce a lower yield and lower quality forage than grass species like perennial ryegrass (*Lolium perenne*). Perennial ryegrass. however, does not tolerate flooding well.

In the valley of the Oder in Germany, where a similar situation occurs, different species and management practices were compared by Schuscke and Bischoff (1980a) to find out how grassland production on winter flooded pastures can be improved. Also, in experiments in the Netherlands by Willemsen (1976), different species for frequently flooded grasslands were compared. In these different experiments, tall fescue (*Festuca arundinacea*) proved to tolerate winter floodings well, once established. Although tall fescue has a lower digestibility than perennial ryegrass when cut at the same frequency, its greater yield potential makes it an interesting species for intensive dairy farms (Cougnon *et al.*, 2014).

Based on the results of these experiments, we hypothesised that tall fescue is a valuable alternative for perennial ryegrass on winter flooded grasslands in the IJzer valley. Both a lysimeter experiment and a field trial were established to compare the two species.

Materials and methods

In the summer of 2017, six lysimeters measuring 1.5×1.5×1.5 m were filled with peat and clay soil from the IJzer valley. The lysimeter can be seen as a metal soil tank, in which the ground water level can be controlled by adding or removing water from a communicating vessel (a smaller tank of 0.3×0.3×1.5 m deep). Each lysimeter was divided in two: one half was sown with perennial ryegrass cv. Melonora (Lp), the other half was sown with tall fescue cv. Barolex (Fa), both at a density of 1,700 seeds m^{-2}. Three lysimeters were assigned to a low ground water level 'LOW', where winter ground water level (mid-December till mid-February) was kept around -30 cm relative to the ground level; the other lysimeters were assigned to a high ground water level 'HIGH' in which the winter ground water level was kept at 0 cm to +5 cm. In summer (June, July, August), ground water level was kept at -100 cm both in the HIGH and the LOW lysimeters. In 2018 and 2019, 5 cuts per year were harvested by clipping the grass at a height of circa 5 cm. Each half of each lysimeter was harvested separately. In 2018, only the yield of the first cut was registered. In 2019, all cuts were weighed. Annual fertilisation amounted to 250 kg N ha^{-1}, 85 kg P_2O_5 $ha^{-1}$and 250 kg K_2O ha^{-1}, distributed evenly over the different cuts. Effect of species and ground water level on the grass yield were estimated using two-way Anova.

On a pasture in Stavele, known to be flooded frequently in winter, six strips of 6×60 m were killed using glyphosate (1,800 g ha^{-1}) on a winter flooded pasture, dominated by couch grass. These strips were rotavated, resown with either tall fescue (Fa: cv. Barolex) or with perennial ryegrass (Lp: cv. Achat' (cv. Melonora was not available in sufficient quantity)) both at 50 kg ha^{-1} and finally rolled. From 2018 on the resown strips were managed like the remainder of the pasture: 3-4 cuts per year with a fertilisation of 170 kg N ha^{-1} from cattle slurry and 150 kg N ha^{-1} from mineral N. In November 2018 and 2019, a botanical analysis was performed on the resown Fa and Lp strips. The relative ground cover of the species present within a 0.1×0.1 m frame was estimated on 20 randomly selected spots in each strip.

Results and discussion

In the first cut of 2018, the negative effect of the high winter water level in the lysimeters on yield was greater for Fa than Lp, resulting in a significant interaction between water level and species (Figure 1A). The yield of Fa however was greater than that of Lp in both ground water levels. In the first cut of 2019, Fa significantly again outyielded Lp, but contrary to our expectation, the yield was greater in the high

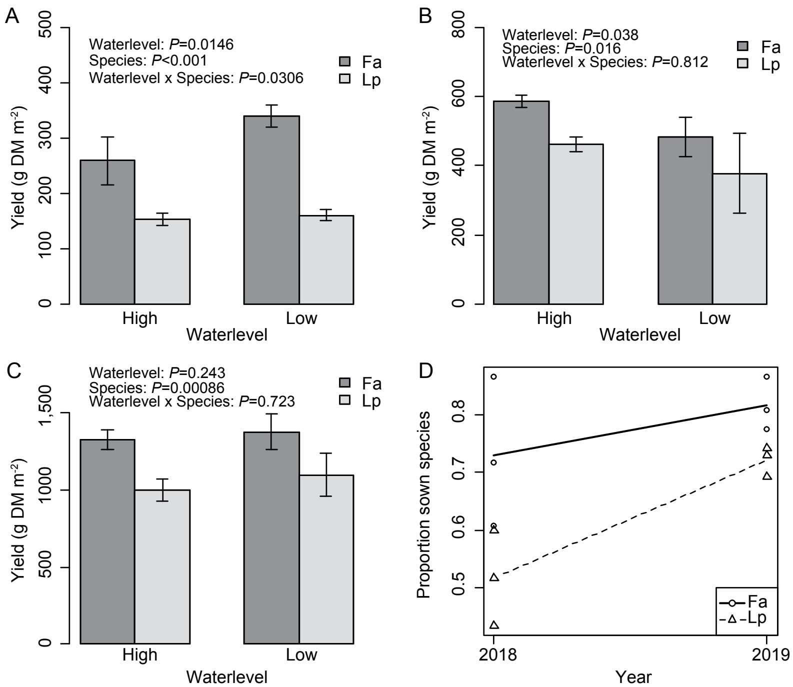

Figure 1. Dry matter yield of tall fescue (Fa) and perennial ryegrass (Lp) measured in lysimeters with either high or low winter ground water levels in the first cut of 2018 (A), the first cut of 2019 (B), the five cuts of 2019 (C). (D): The evolution of the proportion of the sown species in field plots resown with either Fa or Lp.

ground water treatments (Figure 1B). Over all cuts in 2019, there was no longer an effect of ground water level, but Fa outyielded Lp by 29%, which is in line with the values found in literature under similar growing conditions (Figure 1C) (Cougnon *et al.*, 2014). The absence of the expected negative effect of the high ground water level may be due to several reasons. Whereas submerged plants are in a rather anaerobic environment, swards in the lysimeters were never completely submerged, ensuring certain access to oxygen. After a flooding, a layer of sediment remains on the leaves, which is not supported well by all grass species (Schuschke and Bischoff, 1980b), whereas in the lysimeters this effect was not present.

In the field experiment, it was clear that Fa plots contained fewer unwanted species than in the Lp plots (Figure 1D). In the absence of flooding in the winter of 2019-19, the proportion of unwanted species in the Lp plots, however, decreased. Given the high yield potential of Fa, there is no reason to doubt that the Fa plots will be more productive compared with both the Lp and the original vegetation.

Conclusions

The supposed better flooding resistance of tall fescue compared with perennial ryegrass could not be proved in the lysimeters. Tall fescue, however, had a higher yield than Lp. In a field trial on a frequently flooded grassland, plots resown with Fa contained fewer unwanted grass species than plots resown with Lp.

References

Cougnon M., Baert J., Van Waes C. and Reheul D. (2014) Performance and quality of tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) and mixtures of both species grown with or without white clover (*Trifolium repens* L.) under cutting management. *Grass and Forage Science*, 69(4), 666-677.

Schuschke F. and Bischoff H.-M. (1980a) Untersuchungen über den Einfluss von Überschemmungen auf Gräser – 1. Mitteilung: Der Einfluss der Überschemmungsdauer. *Archiv für Acker- und Pflanzenbau und Bodenkunde*, 521-529.

Schuschke F. and Bischoff H.-M. (1980b) Untersuchungen über den Einfluss von Überschemmungen auf Gräser – 3. Mitteilung: Die Wirkung von speziellen Pflegmassnahmen. *Archiv für Acker- und Pflanzenbau und Bodenkunde*, 539-544.

Willemsen W. (1976) *Herinzaai van grasland in uiterwaarden*. Lelystad, the Netherlands: Proefstation voor de rundveehouderij.

Ice encasement tolerance of Norwegian timothy (*Phleum pratense*) cultivars

Dalmannsdottir S., Jørgensen M. and Elverland E.
Norwegian Institute of Bioeconomy Research, Holt 9016 Tromsø, Norway

Abstract

Farmers in Northern Norway frequently experience winter damaged fields caused by ice encasement. The economic consequences are severe due to loss of fodder and costs with reestablishment of swards. It is therefore important to choose the best available varieties for the local climatic and environmental conditions. We tested eight Norwegian cultivars of timothy (*Phleum pratense*), for tolerance to ice encasement and their regrowth capacity. Both old and new cultivars, and cultivars with good overwintering capacity and less biomass production were tested against more productive cultivars with less overwintering capacity. The experiment was a semi-field setup and plants were established in pots which were placed outside. Half of the pots were covered with ice and half were kept under snow cover. During four months, pots were brought, once per month, into a greenhouse for thawing and measurement of biomass production under normal growth conditions. The results indicate that the old winter hardy cultivar 'Engmo' is least affected by ice encasement but produces little biomass. The joint Nordic cultivar 'Snorri' produced most biomass of all the cultivars after a treatment with ice cover. In conclusion, there is a large difference between cultivars in ice encasement tolerance, and ice cover affected regrowth capacity far more than snow cover.

Keywords: ice encasement tolerance, snow cover, regrowth capacity, Northern Norway

Introduction

In recent years, winter damage in grass fields caused by ice encasement has increased in frequency in Northern Norway, resulting in economic losses for farmers. Winter survival of forage grasses is the main challenge for perennial forage production in high latitude areas. Especially changing conditions leading to snow melting and again freezing may result in long lasting ground ice cover and stressful anoxic conditions for the plants. Accumulation of metabolites to toxic concentrations during anoxic conditions under the ice, and/or impact of reactive oxygen species when the ice melts, may lead to plant cell death (Gudleifsson and Bjarnadottir, 2014). Tolerance to anoxic conditions is clearly species- and cultivar-dependent (Höglind *et al.,* 2010). Due to climate change, winter damage caused by ice encasement will probably decrease in some areas, but increase in other areas where snow cover now is stable (Gudleifsson and Bjarnadottir, 2014). Further, warmer autumns may reduce the cold acclimation ability of grasses and thus reduce their winter hardiness (Dalmannsdottir *et al.*, 2014). Timothy (*Phleum pratense*) is the most important forage grass in Northern Norway. The old winter-hardy landrace 'Engmo' was the most-used cultivar until 2005; since then it has been replaced by 'Noreng', a cultivar bred by Graminor in Norway. Farmers report that the current timothy cultivars used are less robust against winter stresses. The more winter-hardy cultivars usually give less biomass in the second harvest compared with the more productive and less winter-hardy cultivars. With this study we aimed to identify if cultivars differ in long-term tolerance to ice encasement. Furthermore, we studied the regrowth capacity of the cultivars after a treatment of a natural snow cover versus ice encasement.

Materials and methods

Seedlings of eight cultivars of timothy ('Engmo', 'Grindstad', 'Noreng', 'Lidar', 'Snorri', 'Varg', 'Gunnar' and 'Liljeros') (Table 1) were planted in 12 cm pots with a soil mixture of sphagnum with fertiliser and perlite (2:1), five plants per pot. Pots were placed in a greenhouse at 18 °C with natural daylight in

Table 1. Description of the timothy cultivars used in the study

Cultivar name	Origin
Engmo	An old landrace, northern-adapted, excellent winter survival, low yield in second harvest
Grindstad	An old landrace, southern-adapted, high yielding, most commonly used in Norway
Noreng	Northern-adapted, higher yielding than Engmo
Lidar	Relatively winter hardy
Snorri	A joint Nordic cultivar based on genotypes originating from the northern part of the nordic countries aiming to increase the yield in second harvest without sacrificing the good winter survival ability
Varg	New cultivar, based on Grindstad
Gunnar	New cultivar, based on Grindstad
Liljeros	New on the market, southern-adapted, higher yielding than Grindstad

Tromsø, Norway (69.68°N) and watered regularly. In the beginning of August, plants were placed outside to harden under natural conditions, and fertilised with NPK (18:3:15), 20 g per pot. On 1 September the grass biomass was cut at 5 cm and the treatments started in middle of December. A solid ice cover was formed by placing crushed ice on the top of half of the pots, and then watered with ice water to cover plants with ice. The pots were randomly placed in boxes, eight pots per box. Half of the pots were not covered with ice and put under a thin mat, which gradually was covered with natural snow cover. Four pots per cultivar (20 plants) per treatment were brought into the greenhouse at monthly intervals: 19 January, 19 February, 19 March and 19 April. The temperature during winter was relatively stable below 0 °C and the ice was not affected by the few peaks above zero. Pots were first thawed at 6 °C for one week before they were placed in the greenhouse at 18 °C and 24 h light in addition to natural light. Survival and biomass production were registered after 3 weeks at growth temperature. The regrowth was harvested and dried at 60 °C for 24 h before weighing. Results were analysed by the Glimmix procedure of SAS with duration of treatment and cultivar as fixed effects and replicate as random.

Results and discussion

All cultivars produced significantly less biomass after a treatment with ice cover compared with the natural snow cover (P<0.001) (Figure 1). 'Engmo' had least difference between the two treatments due to its excellent ice encasement tolerance (Figure 1 and 2). Of all the cultivars, 'Engmo' was the least affected by ice encasement during the whole period of four months (Figure 2). 'Snorri' and 'Noreng' produced more biomass than all other cultivars as a mean of both treatments and all test periods (P<0.05). After three months under natural snow cover 'Noreng' produced more biomass than 'Engmo', but 'Engmo' produced more biomass than 'Noreng' after the ice cover treatment (Figure 1).

Conclusions

'Engmo' was least affected by ice encasement but produced little biomass. 'Snorri' lost more than half of its biomass yield due to ice encasement, but due to its high regrowth capacity and reasonable ice encasement tolerance, its final yield was higher than that of the other tested cultivars. The regrowth capacity was dependent on the cultivar, but it was generally less after a treatment with long lasting ice encasement versus snow cover.

Acknowledgements

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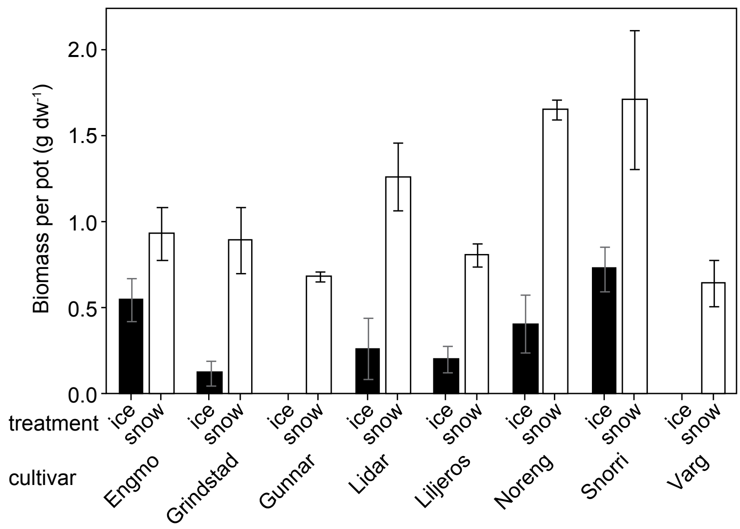

Figure 1. Biomass production per pot of all cultivars after 3 months treatment with ice cover or natural snow cover. Bars indicate standard error of the mean.

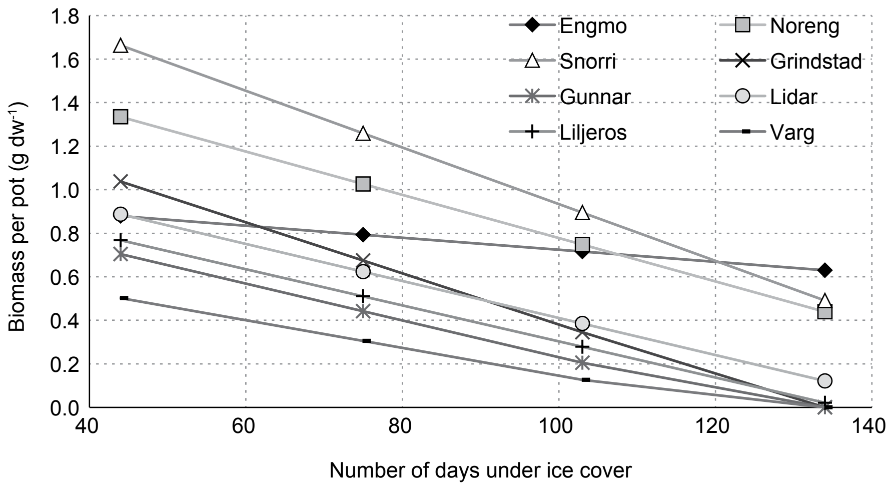

Figure 2. Biomass production of 8 timothy cultivars after 1 to 4 months treatment with ice cover.

References

Dalmannsdottir S., Jørgensen M., Rapacz M., Østrem L., Larsen A., Rødven R. and Rognli O.A. (2017) Cold acclimation in warmer extended autumns impairs freezing tolerance of perennial ryegrass (*Lolium perenne* L.) and timothy (*Phleum pratense* L.). *Physiologia Plantarum*, 160, 266-281

Gudleifsson B.E. and Bjarnadottir, B. (2014). Estimating ice encasement tolerance of herbage plants. In: *Plant Cold Acclimation* (pp. 225-240). Humana Press, New York, NY.

Helgadóttir A. and Kristjansdóttir T. (2006) Snorri – a new Nordic timothy variety for areas around the arctic circle. In: T. Sveinsson (ed.) *Timothy productivity and forage quality – possibility and limitations*. Agricultural University of Iceland, Iceland, pp. 9-14.

Höglind M., Bakken A.K., Jørgensen M. and Østrem L. (2010) Tolerance to frost and ice encasement in cultivars of timothy and perennial ryegrass during winter. *Grass and Forage Science* 65, 431-445

Some remarks on evaluating nitrogen nutrition index in pastures under low intensity of defoliation

Duchini P.G.[1], Guzatti G.C.[2], Americo L.F.[1], Winter F.L.[1] and Sbrissia A.F.[1]
[1]Santa Catarina State University (UDESC/CAV), Avenida Camões, 2090, 88520-000, Lages, SC, Brazil; [2]Santa Catarina Federal Institute (IFSC), April 22nd Street, 2440, 89900-000, São Miguel do Oeste, SC, Brazil

Abstract

Nitrogen nutrition index (NNI) is an important tool to evaluate nitrogen status of pastures. However, the nitrogen content (N%) dilution curves in plant aerial biomass result from biomass increments from young tissues and very low mass values (from 1 Mg DM ha^{-1}). In this sense, pastures managed with low intensity of defoliation maintain high residual biomass, which consists mainly of old and structural plant mass with low N contents. We hypothesised that pastures managed under low intensity of defoliation present a quadratic relationship between green aerial biomass and N% content during regrowth. *Arrhenatherum elatius*, *Dactilys glomerata* and *Festuca arundinacea* pastures were managed for two years with pre-grazing heights of 20 cm and cut to half this height. At the end of this period, half of the plot areas were cut by 50% of sward height and the other half were cut at ground level, and both were fertilised with 400 kg of N ha^{-1}. The three species presented dilution curves ($N\% = a(W)^{-b}$) when cut at ground level but different quadratic relationships were observed when cuttings were performed from 10 cm. Our results suggest that perennial grasses managed with low intensity of defoliation should have their nitrogen status measured and interpreted with caution so as not to underestimate the actual nitrogen status of the pasture herbage.

Keywords: critical nitrogen, dilution curve, grasses, high residue, lenient defoliation

Introduction

Fertilisation programmes based on technical indices, mainly for nitrogen (N), have been used to maximise the productive potential of pastoral systems and optimise resources. In this sense, the nitrogen nutrition index (NNI), determined by the relationship between N concentration in crops (N%) and their critical N (N_{crit}) (Lemaire and Gastal, 1997) has been considered as an effective tool to indicates real N status of pasture herbage. N% can be estimated directly by analysing forage samples, whereas the N_{crit} are determined from estimates of biomass (W) through N% dilution curves (Lemaire and Gastal, 1997). The N% dilution throughout forage accumulation/regrowth process is based on inverse relationship between metabolic (W_m) and structural (W_s) tissues, so that the greater biomass the greater W_s. Pastures managed under intermittent stocking method with low intensity of defoliations retain high residual biomass, composed mostly of W_s and old tissues which have low N% values (Da Silva *et al.*, 2009). Under these types of management, firstly there is an increment in biomass from W_m to recover pasture leaf area index (LAI) (Parsons and Penning, 1988) and, thus, an increase in N% would be expected with subsequent dilution. Therefore, this study tested the hypothesis that there is a difference in N% dilution pattern throughout pasture regrowth according to the adopted residual height.

Materials and methods

Pastures of *Arrhenatherum elatius* 'SCS314 Santa Vitória' (exploitative species), *Festuca arundinacea* 'Quantum II' (conservative species) and *Dactylis glomerata* 'Ambar' (moderately exploitative species) were sown on 13 June 2013, at 18 kg ha^{-1} of pure viable seeds, in Lages, Brazil (27°47' S, 50°18' W; altitude: 960 m above sea level). Plots measured 9×5 m with three replicates in a completely randomized design. From May 2014 to June 2016, cuts were performed when pre-cutting height sward heights reached 20 cm, and were lowered to 10 cm. The pre-cutting height corresponded to the average canopy condition that intercepted around 95% of the incident radiation during the full vegetative development stage of plants. After soil analyses, phosphorus and potassium fertilisers were applied each autumn to maintain a high soil fertility and plots were fertilised every 40 days with nitrogen, as urea, with 30 kg of N ha^{-1} (270 kg ha^{-1} yr^{-1}) during the first experimental year (2014/2015), and 50 kg of N ha^{-1} (450 kg ha^{-1} yr^{-1}) during the second experimental year (2015/2016). After this period, on 25 August 2016, plots were divided in half (experimental units of 9×2.5 m) and cut at 10 cm or at ground level. Immediately after cutting, each pasture received a single fertilisation with 70 kg of P_2O_5 ha^{-1}, 70 kg of K_2O ha^{-1}, and 400 kg of N ha^{-1}. To ensure that N was non-limiting for plant growth, a surplus of N of 200 kg of N ha^{-1} was applied on the last linear metre of each experimental unit. Live biomass and its N% were evaluated five times throughout regrowth (Table 1).

Samples were taken using two 1.40×0.40 m frames. The biomass inside each frame was cut to ground level, then dried in a forced air oven (65 °C for 72 h) and weighed. Samples were ground in a Willey mill to determine N% by the Kjeldahl method. Regressions analysis were made using SigmaPlot, version 11, considering α=0.05.

Results and discussion

The changes in N% in relation to an increase in biomass differed among the pastures and the regrowth from different cutting regimes (Figure 1). Biomass of pastures where regrowth was taken from ground level showed a typical exponential N% dilution pattern (Figure 1A), with the same dilution coefficient observed for C_3 species (Lemaire and Gastal, 1997). However, when regrowth occurred from pre-existing residue structures created over time (pastures cut at 50% of initial height for 25 months) a quadratic pattern of changes in N% with increasing biomass was observed. This is because, after defoliation, high residue retains a large amount of 'old' biomass composed mostly of stems and old leaves, structures with low N% and higher fibre content (Da Silva *et al.*, 2009). However, with the emergence of new leaves through regrowth and therefore production of W_m, there was an increase in N% in the total biomass.

Table 1. Canopy heights in which the live biomass and its N% were evaluated throughout forage regrowth.

Treatments	Live biomass and N% evaluations				
	1st	2nd	3rd	4th	5th
Cut down to 10 cm	10 cm	15 cm	20 cm	25 cm	Two weeks after 25 cm
Cut to ground level	5 cm	10 cm	15 cm	20 cm	25 cm

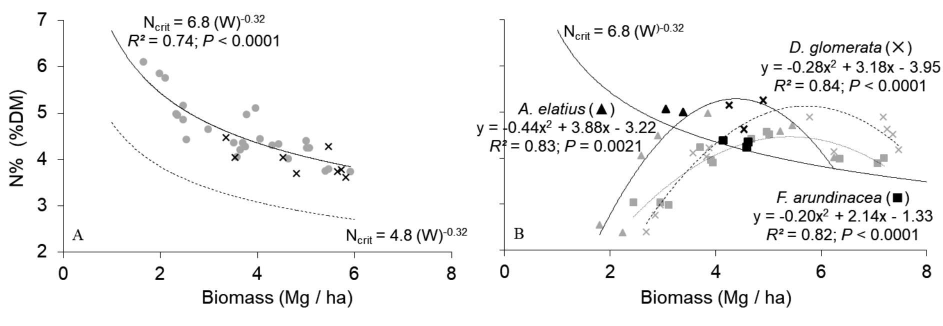

Figure 1. Biomass × N% relationships throughout pastures regrowth from different residue height. (A) Ground level; dotted line represents the dilution curve proposed by Lemaire and Gastal, 1997; data set came from all species; and '×' indicate samples collected in pastures that received 600 kg of N ha^{-1}. (B) Ten cm height; black symbols indicate samples collected when pastures reached a pre-cutting height of 20 cm.

Only after the N% has reached a ceiling value does the N% dilution process start, due to the production of W_s and ageing of the accumulated material. *A. elatius* pastures reached NNI equal to 1.0 with the lowest biomass, while the maximum point in *F. arundinacea* pastures was lower than in other pastures (Figure 1B). It is noteworthy that the three pastures reached NNI greater than 1.0 when they reached the pre-cutting height targets (20 cm). These differences could be linked to the plant/tiller longevity, as more conservative species (those presenting longer-lived tillers) support more old tissue compared with the more exploitative ones (Duchini *et al.*, 2018) and, then, need greater amount of new W_m to reach an equal N% biomass. NNI below N_{crit} at the beginning of regrowth was only a result of the structure and age of the residue, not reflecting the actual nitrogen status of the pastures. Therefore, it is necessary that the N% is evaluated only under conditions close to the maximum point, as presented in Figure 1B. Moreover, no differences were observed for N% and biomass when 400 or 600 kg of N ha^{-1} were applied.

Conclusions

Perennial grasses managed with low intensity of defoliation presented NNI greater than 1.0 when they reached a light interception of around 95% in the full vegetative stage, which corresponded to a canopy height of around 20 cm for the studied species. Therefore, in pastures subjected to lenient cutting/grazing, the NNI should be measured and interpreted with caution in order not to underestimate the actual nitrogen status the pasture herbage.

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References

Da Silva S.C., Bueno A.A.O., Carnevalli R.A., Uebele M.C., Bueno F.O., Hodgson J., Matthew C., Arnold G.C. and Morais J.P.G. (2009) Sward structural characteristics and herbage accumulation of *Panicum maximum* cv. Mombaça subjected to rotational stocking managements. *Scientia Agricola* 66, 8-19.

Duchini P.G., Guzatti G.C., Echeverria J.R., Américo L.F. and Sbrissia A.F. (2018) Experimental evidence that the perennial grass persistence pathway is linked to plant growth strategy. *PLoS ONE* 13(11), e0207360.

Lemaire G. and Gastal F. (1997) N Uptake and distribution in plant canopies. In: Lemaire G. (ed.) *Diagnosis of the Nitrogen Status in Crops*, Springer, New York, USA, pp. 3-43.

Parsons A.J. and Penning P.D. (1998) The effect of the duration of regrowth on photosynthesis, leaf death and the average rate of growth in a rotationally grazed sward. *Grass and Forage Science* 43, 15-27.

Yield performance and competitiveness of different grass species mixtures

Ehrhard D., Poyda A. and Taube F.
Christian-Albrechts-University of Kiel, Institute Crop Science and Plant Breeding, Grass and Forage Science/Organic Agriculture, Hermann-Rodewald-Str. 9, 24118 Kiel, Germany

Abstract

As a consequence of specialisation and regional segregation of agricultural production systems in northwest Europe, permanent grassland is nowadays almost exclusively attributed to dairy farming regions. Concurrently, the intensification of these systems has resulted in large nutrient inputs on grasslands, which are utilised via cutting in most cases. In combination with high traffic frequency on grasslands, this situation threatens the quality of grassland swards due to shifting botanical compositions towards undesired species. The main objective of this study was to identify species interaction effects and overyielding potentials of different grassland mixtures. Therefore, six grass species were sown in September 2018 in monocultures and mixtures and fertilised at two different levels (250+207.5 and 500+415 kg N + K ha^{-1} yr^{-1}, respectively). The annual yield was sampled during five cuts in 2019 and dry matter was determined. The data were evaluated with a linear regression model. The results indicated a positive effect in biomass production by an increase in diversity as described in literature, especially in the higher fertilised treatment.

Keywords: overyielding, *Poa trivialis*, biomass productivity

Introduction

Several grassland diversity experiments have demonstrated positive effects of increasing diversity on yield potential (overyielding). These effects have been explained by complementary effects or facilitative interactions among species, e.g. resource uptake, time, space or protection against biotic and abiotic stress factors (Hector *et al.*, 2002; Tilman *et al.*, 1996). Nevertheless, the possibility of complementarity is limited due to equal requirements on resource use.

Materials and methods

As a part of the European Innovation Partnership (EIP) project 'rough stalked meadow-grass', a field trial with 144 plots of 7×3 m and six grass species, representing different functional groups, was established. The six species were: rough stalked meadow-grass (*Poa trivialis* (G1)) and smooth stalked meadow-grass (*Poa pratensis* (G2)) – shallow rooting; perennial ryegrass (*Lolium perenne* (G3)) and hybrid ryegrass (*Lolium hybridium* (G4)) – high tiller density; tall fescue (*Festuca arundinacea* (G5)) and cocksfoot (*Dactylis glomerata* (G6)) – deep rooting. In total, 39 different communities (C) were established with C1-C6 the monocultures and C7-C39 different binary and multi-species mixtures. The trial was set up at Ostenfeld near Kiel in a simplex design (Kirwan *et al.*, 2009) in September 2018. Two fertilisation levels (250+207.5 and 500+415 kg N + K ha^{-1} yr^{-1}, respectively) were integrated in the experimental setup and randomly distributed among the plots. The grasses were cut five times with a plot harvester, to a stubble height of 5 cm, between May and October 2019. The samples were dried at 60 °C for 48 h and the dry matter content was determined for each plot.

To determine the overyielding potential of the mixtures, we executed a two-step evaluation of the data. For the first step a linear model was designed. The residuals were assumed to be normally distributed and homoscedastic. After that, a model selection was conducted in order to skip unnecessary effects from the model. For the second step, a linear model with the community as a fixed factor was defined.

The residuals were assumed to be normally distributed and homoscedastic too. Based on this model, an analysis of variances (ANOVA) was conducted, followed by multiple contrast tests in order to test for significant overyielding effects as defined by a greater mixture yield compared to the mean monoculture yield of grasses within the mixture weighted by their sown fractions.

Results and discussion

The annual yields of grass mixtures were in most cases higher compared to the mean values of the monocultures, as shown in Figure 1. Especially on the higher fertilisation level, this effect became apparent and was significant for several mixtures. However, communities fertilised with 250 kg N (and 207.5 kg K) ha^{-1} yr^{-1} indicated significant overyielding potentials in only two mixtures.

In Table 1, communities with significant overyielding effects are summarized and the sown grasses indicated. The community including all six species (C39) at both fertilisation levels, as well as communities with *L. hybridium* (G4) and *D. glomerata* (G6), demonstrated the highest overyielding potentials. This is mainly due to the fact that the mean calculated yield of communities was based on the sown proportions from the previous year. In contrast, the yield fractions of the meadow grasses (G1, G2) can be expected to be particularly lower than at the beginning of the experiment due to their low competitiveness (significant negative interaction effects with other species). Thus, community specific determination of yield fractions and overyielding potentials for the second year will be improved by using the exact species proportions and thereby correcting the weighted monoculture yields by species-specific competitiveness.

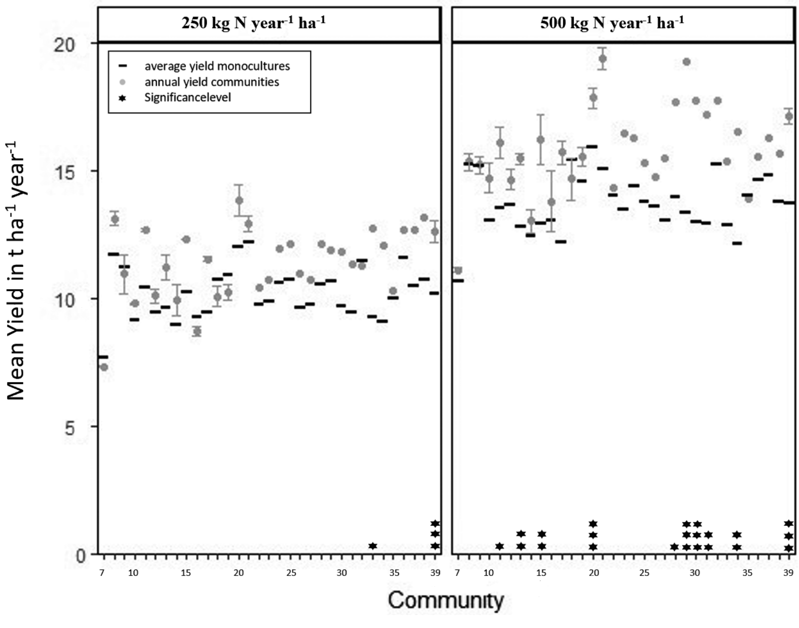

Figure 1. Mean annual yields for the communities 7-39 (circles) at the two fertilisation levels in 2019. The lines indicate the average monoculture yields of species sown in the respective mixture. Error bars represent standard error. Stars at the bottom describe different levels of significance * = *P*<0.05, ** = *P*<0.01, *** = *P*<0.001).

Table 1. Communities of sown grass species with significant overyielding potential.

Community	Fertilisation (kg N ha^{-1} year^{-1})	Species	Pr (>|t|)	Significance[1]
33	250	G1, G3, G4, G5, G6	0.031	*
39	250	G1, G2, G3, G4, G5, G6	<0.001	***
11	500	G1, G6	0.048	*
13	500	G2, G4	0.003	**
15	500	G2, G6	0.001	**
20	500	G4, G6	<0.001	***
28	500	G2, G4, G5	0.012	*
29	500	G2, G4, G6	<0.001	***
30	500	G1, G2, G3, G4	<0.001	***
31	500	G1, G2, G5, G6	0.001	**
34	500	G2, G3, G4, G5	0.001	**
39	500	G1, G2, G3, G4, G5, G6	<0.001	***

[1] * = P<0.05, ** = P<0.01, *** = P<0.001.

Conclusions

Positive mixture effects in biomass productivity and overyielding potential, as shown previously in several biodiversity experiments, can also be confirmed for intensively managed sown grasslands. The effects were even stronger for the very high fertilisation levels as the predominance of high-yielding species was more pronounced. However, for the analysis of the second year, weighted monoculture yields will be corrected for the competitiveness of species by using the species proportions at the end of the first year instead of the sown proportions.

Acknowledgements

The presented results were obtained within the project 'Productive grasslands by stable swards: Development of an online tool to avoid the spread of undesired species (Rough stalked meadow-grass)', funded in the frame of the European Innovation Partnership (EIP).

References

Hector A., Bazely-White E., Loreau M. and Schmid B. (2002) Overyielding in grassland communities: testing the sampling effect hypothesis with replicated biodiversity experiments. *Ecology Letters* 5, 502-511.

Kirwann L., Connolly J., Finn, J.A., Broph C., Lüscher A., Nyfelder D. and Sebastià M.T. (2009) Diversity-interaction modelling: estimating contributions of species identities and interactions to ecosystem function. *Ecology* 90, 2032-2038.

Tilman D., Weding D. and Knops J. (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379, 718-720.

Lignin concentration and digestibility in grassland forb and legume species

Elgersma A.
Independent scientist, P.O. Box 323, 6700 AH Wageningen, the Netherlands

Abstract

In dicotyledonous species, the relationship between chemical composition and digestibility differs from that of grass. Therefore, predictions of the digestibility of species-rich forage can be inaccurate. Data were used from a two-year cutting experiment with three legume species, four non-leguminous forbs, and a perennial ryegrass-white clover mixture. Relations between *in vitro* organic matter digestibility (IVOMD) and compounds of neutral detergent fibre, i.e. the lignin concentration (acid detergent lignin) and the degree of cell wall lignification, might increase insight in IVOMD differences among species or between functional groups. This paper aims to show such relations in legumes, non-leguminous forbs, and a perennial ryegrass-white clover mixture.

Keywords: cell wall compound, digestibility, forb, grass, legume, lignin

Introduction

In dicotyledonous species the relationship between chemical composition and digestibility differs from that of grass, which is often used as a research standard. Therefore, predictions of the digestibility of species-rich forage can be inaccurate. Søegaard *et al.* (2011) and Elgersma *et al.* (2014) showed that in spring, *in vitro* organic matter digestibility (IVOMD) for chicory (*Cichorium intybus* L.) was comparable to that of grass-clover (*Lolium perenne* L. – *Trifolium repens* L.), but they found a relatively large decrease towards summer. An even greater decline was found in ribwort plantain (*Plantago lanceolata* L.). In contrast, caraway (*Carum carvi* L.) maintained its high IVOMD from spring to summer.

Averaged over four cuts during two years, ribwort plantain, salad burnet (*Sanguisorba minor* Scop.) and lucerne (*Medicago sativa* L.) had the lowest IVOMD (636, 641 and 660 g kg^{-1} OM, respectively), while grass-clover and caraway ranked highest (749 and 743 g kg^{-1} OM, respectively) (Elgersma *et al.,* 2014). Relations between IVOMD and neutral detergent fibre (NDF) were shown earlier, but not with NDF compounds such as acid detergent lignin (ADL). This paper aims to show such relations, as they might increase insight in IVOMD differences among forb species. It was hypothesized that relations might differ among species and between functional groups, i.e. legumes and non-leguminous forb species.

Materials and methods

Pure stands were established with salad burnet, caraway, chicory, ribwort plantain, yellow sweet clover (*Melilotus officinalis* (L.) Lam.), lucerne, birdsfoot trefoil and a grass-white clover mixture in 2008. Swards were cut on 29 May, 9 July, 21 August and 23 October 2009 and 31 May, 13 July, 19 August and 21 October 2010. Data on concentrations of lignin, cellulose and hemicellulose (Table 1) were published in Elgersma *et al.* (2014) and general background information and statistical details can be found there. This paper further analyses IVOMD data in relation to lignin concentration of DM and to lignin proportion of cell walls.

Results

In all species, IVOMD and ADL concentrations were negatively related (Figure 1). Among non-leguminous forbs, slopes of regression lines were similar, while the intercept was lower for salad burnet than for caraway and ribwort plantain (P<0.05). The ADL concentration in ribwort plantain was higher

Table 1. Mean IVOMD, NDF, ADF and ADL concentrations and cell wall composition (% cellulose, hemi- cellulose and lignin of NDF) for salad burnet (sb) chicory (chi), caraway (ca), ribwort plantain (rp), yellow sweet clover (ysc), lucerne (lu), birdsfoot trefoil (bt) and a perennial ryegrass-white clover mixture (g-c) (Elgersma *et al.*, 2014).[1]

Trait	sb	chi	ca	rp	ysc	lu	bt	g-c	Sign
IVOMD	641^{a}	715de	743^{ef}	636^{a}	703^{cd}	660^{ab}	676^{bc}	749^{a}	***
NDF	295^{a}	329^{b}	322^{ab}	402^{e}	334^{bc}	383^{a}	328^{ab}	362^{cd}	**
ADF	240^{a}	275^{a}	270^{a}	339^{b}	271^{a}	321^{b}	272^{a}	275^{a}	**
ADL	44^{a}	42^{a}	51^{a}	78^{b}	45^{a}	67^{b}	62^{b}	49^{a}	***
% cell.	66	70	68	65	68	66	63	61	NS
% hemi.	19	17	16	15	18	17	18	26	NS
% lignin	15^{a}	13^{a}	16^{bc}	19^{d}	13^{ab}	17^{cd}	19^{d}	13^{ab}	**

[1] Significance of species effect (NS: not significant; ** P<0.01; *** P<0.001).

[2] IVOMD = *in vitro* organic matter digestibility; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin.

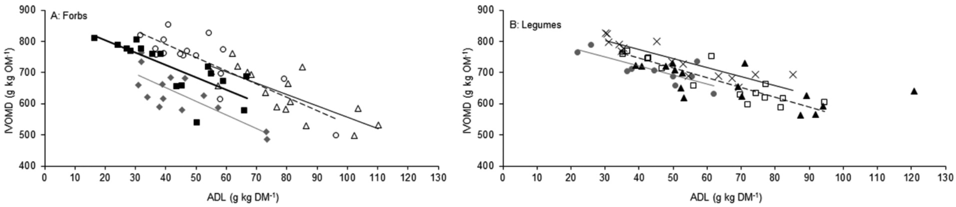

Figure 1. Relations between IVOMD and lignin concentration for (A) four non-leguminous forb species [(salad burnet: ◆, grey line; chicory: ■, bold line; caraway: ○, dashed line; ribwort plantain: △, black line); R^2 of regression lines: 0.64, 0.59, 0.67 and 0.58, respectively (P<0.05), slopes: -4.4, -4.0, -4.3, -3.6] and (B) three legumes (yellow sweet clover: ●, grey line; lucerne: ▲; birdsfoot trefoil: □, dashed line) and a perennial ryegrass – white clover mixture (×, black line); R^2 of regression lines: 0.62, NS, 0.79, 0.79, respectively (P<0.05), slopes: -2.9, NS, -3.1, -2.9.

(P<0.05) than in other forbs (Figure 1A), as was the degree of cell wall lignification (P<0.05; Figure 2A). In legumes, the IVOMD and ADL concentrations were negatively related (Figure 1B); slopes of regression lines were similar among birdsfoot trefoil, yellow sweet clover and grass-clover. The ADL concentration in lucerne and birdsfoot trefoil was similar to that in ribwort plantain and higher (P<0.05) than in yellow sweet clover and grass-clover, as was the degree of cell wall lignification (P<0.05; Figure 2B). The IVOMD declined faster, having more negative slopes with increasing ADL concentration in non-leguminous forbs (Figure 1A) than in legumes and grass-clover (Figure 1B) (P<0.01). Figure 2 illustrates ADL in relation to NDF concentration; most species showed linear relationships, indicating ADL concentrations to be proportional to NDF within that species across seasons. The mean ADL

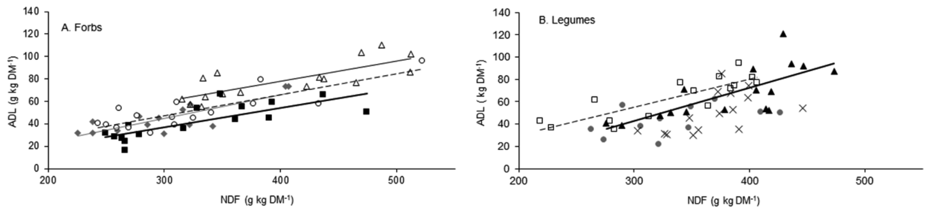

Figure 2. Relations between ADL and NDF concentrations for (A) four non-leguminous forb species [(salad burnet: ◆, grey line; chicory: ■, bold line; caraway: ○, dashed line; ribwort plantain: △, black line); R^2 of regression lines: 0.67, 0.60, 0.71 and 0.62, respectively (P<0.05), slopes: 0.21, 0.17, 0.18, 0.18] and (B) three legumes (yellow sweet clover: ●; lucerne: ▲, bold line; birdsfoot trefoil: □, dashed line) and a grass – clover mixture (×); R^2 of regression lines: NS, 0.54, 0.70, NS, respectively (P<0.05); slopes NS, 0.29, 0.25, NS.

concentrations of ribwort plantain, lucerne and birdsfoot trefoil were higher than in other species. Ribwort plantain and lucerne also had the highest NDF concentrations (P<0.001); birdsfoot trefoil had a low NDF concentration but owing to its high ADL concentration, thus a high lignification (19%) of the cell wall.

The grass-clover mixture showed a similar trend and slope (0.28) as lucerne (Figure 2B), but the relationship between ADL and NDF was not significant. In yellow sweet clover, the range in NDF was lowest and there was no relationship with ADL concentration.

Discussion

On average, the degree of cell wall lignification in non-leguminous forbs was highest in ribwort plantain (19% of NDF) and lowest in chicory. In legumes, birdsfoot trefoil and lucerne out-valued yellow sweet clover and grass-clover (Elgersma *et al.* (2014), Table 1). This study illustrates ADL concentrations in relation to NDF concentrations and IVOMD across samples for various species of biodiverse grasslands and fodder crops (lucerne).

Among non-leguminous forbs, ribwort plantain had, on average, lower IVOMD than caraway and chicory (P<0.05) (Figure 1A), but a similar IVOMD to salad burnet (636, 743, 715 and 641 g kg^{-1} OM, respectively) (Elgersma *et al.,* 2014). The higher IVOMD of caraway (P<0.05) than of ribwort plantain and salad burnet could not be explained by the degree of cell wall lignification as there were no relations, either among or within species. Thus, in salad burnet other factors than lignin or lignification must be associated with IVOMD.

Among legumes, lucerne and birdsfoot trefoil had on average a lower IVOMD than yellow sweet clover, while grass-clover had the highest IVOMD. Among all species, IVOMD was highest in the grass-clover mixture and caraway (749 and 743 g kg^{-1} OM, Table 1). Both had a low ADL concentration. The degree of cell wall lignification (13%) in grass-clover was on average not different from that in caraway (16%), despite the lower NDF concentration in caraway than in grass-clover (P<0.01).

At comparable levels of IVOMD, caraway had more cell wall lignification than chicory, and ribwort plantain more than salad burnet, and birdsfoot trefoil more than yellow sweet clover. Assuming that lignin is indigestible, this implies that in caraway and ribwort plantain and birdsfoot trefoil, the lignin present in the cell walls is less an obstruction to fermentation than in chicory and salad burnet and yellow sweet clover, respectively. This could be due to physical/spatial reasons, e.g. a different anatomical distribution of lignin in cell wall tissues, and/or to chemical factors, e.g. a different bonding of lignin to other compounds. This study showed that IVOMD declined faster (P<0.01) with increasing ADL concentration in non-leguminous forbs than in legumes and grass-clover (Figure 1), confirming the hypothesis.

Conclusions

In conclusion, differences in IVOMD among forb species could not be fully explained by lignin concentration or degree of cell wall lignification. More research is needed to understand the lignification and composition of cell walls in grassland species.

References

Elgersma A., Søegaard K. and Jensen S.K. (2014) Herbage dry-matter production and forage quality of three legumes and four non-leguminous forbs grown in single-species stands. *Grass and Forage Science* 69, 705-716.

Søegaard K., Eriksen J. and Askegaard M. (2011) *Proceedings of the Third Scientific Conference of ISOFAR*, Vol. 1 Organic Crop Production, pp. 190-193. Gyeonggi Paldang.

Nitrogen and phosphorus balances in dairy cattle feeding systems in the north-west of Spain

García-Pomar M.I., Báez D. and Santiago C.

Agricultural Research Center of Mabegondo CIAM-AGACAL, Carretera Betanzos-Mesón do Vento, km 8. Abegondo, A Coruña, Spain

Abstract

In Galicia, the main cow milk-producing region in Spain, nitrogen (N) and phosphorus (P) are lost from dairy farms to the environment. In this study, N and P balances were calculated in order to assist farm management and identify ways of improving the balances, thereby reducing losses to the environment and making better use of farm resources. Sixteen dairy cattle farms were classified according to the type of feeding system: ecological grazing; conventional grazing; grass silage; maize and grass silage; and maize silage. N and P surpluses both increased with intensification of the farm practices (from 123 to 353 kg of N ha^{-1} and from 13 to 41 kg of P ha^{-1}). P use efficiency varied widely within each group and there were no notable differences in relation to the feeding system. Considering the contribution of inputs and outputs to the N and P balances in the dairy farm systems, the main potential improvements identified were related to fertilisation (slurry management, reduction in the use of mineral fertilisers) and feeding (consumption of fodder and legume crops produced on the farm, and control of urea level in milk).

Keywords: surplus, nutrient use efficiency, inputs, outputs, concentrates, fertilisers

Introduction

Surplus amounts of nitrogen (N) and phosphorus (P) on agricultural land indicate potential N and P losses to the environment, either to the hydrosphere through run-off, leaching and erosion, or to the atmosphere (in case of N) – through gaseous emissions. In Galicia (NW Spain), the main dairy producing region in Spain, forested land represents 48% of the total area of the region, which contributes to the non-presence of 'vulnerable zones' (Nitrates Directive 91/676/EC). However, nitrate concentrations in water have been found to be correlated with the stocking rate and maize forage area in dairy farming areas. Moreover, P is strongly accumulated in dairy farm soils and is transported to waters, mainly by run-off and erosion, as well as by leaching at Olsen P>60 ppm (Heckrath *et al.*, 1995).

The objective of the present study was to determine N and P balances in dairy farm systems in order to assist farm management and identify pathways for improving the balance, reducing losses to the environment and making better use of farm resources entailing a lower cost.

Materials and methods

A total of sixteen dairy farms in Galicia, characteriszd by different levels of production intensity, were selected. The farms were chosen to represent the five types of Galician feeding systems: ecological grazing (EcoG, n=2), conventional grazing (ConvG n=3); grass silage (GS n=3); maize and grass silage (MGS n=4); and maize silage (MS n=4). N and P mineral balances (inputs-outputs) on each farm in 2017 were calculated as the difference between nutrient inputs (legume symbiotic nitrogen fixation, mineral and organic fertilisers, feed and animals) and nutrient outputs (milk, animals, organic fertilisers and crops).

An adapted version of the Resource Efficiency Tool developed in Dairyman Project, modified in the Eurodairy project (Foray and Oenema, 2017), was used to calculate the N and P balances.

Results and discussion

Stocking rate expressed as livestock units per hectare, milk production and feed concentrates (Table 1) increased with the intensity of the farming systems (from ecological grazing to maize silage system). N surplus increased with intensification of the farming systems, with notably larger values in the MGS and MS groups. The N inputs increased via concentrates and decreased via symbiotic fixation of legumes as the systems intensified. N inputs via fertilisers were very similar in the ConvG, GS and MGS groups, and they were lowest for EcoG and highest for MS. The highest N output (81-92%) was in milk. Significant N outputs occurred through slurry in the GS (31%) and MS (11%) groups, although they were variable. The value of N use efficiency (outputs/inputs) was 27.6%, and the value was lowest in the ConvG group and highest in the GS group (highly variable), followed by the MS group. P surplus on the farms was similar in the ConvG, GS and MGS groups, and it was higher in the MS group than in the EcoG and

Table 1. Characteristics and components of the N and P balance.[1]

		EcoG	ConvG	GS	GS+MS	MS	Mean
No. of cows		58±4	56±17	62±7	83±33	213±190	103
Agricultural area (ha)		63.1±8.3	40.3±13.4	49.4±14.6	50±21.3	96.5±88.9	61.3
Livestock unit ha^{-1}		1.2±0.1	1.7±0.1	1.6±0.2	2.2±0.5	2.8±0.3	2
L cow^{-1} $year^{-1}$		4,979±1,406	5,762±1,291	8,136±932	9,903±1,355	10,522±755	8,334
kg concentrates cow^{-1} $year^{-1}$		698±127	1,599±369	2,860±1,214	3,020±457	4,070±1,228	2,696
Olsen phosphorus (mg kg^{-1})		24±17	54±37	67±49	53±29	46±29	50
N balance	Animals	0±0	0±0	0±0	2±3	1±1	1
(kg N ha^{-1} $year^{-1}$)	Mineral fertilisers	0±0	94±2	78±26	83±25	117±34	82
	Organic fertilisers	33±23	0±0	0±0	0±0	0±0	4
	Forage bought	1±2	5±7	0±0	4±4	23±28	8
	Concentrates	25±7	90±26	133±58	256±62	355±48	198
	Symbiotic fixation	93±51	82±20	47±61	57±22	16±30	54
Total inputs		152±65	271±30	259±98	402±48	511±113	346
	Milk	23±8	45±7	56±12	95±18	130±17	78
	Animals	5±0	9±0	7±2	8±3	11±3	8
	Crops	0±0	0±0	0±0	0±0	0±0	0
	Slurry	0±0	0±0	28±49	0±0	17±34	10
Total outputs		29±8	54±9	91±63	103±21	158±45	96
Surplus		123±72	217±22	168±109	299±29	353±98	250
Efficiency (%)		22.0±14.5	19.7±1.9	37.0±22.3	25.5±2.7	31.3±7.2	27.6
P balance	Animals	0±0	0±0	0±0	0±1	0±0	0
(kg P ha^{-1} $year^{-1}$)	Mineral fertilisers	3±4	14±12	20±7	19±18	17±6	16
	Organic fertilisers	10±4	0±0	0±0	0±0	0±0	1
	Forage bought	0±0	1±1	0±0	1±1	2±0	1
	Concentrates	6±1	18±5	29±13	31±8	50±7	30
Total inputs		19±7	33±16	49±6	52±26	70±14	48
	Milk	5±1	9±1	10±0	17±4	23±2	14
	Animals	2±0	3±1	2±1	2±1	3±1	3
	Crops	0±0	0±0	0±0	0±0	0±0	0
	Slurry	0±0	0±0	6±10	0±0	3±6	2
Total outputs		6±2	11±2	18±13	19±4	29±9	19
Surplus		13±8	22±14	30±9	32±25	41±11	30
Efficiency (%)		35.8±20.3	39.9±18.1	36.4±21.7	44.6±23.3	42±9.1	40.4

[1] EcoG = ecological grazing; ConvG = conventional grazing; GS = grass silage; MGS = maize and grass silage; MS = maize silage.

ConvG groups. Inputs of P via mineral and organic fertilisers accounted for 50% of the total P inputs in the grazing systems. This proportion decreased as the production intensified, by increasing P inputs via concentrates and represented only 25% of the inputs in the MS group. The highest P output was in milk (56-87%), followed by P in animals (11-26%). Nevertheless, P output through slurry export was high in the GS group (32%) and the MS group (10%). The P use efficiency did not differ greatly in relation to feeding system and varied widely between farms within each group. In a similar study carried out between 2003 and 2005 on eighteen dairy farms in Galicia, N surplus was 332 kg ha^{-1} and N use efficiency, 27% (García *et al.*, 2006) and P surplus was 71 kg ha^{-1} and P use efficiency, 24% (Raison *et al.*, 2006). The N and P surpluses and P use efficiency thus improved during the last few years, but N use efficiency only very slightly.

Farms generally have a significant margin for improvement, taking into account similar dairy systems (De Klein *et al.*, 2016). Considering the contribution of inputs and outputs to the balance, the main improvements were established for each group, mainly in fertiliser use. The soil Olsen P was generally very high (>45 ppm ha^{-1}), except in soils in the EcoG group. Thus, P mineral fertilisation could be greatly reduced or eliminated on the farms. To reduce N inputs via fertilisers (21-35% of the inputs) in each group, we recommend taking legume crops into account when calculating the amount of N that should be applied, and establishing the dose of organic fertilisers according to the analytical content of slurry N, and also injecting the slurry into the soil or covering it immediately after application. Legumes could be introduced in rotation with maize in the GS, MGS and MS groups, or increased in grasslands. In the MS group slurry could be exported. To decrease inputs through concentrates, we recommend increasing the use of own-grown crops by increasing the agricultural area or rotations, including legumes and reducing the supply of excess protein in feedstuff accordingly to urea levels in milk.

Conclusions

There is a wide margin for improvement in N and P balances in dairy farm systems in Galicia. Management practices are recommended for different types of feeding systems. The main recommendations are to reduce P and N mineral fertilisation, calculate the agronomic N balance, incorporate slurry into the soil and increase cultivation of legumes crops.

Acknowledgements

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References

De Klein C.A.M, Monaghan R.M., Alfaro M., Gourley C., Oenema O., Powell J.M. (2016) Realistic nitrogen use efficiency goals in dairy production systems: a review and case study examples. *Proceedings of the 2016 International Nitrogen Initiative Conference*, Melbourne, Australia, pp. 1-9.

Foray S. and Oenema J. (2017) *Webinar: How to measure the resource efficiency*. https://eurodairy.eu/resources/webinar-how-to-measure-the-resource-efficiency/

García M.I., Castro J., Novoa R., Báez D. and López J. (2007) Improving nitrogen mineral balance and conversion rate in dairy farms in Galicia (Spain). In: Bosh A.D., Teira M.R. and Villar J.M. (eds.) *Towards a better efficiency in N use.* 15th Nitrogen Workshop, Editorial Milenio, Lleida, Spain, pp. 401-403.

Heckrath G., Brookes P., Poulton P. and Goulding K. (1995). Phosphorus leaching from soils containing different P concentrations in the Broadbalk experiment. *Journal of Environment Quality* 24, 904-910.

Raison, C., Pflimlin, A. and Le Gall, A. (2006). Optimisation of environmental practices in a network of dairy farms of the Atlantic Area. In: Jarvis S. and Plimlin A. (eds) Proceedings of the final Seminar of Green Dairy Project: Interreg Altantica Area III B No. 100. Institut de l´Élevage, Rennes, France, pp.43-65.

Species mixtures containing plantain produce higher biomass and herbage quality compared to monocultures

Golińska B., Paszkowski A. and Goliński P.
Poznań University of Life Sciences, Department of Grassland and Natural Landscape Sciences, Dojazd 11, 60-632 Poznań, Poland

Abstract

The use of herbs in mixtures with grasses and legumes is an innovative approach implemented in grassland-based forage systems in some European countries. The aim of this study was to determine the effects of the inclusion of plantain in grass-legume mixtures on the yield and sward chemical composition of temporary grassland at two sites in the Wielkopolska region in Poland. The experiment was established in 2015 in Brody (Luvisols soil) and Szelejewo (Cambisols soil) on 10 m^2 plots in a simplex design to define four monocultures and eight mixtures of four species (*Lolium perenne* L., *Trifolium pratense* L., *Trifolium repens* L. and *Plantago lanceolata* L.). During study years (2016-2017) herbage biomass from the whole plot was harvested three times per year to determine annual dry matter yield. Concentration of crude protein, water-soluble carbohydrates, crude ash, calcium and magnesium in herbage were also determined. It was found that the above-ground biomass of two and four-species mixtures was significantly higher than that obtained from monocultures. The herbage yield from leys containing plantain in the sward was higher on Luvisols than on Cambisols.

Keywords: grass-legume mixture, plantain, ley farming, herbage quality

Introduction

Grass-legume mixtures such as ryegrass-clover swards are commonly used in temporary agricultural grassland, because the herbage yield of a mixture can exceed that of its best-performing species when grown in a pure stand (transgressive overyielding) (Finn *et al.*, 2013). One innovative approach in multi-species swards is the addition of herbs into grass-legume mixtures. Recent studies have identified that mixtures containing plantain can improve productivity, and thus be a promising strategy for enhancing agricultural output and forage quality in European temporary grasslands (Cong *et al.*, 2016). The productivity of the grass-legume mixtures is affected by environmental, biotic and management factors. One is the soil type. The aim of this study was to determine the effects of inclusion of plantain in grass-legume mixtures on productivity and herbage quality of leys under conditions of two soil types.

Materials and methods

A study was carried out during 2016-2017 at two sites in the Wielkopolska region in Poland: Brody Experimental Station (52°43'24'N, 16°30'31'E) of the Poznan University of Life Sciences, and Szelejewo Breeding Station (51°86'34'N, 17°15'18'E) of Danko Plant Breeding Ltd. The experiment was established in early autumn 2015 on two soil types: (1) Luvisols at Brody (pH_{KCl} 6.2, total N of 0.13%, P_2O_5 29.4 mg 100 g^{-1}, K_2O 17.9 mg 100 g^{-1}, Mg 5.8 mg 100 g^{-1}) and (2) Cambisols in Szelejewo (pH_{KCl} 5.9, total N 0.10%, P_2O_5 22.0 mg 100 g^{-1}, K_2O 10.2 mg 100 g^{-1}, Mg 6.7 mg 100 g^{-1}). Experimental plots were 10 m^2 (1×10 m) in a simplex design (Ramseier *et al.*, 2005) to define four monocultures (Mono) and eight mixtures consisting of varying proportions of the four species (*Lolium perenne – Lp*, *Trifolium pratense – Tp*, *Trifolium repens – Tr* and *Plantago lanceolata – Pl*). The 8 mixtures consisted of 4 mixtures dominated in turn by each species (Domi 66.7%, of dominant and 11.1% of each of the other species), 3 mixtures composed of two species (Bi-50% *Pl* and 50% *Lp*, *Tr* or *Tp*) and the Centroid community (25% of each species). The monocultures and mixtures were sown according to seed rate in pure stands as recommended in Poland (*Lp*-30, *Tp*-20, *Tr*-15, *Pl*-10 kg ha^{-1}). The sward was managed by cutting three times. Fertiliser was applied each year at: 90 kg N ha^{-1} (30 kg ha^{-1} before each regrowth), 60 kg P ha^{-1}, and 90 kg K ha^{-1}. The yearly mean temperature and total precipitation for 2016 and 2017 at Brody were 9.7, 9.0 °C and 622, 764 mm, and at Szelejewo 10.0, 9.2 °C and 721, 765 mm, respectively. For each plot, biomass of aboveground vegetation was measured at each harvest. This was done by cutting the whole plot to a height of 5 cm and determining the fresh weight of the sward. A subsample of this material was taken, its fresh weight determined, and the material dried at 65 °C to constant weight to measure dry matter (DM). The samples collected for DM were ground to pass through a sieve of 1 mm of mesh size and used for forage quality analysis. Crude protein (based on total N content by Kjeldahl ×6.25), water-soluble carbohydrate (WSC; colorimetric method by Dubois *et al.,* 1956), crude ash, calcium and magnesium in dry matter of herbage were analysed. Statistical analysis of the total annual yield data was carried out according to a simple model (Finn *et al.*, 2013). The analysis of evenness gradient was performed. This is based on classifying the 12 mono/mixture plots into 3 groups: L – low (monocultures, 4 plots), M – medium (mixtures dominated by one species, 4 plots), H – high (two species and Centroid mixtures, 4 plots). Differences in herbage quality between mono/mixtures levels were tested using Tukey's *post hoc* test.

Results and discussion

The mixtures generally had a significantly higher annual yield compared with monocultures at both study sites, on Luvisols by 29.7% and on Cambisols by 25.2% (Table 1). The annual DM yield on Luvisols over the two years was largest in Bi *Tp-Pl* and Centroid mixtures, 17,608 and 16,969 kg ha^{-1}, while on Cambisols, Domi *Tp* and Bi *Tp-Pl* performed well, 14,450 and 13,300 kg ha^{-1}, respectively. The soil type influenced the productivity of plantain-containing grass-legume mixtures. Higher DM yield was obtained on Luvisols in comparison with Cambisols. On average for all *Pl* mixtures, in 2016 the DM yield was 12,524 vs 7,760 kg ha^{-1} and in 2017 it was 17,373 vs 14,976 kg ha^{-1}.

The results in Table 1 show consistent positive effects of increasing plant evenness. In our study, the yield of four- and two-species mixtures exceeded that expected from monoculture performances from either Luvisols or Cambisols (P<0.05). This effect has also been reported by several authors, e.g. Tilman *et al.* (1996) and Finn *et al.* (2013). There was also a high significant effect of year for both soil (P<0.001), but no effect of evenness × year interaction.

Species composition affected contents of protein, WSC, crude ash, calcium and magnesium in herbage (Table 2). The protein content was higher in swards containing clovers, but only Mono *Tr* differed significantly between all treatments. In the *Lp* swards (Mono, Bi and Domi) there was higher WSC content in herbage than in clover-containing swards. Clovers and plantain mono/mixtures produced higher mineral concentrations (in total, Ca and Mg) than *Lp*. This was also found by Pirhofer-Walzl *et al.* (2011), who suggested that including forbs in ryegrass-clover mixtures may not only enhance herbage production but also improve animal nutrition by providing sufficient dietary mineral supply to ruminants.

Table 1. Total annual DM yield (kg ha^{-1}) at three levels of evenness for two soil types (averaged over years 2016-2017).[1]

Item	Luvisols				Cambisols			
Evenness	L	M	H	Mixed	L	M	H	Mixed
Mean	11,517	14,866	15,015	14,941	9,087	11,429	11,320	11,375
Values for comparing means of evenness	L vs M	L vs H	M vs H	L vs Mixed	L vs M	L vs H	M vs H	L vs Mixed
SED	1,357	1,273	1,411	1,026	1,125	1,055	1,169	850
t value	2.47	2.75	0.11	3.34	2.08	2.12	0.09	2.69
P-value	*	*	ns	**	*	*	ns	*

[1] SED = standard error of difference between two means; ns = non-significant; * P<0.05, ** P<0.01.

Table 2. Concentration of selected elements in the herbage (g kg^{-1} DM) depending on the species composition (averaged over years 2016-2017, harvests and soil types).[1]

Treatment	Crude protein	WSC	Crude ash	Calcium	Magnesium
Mono *Lp*	126.9b	123.7a	78.8c	10.3c	2.1ab
Mono *Tp*	164.4b	73.6de	78.7c	20.0a	2.8a
Mono *Tr*	237.8a	64.3e	93.3a	15.9ab	2.3ab
Mono *Pl*	123.0b	75.1cde	88.1ab	18.0ab	2.3ab
Bi *Lp-Pl*	120.5b	111.3ab	79.6c	14.4bc	2.0ab
Bi *Tp-Pl*	155.9b	75.1cde	84.0bc	18.5ab	2.5ab
Bi *Tr-Pl*	144.4b	88.7bcde	93.3a	18.6ab	1.9b
Centroid	151.6b	87.0bcde	79.7c	14.7bc	2.4ab
Domi *Lp*	134.4b	100.3abcd	79.7c	15.5ab	2.4ab
Domi *Tp*	153.8b	87.9bcde	89.8ab	15.3abc	2.3ab
Domi *Tr*	149.4b	104.6abc	83.2bc	10.3c	2.2ab
Domi *Pl*	135.0b	85.0bcde	82.9bc	16.1ab	2.2ab

[1] Means with different lowercase letters are significantly different ($P<0.05$) using Tukey's *post hoc* test.

Conclusions

The above-ground biomass of two and four-species mixtures containing plantain were significantly higher than that obtained from monocultures. The herbage yield collected from leys with plantain in the sward was higher on Luvisols than on Cambisols. The herbage quality was affected by species diversity. We conclude that increasing species diversity by selecting appropriate grass-legume mixtures with inclusion of plantain adapted to specific soils is key to achieving both high yields and good herbage quality in ley farming system.

References

Cong W-F., Søegaard K. and Eriksen J. (2016) Diversity promotes production of ryegrass-clover leys through inclusion of competitive forb species. *Grassland Science in Europe* 21, 557-559.

DuBois M., Gilles K.A., Hamilton J.K., Rebers P.A. and Smith F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28, 350-356.

Finn J.A., Kirwan L., Connolly J., Sebastià M.T., Helgadóttir A., Baadshaug O.H., Bélanger G., Black A., Brophy C., Collins R.P., Čop J., Dalmannsdóttir S., Delgado I., Elgersma A., Fothergill M., Frankow-Lindgberg B.E., Ghesquiere A., Golińska B., Goliński P., Grieu P., Gustavsson A.M., Höglind M., Huguenin-Elie O., Jørgensen M., Kadžiuliene Z., Kurki P., Llurba R., Lunnan T., Porqueddu C., Suter M., Thumm U. and Lüscher A. (2013) Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: a 3-year continental-scale field experiment. *Journal of Applied Ecology* 50, 2, 365-375.

Pirhofer-Walzl K., Søegaard K., Hogh-Jensen H., Eriksen J., Sanderson M.A., Rasmussen J. and Rasmussen J. (2011) Forage herbs improve mineral composition of grassland herbage. *Grass and Forage Science* 66, 415-423.

Ramseier D., Connolly J. and Bazzaz F. (2005) Carbon dioxide regime, species identity and influence of species initial abundance as determinants of change in stand biomass composition in five-species communities: an investigation using a simplex design and RGRD analysis. *Journal of Ecology* 93, 502-511.

Tilman D., Wedin D. and Knops J. (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379, 718-720.

Density and competitiveness of selected ryegrass species and *Festulolium* in mixtures with *Trifolium repens* under N and S fertilisation

Grygierzec B.[1], Szewczyk W.[1], Luty L.[1] and Musiał K.[2]
[1] *University of Agriculture in Krakow, al. Mickiewicza 21, 31-120 Kraków, Poland;* [2] *National Research Institute of Animal Production, ul. Krakowska 1, 32-083 Balice, Poland*

Abstract

Relationship between density and competitiveness among selected species of ryegrasses and *Festulolium* cultivated in mixtures with *Trifolium repens* (TR) under varied N and S fertilisation were assessed in a field experiment carried out in 2016-2017. The interactions between the individual components are described in the available indicators: land equivalent ratio and competitiveness ratio (CR). The CR values for grasses showed that they were highly competitive with respect to TR, especially in the first year of the study.

Keywords: fertiliser, *Lolium*, *Festulolium*, *Trifolium*, competitive ratio

Introduction

Interactions occurring in the cultivation of mixtures between components (Lazzarotto *et al.*, 2009) cause the yield of mixtures, their structure and chemical composition to differ from that of pure sowings (Smith *et al.*, 2014). The yield of mixtures is thus the result of the species' mutual reaction to each other and to the habitat conditions. Therefore, the proper selection of components for mixtures and their proportions is a condition for obtaining high and qualitatively good yields (Annicchiarico and Proietti, 2010). Yu *et al.* (2000) report that intra-species interactions in pure sowings can reduce plant productivity. Competition between species is influenced by agrotechnical factors such as mineral fertilisation and sowing rates. The relationships between the individual components are described in the available indicators: land equivalent ratio (LER) (Mead and Willey, 1980) and competitiveness ratio (CR) (Willey and Rao, 1980). The aim of the study was to assess the relationship between density and competitiveness among selected species of perennial ryegrass and *Festulolium* cultivated in mixtures with *Trifolium repens* (TR) and grown under varied N and S fertilisation treatments.

Materials and methods

A field experiment was carried out during 2016-2017 in a split-plot design with in four replicates of 12 m^2 plots. Tetraploid grass species in the study were: *Lolium boucheanum* (LB) (cv. Gala), *Lolium multiflorum* (LM) (cv. Temida), and *Festulolium* (F) (cv. Agula). The grasses were grown in two species mixtures with TR (cv. Romena) in proportions 75:25 and 50:50. Nitrogen fertilisation treatments were 30 and 50 kg N ha^{-1}. Phosphorus (38 kg P ha^{-1}) was applied once in early spring. Potassium (82 kg K ha^{-1}) and nitrogen were applied in two equal doses in early spring and after the first cut. Sulphur fertilisation in the form of liquid fertiliser was applied in a single application before the start of vegetative growth, with three doses rates: 5, 10 and 15 kg S ha^{-1}. Before harvests, the number of shoots of each of the analysed species was counted on an area of 1 m^2.

Obtained results were used to calculate the following indicators:

- land equivalent ratio LER: $LER = LER_a + LER_b$, so that: $LER_a = Y_{ab} / Y_{aa}$, $LER_b = Y_{ba} / Y_{bb}$,
- competitiveness ratio CR of a and b species respectively: $CR_a = (LER_a / LER_b)\,(Z_{ba} / Z_{ab})$,
  $CR_b = (LER_b / LER_a)\,(Z_{ab} / Z_{ba})$,

where: Y_{aa} = species *a* yield in pure sowing, Y_{bb} = species *b* yield in pure sowing, Y_{ab} = species *a* yield when sown in a mixture with species *b*, Y_{ba} = species *b* yield when sown in a mixture with species *a*, proportion of sowing species *a* in cultivation with species *b*, Z_{ab} = proportion of sowing for species *a* in cultivation with the species *b*, Z_{ba} = proportion of sowing for species *b* in cultivation with the species *a*.

Results and discussion

The calculated values of the CR coefficient, which presents the LER ratio for each species (Dhima *et al.*, 2007) were differentiated for grasses in particular treatments, cuts and also in the years of research (Figure 1). A CR coefficient higher than 1.0 indicates that grasses were more competitive than TR. Values lower than 1.0 were obtained in the second year of research for LB and LM sown in mixtures with TR in the ratio 75:25. CR calculated for F sown in mixtures with TR in the ratio 75:25 was always greater than 1.0.

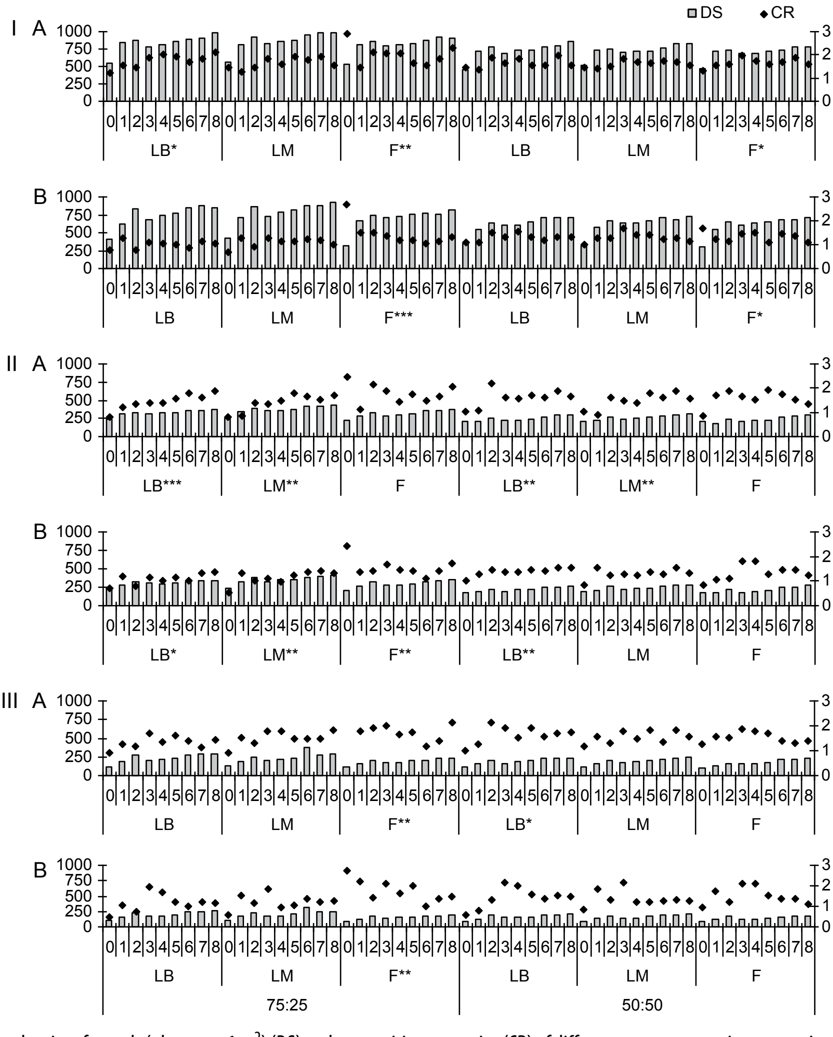

Figure 1. Mean density of swards (plants per 1 m^2) (DS) and competitiveness ratios (CR) of different grasses grown in two species mixtures with *Trifolium repens*. 0: control, 1: N_{30}, 2: N_{50}, 3: $N_{30}S_5$, 4: $N_{30}S_{10}$, 5: $N_{30}S_{15}$, 6: $N_{30}S_5$, 7: $N_{50}S_5$, 8: $N_{50}S_{10}$; LB = *Lolium boucheanum*, LM = *Lolium multiflorum*, F = *Festulolium*; I, II, III: cuts; A: year 2016; B: year 2017; *, **, ***: significant effect at P<0.05, P<0.01, P<0.001, respectively.

The CR values for grasses indicated their high competitiveness in relation to TR, especially in the first year of research. Lower domination in individual treatments and subsequent cuts was shown by grasses in the second year of the study, especially when sown in mixtures with TR in the ratio 75:25, where their density at the beginning of the study was higher. Sowing of the analysed grasses in mixtures with TR in the proportion 50:50, and fertilisation with N and N+S favoured their domination, which was related to the beneficial effect of legumes by enriching soil with nitrogen (Lazzarotto *et al.,* 2009).

Conclusions

The CR indicator values for grasses showed that they were highly competitive with respect to TR, especially in the first year of the survey. Grasses sown in mixtures with TR in the proportion of 50:50 and fertilisation with N and N+S favoured their domination, which was related to the beneficial effect of legumes by enriching the soil with nitrogen. There is a need for further studies in this respect, especially the effect of N and S on grass-clover mixtures cultivated in a 2-3-year cycle.

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References

Annicchiarico P. and Proietti S. (2010) White clover selected for enhanced competitive ability widens the compatibility with grasses and favours the optimization of legume content and forage yield in mown clover-grass mixtures. *Grass and Forage Science* 65, 318-324. https://doi.org/10.1111/j.1365-2494.2010.00749.x

Dhima K.V., Lithourgidis A.S., Vasilakoglou I.B. and Dordas C.A. (2007) Competition indices of common vetch and cereal intercrops in two seeding ratio. *Field Crops Research* 100, 249-256.

Lazzarotto P., Calanca P. and Fuhrer J. (2009) Dynamics of grass-clover mixtures – an analysis of the response to management with the Productive Grassland Simulator (PROGRASS). *Ecological Modelling* 220, 703-724.

Mead R. and Willey R.W. (1980) The concept of a land equivalent ratio and advantages in yields for intercropping, *Experimental Agriculture* 16, 217-228.

Smith R.G., Atwood L.W. and Warren N.D. (2014) Increased productivity of a cover crop mixture is not associated with enhanced agroecosystem services. *PLOS One* 9(5), e97351. https://doi.org/10.1371/journal.pone.0097351.

Willey R.W. and Rao M.R. (1980) A competitive ratio for quantifying competition between intercrops. *Experimental Agriculture* 16, 117-125.

Yu J.Q., Shou S.Y., Qian Y.R. Zhu Z.J. and Hu W.H. (2000) Autotoxic potential of cucurbit crops. *Plant and Soil* 223, 147-151.

Yield of *Festulolium* hybrids at different phenological stages during primary growth

Hoffmann R.[1], Pál-Fám F.[1], Keszthelyi S.[1] and Halász A.[2]
[1]*Kaposvár University, Faculty of Animal Sciences, Department of Botany and Plant Production, Guba S. 40, 7400 Kaposvár, Hungary;* [2]*Szent Istvan University, Faculty of Agriculture and Environmental Studies, Institute of Animal Husbandry, Pater Karoly rd. 1, 2100 Godollo, Hungary*

Abstract

The cultivation of *Festulolium* hybrid grasses is considered novel in European, as well as in Hungarian grassland management. As farmers do not have considerable cultivation experience with *Festulolium* so we set up a comparative experiment with Italian ryegrass (*Lolium multiflorum*), tall fescue (*Festuca arundinacea*) and *Festulolium* hybrids. Our objective was to suggest the most suitable harvest time (phenological state) by monitoring yield changes. The yield change of the first growth was examined for 4 consecutive weeks at four different harvest times, from stem elongation to flowering in 2018. Fresh matter yield and dry matter content, as well as theoretical haylage yields, were measured. Results were evaluated by two-factorial analysis of variance. It was found that the effect of the species and the time of harvest, i.e. the phenological stage, had a significant impact on all the examined parameters. The interaction of the two effects was also significant. Among the hybrids, the yield of the *Lolium* type was found to be higher than that of the *Festulolium* hybrids or those of the Italian ryegrass and tall fescue species. The cultivation of hybrid grasses may open up new perspectives in the forage supply of ruminants.

Keywords: *Festulolium*, green yield, dry matter content, harvesting time

Introduction

In Hungary, increased water scarcity during the growing season has occurred due to weather extremes linked to climate change (less rainfall, longer dry periods and increase of summer heat days). Therefore, there is an increasing need to grow crops with higher drought tolerance, to complement, or be alternatives to, silage maize and other fodder species that currently provide the basis for feeding ruminants. Grass has played a key role in feeding ruminants in the past as well as today. *Festulolium* hybrid grass species have been tested on only a few farms in Hungary, but they have potential to contribute securely and economically to large-scale production of Hungarian fodder. These hybrids are expected to produce higher yields with good winter hardiness and excellent drought tolerance (Zwierzykowski and Naganowska, 1994). In other countries, different studies have been conducted on these hybrids, such as morphological differences from other grasses (Kulik *et al.,* 2005), the effects of grazing and mowing on mixtures containing *Festulolium*, yield trends (Kulik and Wanda, 2008), or yield and assessment of certain feeding parameters (Borsuk and Fijałkowska, 2019). At present, Hungary has little field experience of cultivation technology and forage utilisation of *Festulolium* species hybrids, which are generally considered as novel species for cultivation. In order to improve the security of grass-based forage supply, we used *Festulolium* species hybrids, which are still new in Hungarian for large-scale cultivation. During our experiments, the yields of the high-quality stages for the preparation of haylage were investigated.

Materials and methods

The site of the experiments was the Institute of Forage Production, University of Kaposvár (46 ° 41'03 'N; 18 ° 11'00' E). The soil type in the experimental area is limestone chernozem. In the experiment an Italian ryegrass (IR; *Lolium multiflorum*), a tall fescue (TF; *Festuca arundinacea*) and four different *Festulolium* hybrids (Fh) were tested, two of which were *Lolium* type (Fh1 and Fh2) and the other two were *Festuca* type (Fh3 and Fh4). After soil preparation and fertilisation (300 kg ha^{-1} N:P:K 4:17:30; 150 kg ha^{-1}

CAN 27), the seeds were sown in 1.5×45 m blocks on 14 September 2017. In the following spring, on 12 March 2018, the treatment plots received CAN 27 fertiliser at a dose of 200 kg ha^{-1}. After sowing, the autumn was warmer than average and rather rainy, which favoured the development of the young plants. January (13 mm) and April of 2018 (10.4 mm) were dry months, but precipitation in February (33 mm) and March (34 mm) was 2-2.5 times the average.

Sampling of herbage was performed with a 1 m^2 frame randomly placed at 4 replicates at each of 4 sampling times. In the course of sampling, previously sampled areas were avoided. Sampling started on 19 April, repeated on four occasions at one-week intervals. Mowing times and phenological phases (BBCH scale) were: 19 April, BBCH 34; 26 April, BBCH 41; 4 May, BBCH 49; 5 May, BBCH 59. The stubble height was between 8 and 10 cm. The green mass of the samples was measured at the site, whereas the dry matter content after drying and the theoretical haylage yield was normalised to 30% water content on the basis of 300 g kg^{-1} dry matter. In practical terms, the results were evaluated by two-factorial variance analysis. Variety effects and differences between variants at each sampling time point were assessed by one-way analysis of variance.

Results and discussion

Based on the results of the two-factorial analysis of variance, the effect of cultivar/hybrid and mowing time was significant for the three parameters analysed: fresh matter, dry matter content and estimated haylage yield ($P \leq 0.001$). The combined effect of cultivar and time ($P \leq 0.05$) was also significant. Significant differences were observed in fresh matter yield at each mowing time (Table 1). At the first sampling, Fh2 produced the highest yield, whereas IR and Fh1 did not produce significantly lower yields than Fh3 or Fh4. At the second sampling time, both Fh1 and Fh2 had significantly higher fresh matter yields than *Festuca* type hybrids and TF. The highest yields were obtained at the time of the third mowing, and there was a significant difference between IR and Fh2. It is noteworthy that the yield of Fh4 hardly increased. In all cases, fresh matter yields decreased at the last mowing time, with the greatest decrease in IR, Fh2 and Fh3, the fresh matter yield of which was significantly lower in previous cuts when compared with IR and *Lolium* type hybrids. The dry matter content was significantly lowest at the first sampling of Fh1 and Fh2. In the second cut, the dry matter content of Fh3, Fh4 and Fh5 remained virtually unchanged. Higher growth was observed only for TF (18.4 g kg^{-1}) and Fh1 (14.7 g kg^{-1}). At the third sampling, Fh2 had significantly the lowest DM content; the difference in comparison with IR and Fh5 was discernible, exceeding 40 and 60 g kg^{-1}, respectively. At the fourth mowing time, the dry matter content increased significantly. Growth was most intense in the IR and TF hybrids and in Fh3. The two *Lolium*-type hybrids exhibited significantly the lowest content of dry matter. Estimation of haylage yield is important

Table 1. Changes in fresh matter yield, dry matter content and estimated haylage yield for different grasses at different harvest dates (^{a,b,c,d,e} $P \leq 0.05$). Std. errors refer to the columns.

	Fresh matter yield (Mg ha^{-1})				Dry matter (g kg^{-1})				Estimated haylage yield (Mg ha^{-1}) based on DM of 300 g kg^{-1} DM			
Sampling dates[1]	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Italian ryegrass	33.5ab	38.5a	40.4a	28.7ac	161a	166ad	193.07a	233a	17.9a	21.2a	26.0a	22.3a
Festulolium Fh1	38.1a	39.2a	43.1ab	36.3a	128b	142ab	166.11b	188b	16.1ab	18.5b	24.4a	22.2a
Festulolium Fh2	34.5ab	43.4a	46.5b	33.3a	131b	127b	150.32c	169b	15.0b	18.4b	23.3ab	18.7b
Festulolium Fh3	29.9b	29.5b	33.3c	17.7bd	152a	152ad	185.22a	243a	15.0b	14.8c	20.6b	14.3c
Festulolium Fh4	10.8c	11.9c	15.9d	13.7b	198c	201c	212.20d	229a	7.1c	7.9d	11.2c	9.9d
Tall fescue	31.1b	31.8b	32.7c	23.1cd	148a	167d	170.21b	222a	17.9b	21.2b	26.0c	22.3bc
Std.error	2.147	2.6865	2.5462	3.4960	7.8867	8.5190	6.4164	11.3563	0.87327	0.80577	1.27758	1.56454

[1] Sampling dates: 1-4 are respectively 19 April, 26 April, 4 May and 11 May.

for the practical relevance of the results in terms of farm-scale feed security. We did not opt for presenting the dry matter yield; obviously this can be calculated from the available data (due to the calculation method the result of statistical evaluation is virtually identical). In the calculated yield, for the first 3 sampling times IR gave the highest yield followed by the two *Lolium*-type hybrids. The difference in yield in IR was not significantly higher than that of Fh1. The difference between IR and Fh2 at the first, third and fourth cuts was significant. However, this did not exceed 2.8 Mg ha^{-1} at the third cut, which gave the highest yield. The yield of Fh4 was significantly lower at all times, and the yield of TF was significantly lower only at the third mowing time, compared with that of IR and *Lolium*-type hybrids.

Conclusions

Based on our preliminary results, the cultivation of the Fh4 *Festuca* type hybrid seems less promising due to its slow development and low fresh matter yield. Low yields at early mowing times can also require longer wilting, thereby leading to poorer feed values. Fresh matter yield and dry matter content at the third mowing time was adequate and would have provided an acceptable amount of haylage. In summary, cultivation of *Festulolium* hybrids is promising, especially for *Lolium*-type hybrids, and these are good targets for further investigations. At the last cut, fresh matter yields fell significantly while the dry matter content increased rapidly, indicating that the period of intensive growth is over; therefore, it is worth cutting the remaining herbage prior to the deterioration of its the feeding value.

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References

Borsuk M. and Fijałkowska M. (2019). Effects of cultivar and harvest date on the composition of nitrogen fractions in Festulolium herbage. *Journal of Elementology* 24, 1241-1251.

Kulik M.A. and Warda M. (2008). Yields of the sward depending on utilization method and species tested in mixtures. *Grassland Science in Europe*, 13, 266-268.

Kulik M., Zwierzykowski Z. and Jokś W. (2005) Morphological characteristics to discriminate Festulolium hybrids (*Festuca pratensis* × *Lolium perenne*). In: *XX International Grassland Congress: offered papers*, Dublin, p. 121.

Zwierzykowski Z. and Naganowska B. (1994) The use of *Lolium-Festuca* hybrids in breeding. *Genetica Polonica* 35A, 11-17.

The chlorophyll concentrations in leaves of forage grass species in conditions of water shortage

Janicka M.
Department of Agronomy, Faculty of Agriculture and Biology, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland

Abstract

This study was aimed at determining differentiations in the leaf greenness index (SPAD) values of forage grasses depending on the species, habitat moisture and NPK fertilisation. In addition, the relationship between the total nitrogen content in grass leaf blades and the value of SPAD was assessed. Four grass species used in Poland in mixtures for grassland reseeding or overseeding were tested: *Dactylis glomerata* L., *Festuca pratensis* Huds., *Lolium perenne* L. and *Phleum pratense* L. The weather conditions during the vegetation periods 2005 and 2006 were extremely dry. In such conditions, the highest SPAD readings were found in *D. glomerata* and the lowest in *L. perenne*. An intermediate content of chlorophyll was evident in *P. pratense* and *F. pratensis.* Higher values of SPAD were obtained for grasses growing in a moderately dry habitat compared with a moderately wet site. In all of the tested species growing in fertilised areas, more than a two-fold increase in SPAD was found compared with treatments without fertilisation. Chlorophyll content was highest in the spring (1st regrowth), lower in the autumn (3rd regrowth) and the lowest in the summer (2nd regrowth). There was a close correlation between the total nitrogen content (%) and SPAD.

Keywords: leaf greenness index, SPAD readings, grass species, habitat moisture, fertilisation, drought

Introduction

Chlorophylls play an important role in forage grasses. They are the basis for photosynthesis and thus affect the amount of biomass and its quality. Chlorophylls are also an indicator of the life processes of plants, their viability and responses to habitat, such as stressful thermal and moisture conditions. The chlorophyll content of plants is a genetic feature associated with the species as well as the variety (Kozłowski and Swędrzyński, 2007). It is modified by many different factors, such as weather conditions, and especially precipitation (Olszewska *et al.*, 2010; Staniak, 2013). However, results in this range are contradictory. Due to prolonged droughts occurring more and more often during the growing season, an effect of climate change in central Poland, the content of chlorophyll in forage grass species requires further research. This study was aimed at determining differentiations in the leaf greenness index (SPAD) values of forage grasses depending on the species, habitat moisture and NPK fertilisation. In addition, the relationship between the total nitrogen content in grass leaf blades and the SPAD values were assessed.

Materials and methods

The study was carried out during 2005-2006 at the Experimental Station at Jaktorów (central Poland) at two sites differing in soil moisture: moderately dry and moderately wet. At both sites, four grass species (used for overseeding) were tested: *Dactylis glomerata* L. (cv. Astera), *Festuca pratensis* Huds. (cv. Pasja), *Lolium perenne* L. (cv. Argona) and *Phleum pratense* L. (cv. Kaba). Three cuts were carried out each year. The following doses in kg ha^{-1} of mineral fertilisers were applied: 180 N, 30 P and 100 K (Janicka, 2017). The control object was not subjected to oversowing, nor was it fertilised. SPAD measurements were taken in each regrowth of 2005 and 2006 (first and second years of full utilisation) on undamaged, fully developed flag leaves from four randomly selected shoots of each species, taking six measurements in the middle of each leaf blade. Measurements of relative chlorophyll concentration were taken using a CCM-200 (Opti-Sciences) without destructive sampling. Before making the first cut, the plant material (leaf blades) was taken to determine the total nitrogen content. This was done using the Kjeldahl distillation method in the Laboratory of the Agronomy Department. The data were analysed statistically using variance analysis (ANOVA). Verification of the difference significance was based on Tukey's test ($P \geq 0.05$).

Results and discussion

The two growing seasons in which the studies were carried out were classified as extremely dry and very dry; the hydrothermal index of Vinczeffy (calculated as the quotient of total precipitation to the sum of temperature) was 0.098 and 0.113 mm °C^{-1}, respectively. Optimal conditions for the growth and development of grassland vegetation occur when the hydrothermal index is 0.20-0.25 mm °C^{-1}. In the first year of utilisation, the sum of rainfall during the vegetation period was about 74% of the long-term mean (Table 1). The second year was characterized by very unevenly distributed rainfall during the growing season; very low in June (40% of the long-term sum), low in July and September, while very high in August (203 mm), which accounted for 60% of the rainfall during the whole vegetation period.

The highest SPAD readings, irrespective of habitat, were found in *D. glomerata,* and the lowest in *L. perenne*. An intermediate content of chlorophyll (a + b) was found in *P. pratense* and *F. pratensis* (Table 2). Higher values of leaf greenness index were obtained for grasses growing in a moderately dry habitat. Among the species tested, *P. pratense* and *L. perenne* responded to water shortages the most strongly, and *D. glomerata* the least. This confirms previous reports that *L. perenne* is very sensitive to drought (Janicka,

Table 1. The weather conditions in growing seasons 2005 and 2006.

	Year	Month						Growing season
		Apr	May	June	July	Aug	Sept	Apr-Sept
Average air temperature (°C)	2005	8.8	13.3	15.8	19.7	16.9	15.5	15.0
	2006	9.2	13.9	18.6	24.2	18.2	16.5	16.8
	long-term mean	8.7	14.5	17.5	19.1	18.5	13.7	15.3
Sum of precipitation (mm)	2005	22.7	56.6	39.6	102.4	27.9	22.0	271.2
	2006	51.0	47.6	30.0	6.1	203.1	8.9	346.7
	Long-term mean	39.8	58.2	74.7	79.9	59.7	53.7	366.1

Table 2. Leaf greenness index (SPAD) of grass species depending on the habitat moisture in 2005 and 2006.[1]

	Species	Habitat					
		Moderately wet			Moderately dry		
		2005	2006	mean	2005	2006	mean
	Dactylis glomerata	12.24^a	15.41^a	13.82^a	13.67^a	14.13^a	13.90^a
	Festuca pratensis	10.42^b	8.85^b	9.63^b	11.61^b	9.11^c	10.36^c
	Lolium perenne	4.97^c	2.94^c	3.95^c	5.73^c	3.56^d	4.64^d
	Phleum pratense	11.15^b	9.46^b	10.30^b	13.96^a	11.31^b	12.63^b
Mean for fertilisation	NPK	13.20^a	12.37^a	12.78^a	14.95^a	13.63^a	14.29^a
	control	6.20^b	5.95^b	6.07^b	7.54^b	5.43^b	6.48^b
Mean for year of utilisation	Mean	9.70	9.16	9.43	11.24^a	9.53^b	10.38
Mean for regrowth	1st regrowth	11.91^a	9.61^a	10.76^a	11.09	12.12^a	11.60^a
	2nd regrowth	6.92^c	7.84^b	7.38^b	11.74	8.37^b	10.05^b
	3rd regrowth	10.27^b	10.03^a	10.15^a	10.90	8.09^b	9.49^b

[1] Different letters in the same column present significant differences at $P<0.05$.

2012). It should be noted that in all grass species, much lower values of the leaf greenness index were obtained compared with results provided by other authors (e.g. Gáborćik, 2003, Olszewska *et al.*, 2010; Staniak, 2013). This was probably due to the droughts in the two years prior to the study. The results of our study very clearly confirm the impact of NPK fertilisation on the concentration of chlorophyll in grass leaves. During a period of extreme drought, more than a two-fold increase in SPAD readings was found in all of the tested species. *D. glomerata* reacted most strongly to NPK fertilisation. During the growing season, great changes in the value of the leaf greenness index were found. Chlorophyll content was highest in the spring, lower in the autumn and lowest in the summer. Fertilisation NPK stabilised the chlorophyll content during the growing season, especially in the moderately dry habitat in the first year of utilisation. A close correlation was found between the total nitrogen leaf content (%) and SPAD values (r=0.868) in the first regrowth. This confirms that SPAD measurements could be used in an evaluation of plant nitrogen status and in grass (species and cultivars) selection (Gáborćik, 2003).

Conclusions

In conditions of water shortage, the highest content of chlorophyll (a + b) (SPAD readings) was found in *D. glomerata* and the lowest in *L. perenne*. The intermediate content of chlorophyll (a + b) was found in *P. pratense* and *F. pratensis.* The negative impact of weather conditions was mainly noted in the summer regrowths. Higher SPAD values were obtained for grasses growing in conditions of lower soil moisture. Over a two-fold increase in SPAD readings was found in all of the tested species that grew in fertilised areas. SPAD measurements can be used to assess the plant nitrogen status and grass selection.

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References

Gáborčík N. (2003) Relationship between contents of chlorophyll (a+b) (SPAD values) and nitrogen of some temperate grasses. *Photosynthetica* 41(2), 285-287.

Janicka M. (2017) The effectiveness of different overdrilling technologies of the dry meadows in central Poland. In: Kovačević D. (ed.) *Book of Proceedings Agrosym 2017.* VIII International Scientific Agriculture Symposium 'Agrosym 2017', East Sarajevo, Faculty of Agriculture, Bosnia and Herzegovina, ref. 29, pp. 402-409.

Kozłowski S. and Swędrzyński A. (2007) Variability in the occurrence of chlorophyll and carotenoids dyes in *Lolium perenne* (*Poaceae*). *Fragmenta Floristica et Geobotanica Polonica*, Suppl. 9, 163-171.

Olszewska M., Grzegorczyk S., Olszewski J. and Bałuch-Małecka A. (2010) A comparison of the response of selected grass species to water stress. *Łąkarstwo w Polsce*, *Grassland Science in Poland*, 13, 127-136.

Staniak M. (2013) Response of selected species and cultivars of forage grass to water shortage in the soil. *Monografie i Rozprawy Naukowe*, 38, Wydawnictwo IUNG-PIB, Puławy, pp. 217.

Nitrogen concentrate from slurry digestate reaches mineral nitrogen efficiency as fertiliser for grass

Järvenranta K.[1], Virkajärvi P.[1], Partonen A.-P.[2] and Nousiainen J.[2]

[1]Natural Resources Institute, Halolantie 31 A, 71750 Maaninka, Finland; [2]Valio Ltd., Meijeritie 6, 00370 Helsinki, Finland

Abstract

Nitrogen (N) use efficiency of slurry is often lower than the N utilisation of mineral N, which limits its use as efficient N fertiliser. The aim of this study was to determine the grass N utilisation of separated liquid fraction (N concentrate) of raw and digested cattle slurry compared with mineral N and untreated slurry. The fractionation method was developed by Valio Ltd. and three experiments were accomplished at the Maaninka and Siikajoki sites of the Natural Resources Institute (Finland) during 2017-2019. In fractionation, solid matter, liquid N fraction and water are separated from slurry or biogas reject. In this experiment, liquid fraction from slurry and digestate was used as N fertiliser in experiments compared with untreated slurry and mineral fertiliser (100 kg N ha^{-1} $treatment^{-1}$). The N utilisation of digested slurry N concentrate was as high as that of mineral fertilisers, whereas untreated slurry N concentrate was 12-13% lower. The reason for lower N utilisation was probably because higher carbon content of untreated slurry caused a priming effect in soil and some N was temporarily bound to the microbial biomass. Digested slurry N concentrate offers the possibility to use slurry N efficiently, as the amount is smaller and it contains no phosphorus.

Keywords: slurry, biogas, digestate, nitrogen, fertiliser, grass

Introduction

There are numerous difficulties using slurry as an efficient grass nitrogen (N)-fertiliser. In dairy cattle slurry the proportion of soluble N of total N is not optimal for grass growth – typically only 40-60% of the total N is in mineral form – and thus it is often supplemented with extra mineral nitrogen to enhance the grass DM yield. Meanwhile some of the slurry total N remains in the soil and is mineralized after the growing season, which presents a risk of N leaching. Ammonia volatilization risk may also be high unless the slurry is injected into the soil. Slurry P content may also restrict the use of raw slurry on the fields where soil P concentration is high. With all these challenges it is crucial to find new, more efficient methods for slurry utilisation. Fractionation of solids, nutrients and water into separate pools would be a potential solution for this and ease the use of slurry at farm level. To resolve this challenge Valio Ltd. has developed a new method for slurry fractionation.

The aim of this study was to determine the composition of the liquid nitrogen fraction (N-concentrate) made of raw or digested cattle slurry and evaluate their potential as N fertiliser in grass production, compared with that of mineral fertiliser. The tests were carried out simultaneously at Maaninka and Siikajoki.

Materials and methods

The study was carried out at the Natural Resources Institute Finland (Luke), Kuopio (63°14' N, 27°31' E) and Siikajoki (64°41' N, 25° 9' E). The soils of the experimental area were moderately coarse-textured sandy soil in the plough layer at both sites, with pH 6.3-6.5, soil P_{aac} concentration 9.7-13.5 mg l^{-1}. Soil C/N ratio ranged from 8 at Maaninka to 25 at Siikajoki. The mean annual air temperatures are 3.1 and 2.6 °C, and the mean annual precipitations are 612 and 538 mm at the Maaninka and Siikajoki sites, respectively (FMI's open data). The experiments were accomplished during 2017-2019 on timothy-meadow fescue

(*Phleum pratense-Festuca pratensis*) grass mixture swards at both sites. The first fertilisation was given as mineral fertiliser in May prior to the first silage cut in June (first cut not included in experiment). All experimental treatments were applied after the first cut. Experimental design was a randomised complete block design with four replicates. The experiment comprised 6-7 treatments depending on the year and experimental site (Table 1).

In fractionation, solid matter, liquid N fraction and water are separated from slurry or biogas reject material by a screw separator, after which the flocculating chemicals were added to the liquid fraction and the slurry was further fed to a centrifugal separator. The liquid fraction produced by the centrifuge was stored for final filtration procedures, performed by Valio Ltd.

Dry matter yield (kg DM ha^{-1}) was measured from each cut. The N concentrations of the grass (g kg^{-1} DM) were determined by near infrared spectroscopy (Valio Ltd.). N balance was calculated as a difference between given fertiliser total N and sum of N yield in DM in cuts 2 and 3. Soil samples (0 to 20 cm) were taken from each plot. Soil P_{aac} was determined by Eurofins Oy (Vuorinen and Mäkitie, 1955). Statistical analyses were performed using Mixed procedure in SAS 9.4. Experimental sites and years were analysed separately.

Results and discussion

The weather in 2017 was exceptionally cool during the growing season at both sites; this led to reduced grass growth and DM yields were generally low (Table 2). In 2018 the weather was warm and DM yields were high. In 2019 the summer was exceptionally dry and cool, which suppressed the grass growth again (Table 2) compared with the more favourable year of 2018.

In 2017, the DM yield of the N-concentrate slurry treatment (Tr4) was 16% higher than that of raw slurry (Tr3), but 19% lower than the DM yield of mineral N treatments (Tr2 and Tr5). This was assumed to be due to the carbon (C) content of the N-concentrate. This may have had a priming effect in soil and bound some of the mineral N to the soil microbiome (Kuzyakov *et al.* 2000). As biogas digestion is known to reduce the readily available C in slurry (Möller and Müller, 2012), digestion was added to the procedure and biogas reject was used as base for Tr6 'N-concentrate digestate' in 2018 and 2019. The theory of diminishing effect of raw slurry N-concentrate C content on grass N utilisation was strongly supported by the experiments in 2018 and 2019, as the DM yield and soluble N utilisation (data not shown) of Tr6 was equal to Tr2 and Tr5 on both sites (Table 2). There was no significant difference in grass DM feeding values between the treatments (except 0N; data not shown).

Table 1. Experimental treatments. The figures are mean values for all 3 experiments, sd in parenthesis where relevant.

	Tr		Mg ha^{-1}	Tot N kg ha^{-1}	Slurry or conc. sol. N kg ha^{-1}	Min N kg ha^{-1}	P kg ha^{-1}	Maaninka K kg ha^{-1}	Siikajoki K kg ha^{-1}
1. cut		Whole area		100		100	0	28	55
2. cut	1	0 N					20	30	70
	2	N mineral fertliser		100		100	20	34	74
	3	Slurry + min N	38 (0.5)	167 (27.3)	93 (7.3)	16 (3.5)	26 (2.1)	145 (18.1)	144 (18.7)
	4	N-concentrate slurry	23 (1.7)	127 (14.8)	100 (1.7)		20	235 (8.1)	235 (8.1)
	5	Urea	22 (2.0)	104 (7.8)	104 (7.8)		20	30	70
	6	N-concentrate digestate	19 (0)	116 (12.0)	100 (2.1)		20	202 (15.6)	202 (15.6)
	7	Injected slurry + min N	37	138	86	14	25	123	0

[1] Target N-fertilisation application was 100 kg soluble N for the second cut. Treatment 6 was included in Experiment 2 and 3. Treatment 7 was applied only at Maaninka in Experiment 2.

Table 2. Dry matter (DM) and N yields in Experiments 1, 2 and 3.

Tr	Maaninka 2. + 3. cut						Siikajoki 2. + 3. cut					
	DM kg ha^{-1}			N balance kg ha^{-1}			DM kg ha^{-1}			N balance kg ha^{-1}		
	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
1 0 N	1,120	2,670	1,340	-22	-39	-22	940	2,190	1,190	-19	-29	-18
2 N mineral fertiliser	3,420	5,750	3,960	23	-2	22	3,540	4,660	4,310	16	8	20
3 Slurry + minN	2,370	4,610	2,680	145	79	124	2,360	3,190	2,680	144	101	126
4 N-concentrate slurry	2,870	5,030	3,120	81	29	67	2,630	3,960	3,460	31	37	58
5 Urea	3,370	5,810	3,950	25	6	44	3,260	4,280	3,320	40	18	54
6 N-concentrate digestate		6,030	3,940		5	49		4,530	4,020		18	46
7 Injected slurry + minN		4,970			66							
Mean	2,630	4,980	3,170	51	21	47	2,550	3,800	3,160	28	26	48
SEM	71	228	153	2.0	4.5	2.1	118	144	104	2.2	2.8	2.2

[1] SEM = standard error of the mean.

The N balance between given fertiliser N and N yield in the DM was lowest for Tr2 at both sites and the difference between Tr2 and Tr6 was statistically significant at Maaninka in 2019. However, there was no difference between Tr5 (Urea) and Tr6, for either year or site. As expected, the non-mineral N content of the added substrate affected the utilisation rate of the applied N. Raw slurry non-mineral N content was 40%, N-concentrate made of raw slurry was 20% and N-concentrate made of biogas reject 13%. Warm weather in 2018 favoured slurry N utilisation for grass growth as can be seen from the lower N balance at both sites and also on Tr7 at Maaninka in 2018.

Conclusions

According to this study, N concentrate made from cattle slurry-digestate is as effective as mineral N-fertiliser as a source of N, and thus can be used to replace artificial N in grass fertilisation. The C content of the N-concentrate is crucial when it comes to N utilisation efficiency; the lower the C concentration of the substrate, the better the N utilisation for grass DM production.

References

Kuzyakov Y., Friedel J.K. and Stahr J. (2000) Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry* 32, 1485-1498.

Möller K. and Müller T. (2012) Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. *Engineering in Life Sciences* 12, 1-16. https://doi.org/10.1002/elsc.201100085.

Vuorinen J. and Mäkitie O. (1955) The method of soil testing in use in Finland. *Agrogeological Publications* 63, 1-44.

Maintenance of good soil phosphorus levels is essential for high grassland yield with good quality

Junklewitz P.[1] and Liespuu J.[2]
[1]Research Centre Hanninghof, Yara International ASA, 48249 Duelmen, Germany; [2]Yara Suomi Oy, Kotkaniemi Research Farm, Ojakkala, Finland

Abstract

Adequate phosphorus (P) supply is essential for optimum grass growth and there is an interaction between P derived from soil and from fertiliser. This study aimed to evaluate the effect of soil P level, combined with P fertilisation, under Nordic conditions, in a long-term field trial, conducted at Kotkaniemi Research Farm, Finland. The grassland trial was established in 2017 on high-humus loamy clay, with a soil P gradient ranging from fair to satisfactory. Mineral P fertiliser was applied annually in steps from 0 to 80 kg P ha^{-1} to three subplots, each subplot representing a different soil P level. Biomass yield and P content were evaluated from each plot in both years. Grass quality measurements were taken in 2018 only. Both soil P content and P fertilisation had positive effects on grass yield and P uptake. The effect of P fertilisation was greater at low soil P content, especially at first cutting, when plant-available P from soil was not available at high enough levels to support plant growth. High P fertilisation rates were out yielded by satisfactory soil P levels. However, P fertilisation had a positive effect on yield at later cuttings and on grass P contents.

Keywords: phosphorus, grassland, soil, fertiliser

Introduction

Adequate phosphorus (P) supply is essential for optimum grassland production. Phosphorus supply from soil is controlled predominantly by the concentration of plant-available P in soil solution and the P buffering capacity of the soil. Plant availability can be restricted by fixation to soil particles and by low rates of diffusion in soil. Below a critical level of available P in soil solution, release of less readily available phosphorus compounds might not be sufficient to supply crops for optimum yield. Phosphorus applied via fertiliser can complement soil P supply. However, only a portion of fertilised P is plant available in the year of application. Because of this, fertiliser P also contributes to residual P in the soil and becomes plant-available over several years following application. Long-term studies are necessary to evaluate P release from fertiliser and to calculate an appropriate P application rate at different soil P levels to match crop demand for high yield and to avoid environmental losses (Syers, 2008). This study aimed to evaluate interactions between P fertilisation rate and soil P level, in a long-term trial, under typical grassland management conditions, in southern Finland.

Materials and methods

The experiment was located at Kotkaniemi Research Farm in Finland (60° 35' 90' N; 24° 38' 11' E). The site, characterised by clay soil with 8% organic matter exhibited a gradient in soil P content ranging from fair (4.6 mg P l^{-1}) to satisfactory (11 mg P l^{-1}) according to the Finnish soil P classification (Viljavuuspavelu, 2008). This P gradient was established by differential P supply from 1973 to 2014. Grassland (80% timothy, 20% meadow fescue) was established on this site in spring 2015. The present trial was established in spring 2017.

The site was split into three subplots based on the soil P level (P_{fair} – 4.7 mg P l^{-1}, $P_{fair/sat}$ – 6.6 mg P l^{-1}; P_{sat} – 10.5 mg P l^{-1}). Within each subplot, P (triple superphosphate, 20% P) was applied annually, in spring, at rates of 0, 10, 20, 40, 60 and 80 kg P ha^{-1}. In total, 24 treatments (3 soil levels × 8 P rates) were installed with 2 replicates each in a split-plot design, net plot size was 3×10 m^2. Nitrogen and potassium were applied to all plots at uniform rates of 250 kg N ha^{-1} and 200 kg K ha^{-1} as YaraCAN 27 (27% N) and Kaliumsuola (50% K) as split applications to each cut. Other nutrients were supplied with 167 kg ha^{-1} Greencare Pro Ca/Mg Plus (12% Ca, 7.5% Mg, 18% S, micronutrients) in one annual application in spring. Grass was managed in a 3-cut system. After cutting, dry matter (DM) yield was evaluated and samples of aboveground biomass were analysed for P by microwave digestion in a mixture of nitric acid and hydrogen peroxide followed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Soil P content was analysed using acid ammonium acetate extraction (Saarela, 2002). All data were statistically evaluated by ANOVA at each year × soil P level combination followed by Tukey test, and overall, by multivariate analysis of variance.

Results

Phosphorus supply had a significant effect on yield in both years of the trial (Table 1). On average, DM yield was increased from 5.2 to 7.3 Mg DM ha^{-1}. As expected, the lowest yield occurred in the 'fair' soil P level plots (P_{fair}), and the highest yield was recorded in the 'satisfactory' soil P level (P_{sat}) plots. Yield increased positively over all soil P levels in 2017, but in 2018, P_{fair} and $P_{fair/sat}$ produced similar grass yield and only plots in P_{sat} yielded more. The differences between the years might be attributable to weather conditions, as 2017 was a rather cold and wet year, whereas 2018 was hot and dry. In both years, yield was below the long-term average for Kotkaniemi of 10-11 Mg DM ha^{-1}. The impact of P fertiliser application on total DM yield was statistically significant in a multifactorial analysis over both years and all soil P levels, but not within the single soil P level × year combinations. However, there was a positive effect of P application, as the 0-P control treatment had the lowest yield and P content in most soil P level × year combinations.

The growth response of the different cuts (Figure 1), to P from soil, was highest in cut 1. Dry matter yield was significantly higher at increasing levels of soil P, but was not significantly different with P fertilisation. Despite a statistically significant yield response at later cuts to soil P level and P fertilisation rate, their impact on yield was only slight.

Table 1. Total annual yield and weighted P content of grassland grown at different soil P contents and fertilised at different P rates.[1]

		DM yield (Mg ha^{-1})						P content, weighted (g P kg^{-1} DM)					
	Year	2017			2018			2017			2018		
	Soil P level	P_{fair}	$P_{fair/sat}$	P_{sat}	P_{fair}	$P_{fair/sat}$	P_{sat}	P_{fair}	$P_{fair/sat}$	P_{sat}	P_{fair}	$P_{fair/sat}$	P_{sat}
P rate (kg P ha^{-1})	0	5.38	6.66	7.33	5.03	4.68	5.75	2.26^{a}	2.80^{a}	3.03^{a}	2.81	3.14^{a}	3.12^{a}
	10	6.24	6.53	7.53	5.61	5.21	6.21	2.76^{ab}	2.80^{a}	3.17^{ab}	3.16	3.16^{a}	3.37^{ab}
	20	6.31	6.76	7.70	5.61	4.67	6.41	2.64^{ab}	3.11^{ab}	3.32^{ab}	3.11	3.33^{ab}	3.39^{ab}
	40	5.91	6.76	7.73	5.43	5.76	6.81	2.81^{ab}	3.33^{b}	3.32^{ab}	3.11	3.66^{b}	3.48^{abc}
	60	6.19	6.96	7.86	5.82	5.57	6.16	3.05^{b}	3.40^{b}	3.52^{b}	3.48	3.70^{b}	3.72^{bc}
	80	6.10	6.86	7.50	5.34	5.18	6.80	3.22^{b}	3.34^{b}	3.51^{b}	3.49	3.70^{b}	3.86^{c}
P-value		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	<0.01	<0.05	n.s.	<0.01	<0.01

	DM yield		P content weighted	
	P-value	SEM	*P*-value	SEM
P fertilised (F)	<0.05	0.127	<0.001	0.0389
Soil P level (S)	<0.001	0.090	<0.001	0.0275
Year (Y)	<0.001	0.073	<0.001	0.0224
F × S	n.s.	0.220	n.s.	0.0674
F × Y	n.s.	0.127	n.s.	0.0551
S × Y	<0.01	0.180	<0.05	0.0389

[1] DM = dry matter; SEM = standard error of the mean; n.s. = not significant. Values with different superscript letters in a row are significantly different.

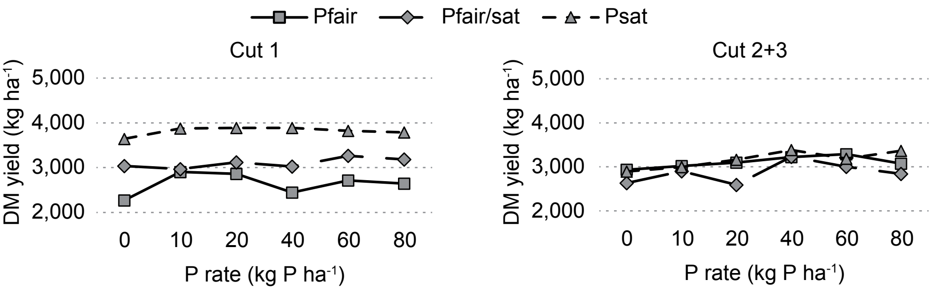

Figure 1. DM Yield of 1st cut (left) and later cuts (right) as influenced by soil P level and P fertilisation.

Discussion

Results of the first two years of this trial show that optimum soil P supply is important for first cut yield but has a lesser effect on yield of the following cuts. A review by Grant (2001) supports the results that plants have a higher requirement for easily available P at low soil temperatures, early in the season. The results of the current study also indicate that soil P supply influences yield more than P fertiliser application. This is only partly supported by a study conducted in Ireland (Sheil, 2016), results from which confirmed the importance of sufficient P supply early in the season. In contrast to the current trial, P fertiliser application positively affected yield in the Irish study.

Phosphorus uptake was significantly higher at greater soil P levels and with P fertilisation in both years. Phosphorus fertilisation increased tissue P content in all soil P levels (Table 1). These findings indicate that, in the year of application, P fertilisation has a positive effect on grass P uptake. It is important to note that P fertiliser was applied to the soil surface and was not incorporated into the soil. As a result, plant roots may have had limited access to applied P. This may also explain why net P uptake from fertiliser was less than 10 kg P ha^{-1} in all plots. The results from the first two trial years suggest that, under low soil temperature regimes, the availability of adequate P soil levels is a decisive factor for optimum crop growth, which cannot be compensated by P fertiliser application within one year.

Conclusions

It is essential to maintain the soil P content at an adequate level to optimise grass growth, especially early in the season. A 'fair' soil P content was not sufficient to achieve high grassland yield. Phosphorus fertilisation could not compensate for less than adequate soil P concentrations in the first two years of the trial. Annual P fertilisation contributes to grass quality by increasing P content and affects yield only later in the season. This trial will continue to further investigate the long-term grass response to soil P level and P fertilisation and to better understand the interaction between the two.

References

Grant C.A. Flaten, D.N., Tomasiewicz D.J. and Sheppard S.C. (2001) The importance of early season phosphorus nutrition. *Better Crops,* 85(2), 18-23.

Saarela I. (2002) Phosphorus in Finnish soils in the 1900s with particular reference to the acid ammonium acetate soil test. *Agricultural and Food Science in Finland* 11, 257-271.

Sheil T.S., Wall D.P., Culleton N. and Murphy J. (2016) Long-term effects of phosphorus fertilizer on soil test phosphorus, phosphorus uptake and yield of perennial ryegrass. *Journal of Agricultural Science*, 154, 1068-1081.

Syers J.K. Johnston A.E. and Curtin D. (2008) Efficiency of soil and fertilizer phosphorus use. *FAO Fertilizer and Plant Nutrition Bulletin*, vol. 18; Rome, Italy.

Viljavuuspavelu (2008) Viljavuustutkimuksen tulkinta peltoviljelyssä; https://docplayer.fi/6586163-Viljavuustutkimuksen-tulkinta-peltoviljelyssa.html, downloaded 31.01.2020

Genetic diversity and distribution of the *VRN1* gene within timothy (*Phleum pratense* L.) accessions

Kalendar R., Vottonen L.L. and Seppänen M.M.
Department of Agricultural Sciences, University of Helsinki, P.O. Box 27 (Latokartanonkaari 5), 00014 Helsinki, Finland

Abstract

Timothy (*Phleum pratense* L.) is a perennial forage grass grown commonly in Europe except in the Mediterranean region. The ability of timothy to adapt to a wide range of environmental conditions is mostly determined by the allelic diversity within genes that regulate the vernalization requirement (VRN). This study explored the genetic diversity in the *VERNALIZATION1* (*VRN1*) gene promoter and intron 1 within timothy accessions. The genetic diversity of the promoter and intron 1 of the *VRN1* gene was analysed in 44 timothy accessions for vernalization responses. Accessions with strong and with intermediate and with weak vernalization were assessed. *VRN1* promoter and intron1 sequences were sequenced for all polymorphic variants amongst different accessions of timothy. We applied the genome walking approach to identify new *VRN1* promoter and intron 1 sequences in the timothy genome. An allele-specific PCR assay was developed to identify *VRN1* promoter and intron1 allelic variation. We observed a high level of polymorphism and copy number variation in the *VRN1* gene. Identification of novel promoter/intron1 polymorphisms will allow quantification of its effect on vernalization response and early flowering, alone and in combination with spring alleles at the *VRN1* loci.

Keywords: *Phleum pratense,* vernalization, genetic diversity, flowering time, homologues

Introduction

Timothy (*Phleum pratense* L.) belongs to *Poaceae* family, which includes some of the world's most important cereal and pasture grasses (Tanhuanpää *et al.*, 2016, Stewart *et al.*, 2011). The production and use of timothy are concentrated in cold temperate regions where it is commonly grown for forage due to its winter hardiness and high forage quality. Forage grasses, including timothy, are characterized by high levels of digestibility, nutritional value, and productivity at the vegetative and juvenile growth stages. Timothy is a perennial cool-season forage grass that is commonly used in grass leys in temperate regions. The adaptability of timothy to a wide range of environmental conditions is mainly controlled by genes that determine the vernalization requirement. These agronomically valuable traits are important to ensure the flowering of timothy in the most favourable climatic conditions and are widely used in modern breeding to obtain high-yielding varieties that are favourably adapted to changes in environmental conditions. In moderate cereals, three well-described genes (*VRN1*, *VRN2*, and *VRN3*) are involved in the vernalization reaction (Fiil *et al.*, 2011; Jokela *et al.*, 2014, 2015). Proteins encoded by *VRN1* and *VRN3* promote flowering and the early flowering allele (spring type) is dominant for both genes. The protein encoded by *VRN2* inhibits flowering and the late-flowering allele (winter type) is dominant. Thus, in timothy, only recessive *VRN1* and *VRN3* alleles and at least one dominant *VRN2* allele must be present to yield effective winter types, while all other allelic combinations can lead to spring types. In timothy, however, the role of *VRN2* is not as clear (Seppanen *et al.*, 2010) and the regulatory gene inhibiting flowering has not yet been characterized. In this study, we identified sequence variation at the promoter and intron 1 regions of the *VRN1* gene in a geographically diverse collection of timothy genotypes, defined their interspecies distribution, and identified new allelic variants. We also tested for correlations between vernalization response and geographic origin of genotypes.

Materials and methods

Forty-four timothy accessions with strong, intermediate, or weak vernalization response were selected for this study. The breeding lines of northern (BOR N) and southern (BOR S) origin and cultivars characterized previously for their agronomic performance in Boreal climate (Grindstad, Donatello, BOR1) were also included. DNA was extracted from leaves using a commercial DNA Kit. The *VRN* plant gene sequences were obtained from the NCBI database. For each of the *VRN* exon1 genes, the sequence accessions were aligned and conservation was assessed with the multiple alignment procedure of MULTALIN (Corpet, 1988). The conserved exon1 segments of the *VRN1* were used for design of PCR primers, which was performed with the program FastPCR (http://primerdigital.com/fastpcr.html) (Kalendar *et al.*, 2017). Several inverted primers were designed for the exon1 genes. The genome walking approach (Kalendar *et al.*, 2019) was applied for newly identification of *VRN1* promoter and intron 1 sequences in the timothy genome. PCR fragments were excised from a 1% agarose gel and purified on silica spin columns (Qiagen) according to the manufacturer's protocol. Each PCR product was cloned into a pGEM-T Easy vector (Promega) and sequenced. Sequencing was performed in Eurofins Genomics. The sequencing confirmed the *VRN1* gene-specific nature of the amplicons. The full-length *VRN1* gene promoter and intron 1 were sequenced from the cloned products and the sequences were deposited in Genbank (MK007525, MK240190-MK240221).

Results and discussion

VRN1 is a well-conserved multiple copy nuclear gene in the grass family. Orthologs of *VRN1* have been identified in temperate grass species such as perennial ryegrass (*Lolium perenne*), darnel ryegrass (*Lolium temulentum*), and meadow fescue (*Festuca pratensis*) (Petersen *et al.*, 2006). Alignment of DNA sequences of *VRN1* gene exons from *Poaceae* members identified well-conserved regions within these exon sequences. Primers targeting the exon 1 (JN969602, GU071076, DQ108934) were tested on all available *Poaceae* species and timothy accessions and universally led to amplification of the desired PCR product. DNA sequencing revealed polymorphisms within the promoter and intron 1 DNA fragments, which allowed the identification of the variability of *VRN1* gene polymorphisms in *P. pratense* accessions. A great majority of timothy accessions carried various deletions within the *VRN1* first intron and in the promoter region. The phylogram shows the cluster distribution of varieties of timothy accessions depending on the content of the *VRN* promoter-exon 1 region (Figure 1). The most common combinations of *VRN1* for timothy accessions were independent of the eco-geographical growing area. We identified many novel variants of the *VRN1* promoter/intron 1 sequences. The control of flowering time in *P. pratense* in the vernalization response genes is diverse, ranging from single-copy to multicopy genes, also greatly varied in their ecology.

Conclusions

P. pratense L. has earlier been characterised as a long-day plant, which neither requires vernalization to induce flowering nor shows a vernalization response (Fiil *et al.*, 2011; Stewart *et al.*, 2011). Identification of novel promoter/intron1 allelic variants provides a valuable genetic resource for practical breeding of timothy. This study clearly demonstrates the presence of considerable genetic variation for vernalization response within timothy, and future studies will elucidate the underlying causative genetic variation.

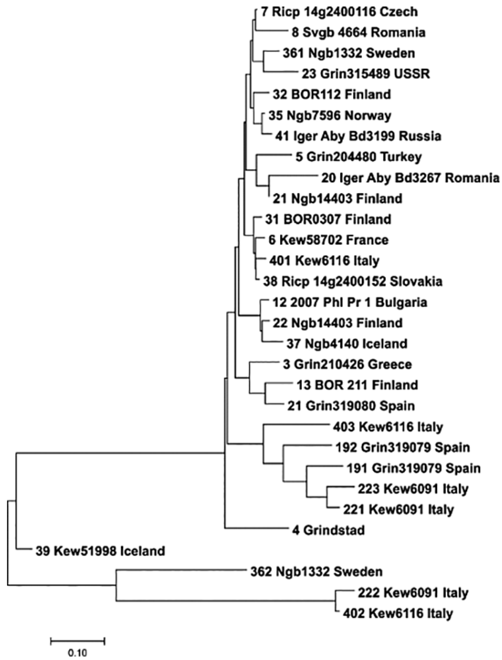

Figure 1. Results of cluster analysis. The dendrogram shows the cluster distribution of timothy accessions depending on the content of *VRN1* promoter-exon 1 variation.

Acknowledgements

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References

Corpet F. (1988) Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Research,* 16, 10881-10890.

Fiil A., Jensen L.B., Fjellheim S., Lübberstedt T. and Andersen J.R. (2011) Variation in the vernalization response of a geographically diverse collection of timothy genotypes. *Crop Science,* 51.

Jokela V., Trevaskis B. and Seppanen M.M. (2015) Genetic variation in the flowering and yield formation of timothy (*Phleum pratense* L.) accessions after different photoperiod and vernalization treatments. *Frontiers in Plant Science,* 6, 65.

Jokela V., Virkajarvi P., Tanskanen J. and Seppanen M.M. (2014) Vernalization, gibberellic acid and photo period are important signals of yield formation in timothy (*Phleum pratense*). *Physiologia Plantarum,* 152, 152-163.

Kalendar R., Khassenov B., Ramanculov E., Samuilova O. and Ivanov K.I. (2017) FastPCR: An i*n silico* tool for fast primer and probe design and advanced sequence analysis. *Genomics,* 109, 312-319.

Kalendar R., Shustov A.V., Seppänen M.M., Schulman A.H. and Stoddard F.L. (2019) Palindromic sequence-targeted (PST) PCR: a rapid and efficient method for high-throughput gene characterization and genome walking. S*cientific Reports,* 9, 17707.

Petersen K., Kolmos E., Folling M., *et al.* (2006) Two MADS-box genes from perennial ryegrass are regulated by vernalization and involved in the floral transition. *Physiologia Plantarum,* 126, 268-278.

Seppanen M.M., Pakarinen K., Jokela V., *et al.* (2010) Vernalization response of P*hleum pratense* and its relationships to stem lignification and floral transition. *Annals of Botany,* 106, 697-707.

Stewart A.V., Joachimiak A.J. and Ellison N.W. (2011) Phleum. In: Kole C. (ed.) *Wild crop relatives: genomic and breeding resources: millets and grasses.* Springer, Berlin, Germany, pp. 257-274.

Tanhuanpää P., Erkkilä M., Kalendar R.., Schulman A.H. and Manninen O. (2016) Assessment of genetic diversity in Nordic timothy (*Phleum pratense* L.). *Hereditas,* 153, 5.

Differences in root morphological features and shoot P accumulation at different soil P levels

Knuutila K.[1], Junklewitz P.[2], Liespuu J.[3], Mäkelä P.S.A.[1], Owusu-Sekuyre A.[1], Tasanko E.[1], Alakukku L.[1] and Seppänen M.[1,4]

[1]Department of Agricultural Sciences, University of Helsinki, Finland; [2]Research Centre Hanninghof, Yara International Ltd., Germany; [3]Kotkaniemi Research Farm, Yara Suomi Ltd., Finland; [4]Yara Suomi Ltd., Finland

Abstract

The morphology and architecture of roots affect plant nutrient uptake. The objective of this study was to investigate how soil initial phosphorus (P) fertility level (Fair P 6.2 mg l^{-1} soil, Good P 17.5 mg l^{-1} soil) and annual P application (20 kg ha^{-1}) affected root morphology, P content of silage grass and concomitantly its nutritive value. Soil P levels were created in a 40-year fertilisation experiment (1973-2013). In a field trial (Kotkaniemi experimental farm) shoot and root samples were taken at the beginning of the growing season of 2018, at the time of the 1st and 3rd harvests and twice after 1st and 3rd harvests. Roots were washed, photographed and root area, mean root length, and diameter were analysed with ImageJ program. Root and shoot nutrient contents were analysed with ICP-OES. P application increased root area during the 1st harvest but it decreased at 3rd harvest. Soil fertility P and P fertilisation had a positive effect on root dry weight. Root area was negatively correlated with increasing shoot P content, which indicates that increase in root area compensated P uptake when less P was available. However, increased P foraging capacity was insufficient to elevate grass P content to an adequate level.

Keywords: grass root, P uptake, root area, root diameter, soil P level, P fertilisation

Introduction

Over 60% of living grass biomass consists of roots (Gibson, 2009). In the roots of most grass species, 70-90% grow at a depth of 0-20 cm (Bolinder *et al.*, 2002). Nutrient deficiency affects root morphology by enhancing root growth and root hair cylinder volume (result of root diameter, length, and root hair length) when nutrients are less available (Haling *et al.*, 2016). When comparing plants with a fine extensive root system to plants with a thick and small root system, P deficiency was found to affect root morphology (Hill *et al.*, 2006). Changes in the root mass fraction and specific root length indicated the adaptation of roots to P stress, although root diameter and the size of the root system had more important roles in describing plant P requirements. Although plants can adapt to different soil P levels, P availability has been shown to increase plant N and P uptake (Perez-Corona and Verhoeven, 1996; Xu *et al.*, 2016). Our aim was to investigate how P availability from soil and fertiliser affect grass root morphology and plant P uptake and to determine whether the changes of root morphological features are correlated with root P uptake.

Materials and methods

Root growth and nutrient uptake of the 3rd year silage grass ley (containing timothy (*Phleum pratense* L.), meadow fescue (*Schedonorus pratensis* Huds.), and tall fescue (*Schedonorus arundinaceus* Schreb.)) were investigated in a field trial at Kotkaniemi experimental farm (Yara Suomi Ltd., Finland). Soil fertility P levels (Fair: 6.2 P mg l^{-1} soil; Good: 17.5 P mg l^{-1} soil) of the grass ley were created in a 40-year, long-term fertilisation experiment with increasing NPK levels and the effect of soil fertility P levels and annual P fertilisation (P0: 0 P kg ha^{-1} and P20: 20 P kg ha^{-1}) were evaluated. In 2018, the ley was fertilised with N-P-K-S fertiliser (250/250–0/20–200/200–0/1.2) and harvested 3 times during the growing period.

Due to a long period of water deficit, the ley was irrigated (30 mm) on 11 June and 31 July. Growth stage during the 1st harvest was 31, and at the time of the 3rd harvest 21 (Simon and Park, 1983).

One plant and root sample (cylinder height 20 cm, ø 10 cm) was taken from each plot (3 replicates) at the beginning of growing season (5 May), 1 week before the 1st (24 May) and 3rd (12 September) harvest, and 2 times after the 1st (19 and 26 June) and the 3rd (24 September and 1 October) harvests. Soil samples were washed, and root samples were photographed and dried as reported by Knuutila *et al.* (2019). Samples were ground (1 mm sieve, Ultra Centrifugal Mill ZM 200, Retsch, GmbH, Haan, Germany) and P was measured with Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, ISO 11885:2007(E)). Root area (m^2 m^{-2}), mean root length (mm), and mean root diameter (mm) were analysed from the images (ImageJ program, ver 1.52c, Wayne Rasband, National Institutes of Health, USA). Analysis of variance (ANOVA) was performed to compare differences between all treatments and sampling dates. Means were compared using Fisher's Least Significant Difference (LSD) when the F-value in the ANOVA was significant (P<0.05). Pearson's correlation was used to determine the correlations between variables.

Results and discussion

Soil P fertility level and P fertilisation affected the root area, mean root length, and mean root diameter. Root area was highest in the spring and decreased during the growing season. In the spring, and around the 1st harvest, root area (Figure 1A) increased when more P was available either from soil or fertilisation. However, at the time of the 3rd harvest and afterwards, the root area decreased with increasing P fertilisation. As an exception, the root area with a Good soil P fertility level increased 1 week and 2 weeks after the 3rd harvest.

After the 1st harvest, grass yield increased with increasing P fertilisation and soil P fertility level, as shown in Knuutila *et al.* (2019). Root area, mean root diameter and mean root length did not correlate with grass yield. However, shoot P content had a negative correlation with root area (r^2=0,215, P<0.000) whereas mean root length and mean root diameter did not correlate with shoot P content. In the 1st harvest shoot P content increased with P availability and was highest in treatment Good P20 (2.97 g kg^{-1} DM) and lowest in Fair P0 (2.37 g kg^{-1} DM). The treatments Fair P20 (2.67 g kg^{-1} DM) and Good P0 (2.80 g kg^{-1} DM) did not differ from each other or from Fair P0 and Good P20 at the 1st harvest. There was no difference in shoot P content between soil P fertility levels or P fertilisations in the 3rd harvest.

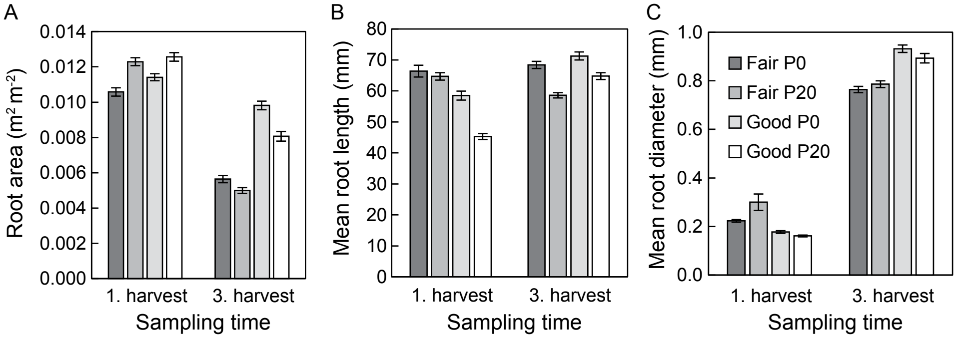

Figure 1. (A) Root area (m^2 m^{-2}), (B) mean root length (mm) and (C) mean root diameter (mm) (soil layer 0-20 cm) means of four treatments (low soil P fertility 6.2 P mg l^{-1} soil with 0 and 20 P kg ha^{-1} fertilisation and high soil P fertility 17.5 P mg l^{-1} with 0 and 20 P kg ha^{-1} fertilisation) 1 week before 1st and 3rd harvest. The data shown are means ± SE; n=3.

P fertilisation decreased mean root length (Figure 1B) at the time of harvests but increased in the spring and after each harvest, which indicated improved root regrowth. Mean root diameter (Figure 1C) was lowest in the spring and increased during the summer. P fertilisation increased root diameter at the Fair soil P fertility level. In addition, the root diameter was larger at higher soil P availability. Waddell *et al.* (2017) also found that increased P availability decreased the fine root (<0.24 mm) portion in comparison with P application for several grass species. However, P application even decreased root diameter at the Good soil P level. Root diameter was unusually large in the summer, which may be due to a long period of water deficit during the spring and summer months. Water deficit has been shown to increase root diameter (Zhou *et al.*, 2014).

Conclusions

P availability modified root morphological properties such as root area, mean root length and root diameter. Soil fertility P and P fertilisation increased root biomass (Knuutila *et al.*, 2019), but available P decreased root area. Increased root area compensated for P uptake at lower P availability but was unable to compensate for adequate shoot P content or yield.

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References

Bolinder M.A., Angers D.A., Bélanger G., Michaud R. and Laverdière M.R. (2002) Root biomass and shoot to root ratios of perennial forage crops in eastern Canada. *Canadian Journal of Plant Science* 82, 731-737.

Gibson D.J. (2009) *Grasses and grassland ecology*. Oxford University Press, New York, USA, 305 pp.

Haling R.E., Yang Z., Shadwell N., Culvenor R.A., Stefanski A., Ryan M.H., Sandral G.A., Kidd D.R., Lambers H. and Simpson R.J. (2016) Root morphological traits that determine phosphorus-acquisition efficiency and critical external phosphorus requirement in pasture species. *Functional Plant Biology* 43, 815-826.

Hill J.O., Simpson R.J., Moore A.D. and Chapman D.F. (2006) Morphology and response of roots of pasture species to phosphorus and nitrogen nutrition. *Plant and Soil* 286, 7-19.

Knuutila K., Junklewitz P., Liespuu J., Mäkelä P.S.A., Owusu-Sekuyre A., Alakukku L. and Seppänen M. (2019) The root growth of forage grasses at different soil P levels. *Grassland Science in Europe* 24, 158-160.

Perez-Corona M.E. and Verhoeven J.T.A (1996) Effects of soil P status on growth and P and N uptake of *Carex* species from fens differing in P-availability. *Acta Botanica Neerlandica* 45, 381-392.

Simon, U. and Park, B.H. (1983). A descriptive scheme for stages of development in perennial forage grasses. In: Smith J.A. and Hays V.W. (eds.) *Proceedings of the XIV International Grassland Congress*, Lexington, Kentucky, USA, pp. 416-418.

Waddell H.A., Simpson R.J., Ryan M.H., Lambers H., Garden D.L. and Richardson A.E. (2017) Root morphology and its contribution to a large root system for phosphorus uptake by *Rytidosperma* species (wallaby grass). *Plant and Soil* 412, 7-19.

Xu B., Gao Z., Wang J., Xu, W., Palta J.A. and Chen Y. (2016) N:P ratio of the grass *Bothriochloa ischaemum* mixed with legume *Lespedeza davurica* under varying water and fertilizer supplies. *Plant and Soil* 400, 67-79.

Zhou Y., Lambrides C.J. and Fukai S. (2014) Drought resistance and soil water extraction of a perennial C_4 grass: contributions of root and rhizome traits. *Functional Plant Biology* 41, 505-519.

Yield and biological N_2 fixation response in grass-clover mixtures to cattle slurry and mineral N

Kristensen R.K.[1], Rasmussen J.[1], Frandsen T.S.[2] and Eriksen J.[1]
[1]*Department of Agroecology, Aarhus University, Denmark;* [2]*SEGES, Aarhus, Denmark*

Abstract

Swards sown with grass-clover mixtures are widely used in dairy production systems worldwide, but grasses and forage legumes differ substantially in their nitrogen (N) acquisition strategies. Nitrogen application to grass-clover gives the grass component a competitive advantage over the clover, but earlier studies have indicated a less pronounced effect for this advantage when N is applied as animal manure compared with mineral N fertiliser. The implications of these findings have yet to be elucidated, and the aim of this study was to see if different amounts of mineral fertiliser and slurry + mineral fertiliser resulted in different DM yield, botanical composition, and N_2 fixation. The experiment was conducted in farmers' fields in two Danish locations for one year. The study showed an expected increase in DM production with increasing fertilisation. The negative response in clover fraction was lower with slurry + mineral fertiliser than mineral fertiliser at one of the sites, in line with the hypothesis. This will greatly impact the estimated N balance of the grass-clover mixtures due to differences in clover biological N_2 fixation.

Keywords: botanical composition, N response trial, field experiment

Introduction

Fertilisation of grass-clover swards for animal feed is common in Danish dairy farming production systems in order to harvest 4-5 times for silage during a growing season. Fertilisation typically consists of slurry from the on-farm cattle supplemented with a mineral fertiliser. Nitrogen application to grass-clover gives the grass component a competitive advantage over clover, but earlier studies have indicated a less pronounced effect for this advantage when the N is applied as animal manure as opposed to mineral N fertiliser (Nesheim *et al.*, 1990; Søegaard, 1998). However, there is a lack of knowledge on the extent of this effect, both on sward botanical composition and on the N_2 fixation by the clover.

The aim of this experiment was to examine the effects of a typical Danish dairy farmer's N-application strategy (slurry and mineral fertiliser combined) versus the typical N-application in experimental trials (solely mineral fertiliser). The hypothesis of this study was that the ability of clover to compete with grass was better when the N is applied as slurry compared with application as mineral N fertiliser.

Materials and methods

The experiment was located in two farmer's fields on sandy soils in the western part of Denmark. The swards were established with white- and/or red clover (*Trifolium repens* L.) (*Trifolium pratense* L.) and perennial ryegrass (*Lolium perenne* L.) in 2017 and the results reported are from the first production year in 2018. Site 1 (Esbjerg) was seeded with white clover and perennial ryegrass, whereas Site 2 (Vildbjerg) also included red clover in the mixture. At each site N-response trials were established with mineral fertilisers (0, 60, 120, 240, 360 and, 480 kg N ha^{-1}) and cattle slurry + mineral fertiliser (120, 240, 360 and, 480 kg N ha^{-1}) in four replicates. The amounts of N in slurry were calculated on the basis of NH_4^+ content at the time of the application. The grass-clover was harvested five times during the growing season with determinations at each harvest of dry matter yield, botanical composition (hand sorting), and biological N_2 fixation. Biological N_2 fixation was estimated using the ^{15}N dilution method (Fried and Middelboe, 1977; McNeill *et al.*, 1994). Statistical analyses were done in R (R Core Team, 2017) performing a two-way ANOVA and comparing the estimated means using the Tukey method.

Results and discussion

The DM yield response showed, as expected, that increasing N fertilisation resulted in increased biomass production, but with a relatively flat response from low N to high N of approximately 7 kg DM kg^{-1} N at both sites (Figure 1). The DM yield responses were similar for the mineral fertiliser and the slurry + mineral fertiliser treatments. The sward with perennial ryegrass and white clover at Site 1 had significantly (P<0.005) lower yields (by around 1 Mg DM ha^{-1} for all N levels) than the perennial ryegrass and red- and white clover sward at Site 2.

The fraction of clover in the harvested sward was highest, at around 50%, without fertilisation and it decreased, as expected, with increasing N fertilisation (Figure 1). However, at Site 2 the slurry + mineral fertiliser treatment resulted in a smaller decrease in the clover fraction, from around 50 to 40%, compared with the mineral fertiliser treatment, which went down from 50 to 25%, whereas at Site 1 no such difference was observed between the fertiliser treatments (P>0.005). Thus, the results from Site 2 confirm earlier findings by Nesheim *et al.* (1990) and Søegaard (1998), although their findings were without red clover in the investigated mixture. It is worth noting that the difference between the mineral fertiliser and slurry + mineral fertiliser treatments were only evident at one of the sites, and this means that unknown site-specific conditions must have affected the N response of the fertiliser types. The underlying mechanisms need to be investigated in more detailed experiments.

The difference in sward botanical composition due to fertiliser type had a strong impact on the N quantity acquired by clover via N_2 fixation (Figure 1). At Site 2, the difference between the two fertiliser treatments corresponded to more than 80 kg N ha^{-1} in harvested clover biomass. This has a significant impact on the estimated N balance for the whole grass-clover systems.

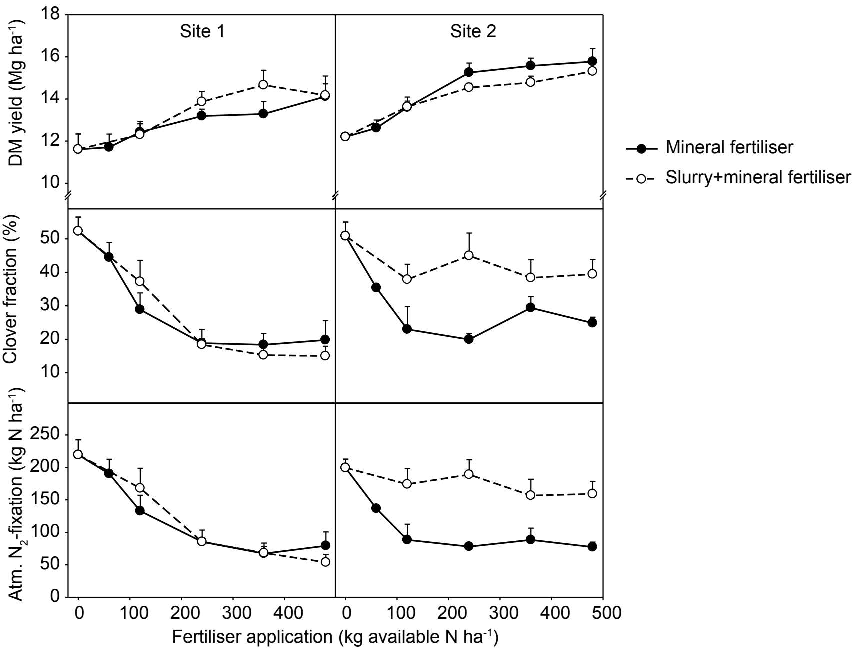

Figure 1. The fertiliser response to mineral fertiliser and slurry + mineral fertiliser in terms of dry matter (DM) yield, clover fraction of DM, and N input via clover N_2 fixation at the two sites: Site 1 (Esbjerg) and Site 2 (Vildbjerg). The available N in slurry was based on the NH_4^+ content at the time of the application.

Conclusions

The study showed an expected increase in DM production with increasing fertilisation. The negative response in clover fraction was lower with slurry + mineral fertiliser than mineral N fertiliser at one of the sites, in line with the hypothesis. This will greatly affect the estimated N balance of the grass-clover mixtures due to differences in clover biological N_2 fixation.

Acknowledgements

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References

Fried M. and Middelboe V. (1977) Measurement of amount of nitrogen fixed by a legume crop. *Plant and Soil* 47(3), 713-715.

McNeill A.M., Hood R.C. and Wood M. (1994) Direct measurement of nitrogen fixation by Trifolium repens L. and Alnus glutinosa L. using 15N2. *Journal of Experimental Botany* 45(6), 749-755.

Nesheim L., Boller B.C., Lehmann J. and Walther U. (1990) The effect of nitrogen in cattle slurry and mineral fertilizers on nitrogen fixation by white clover. *Grass and Forage Science* 45(1), 91-97.

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Søegaard K. (1998) Timing of N-application after defoliation of clover-grass. *Grassland Science in Europe* 3, 979-982.

Effects of increasing plant diversity on yield of grass and grass-legume leys in Finland

Kykkänen S., Korhonen P., Mustonen A. and Virkajärvi P.
Natural Resources Institute Finland (Luke), Halolantie 31 A, 71750 Maaninka, Finland

Abstract

Multi-species forage grass and grass-legume leys with high response diversity are thought to show more resilience in changing environmental conditions than simpler forage mixtures and monocultures. Climate change will increase the occurrence of extreme weather events, such as drought periods, highlighting the importance of increasing resilience of production systems. At the same time, longer growing seasons and milder winters are likely to increase the possibilities for cultivation of new grass and legume species at high latitudes. Field experiments (nested experimental design) were established in Central Finland (2018-2019) to compare nine different seed mixtures (four grass mixtures, G 1-4; and five grass-legume mixtures, L 1-5) with different combinations of timothy, meadow fescue, tall fescue, perennial ryegrass, red clover, white clover, alsike clover and sand lucerne. The compared variables included dry matter (DM) yield (kg DM ha^{-1}), nutritive value (D-value) and botanical composition. Harvest frequency and fertilisation were optimised for G and L leys separately. Annual yield of L and G mixtures varied from 5,700 to 8,500 kg DM ha^{-1} and from 7,200 to 10,000 kg DM ha^{-1}, respectively. Only grass-legume mixtures produced higher yields with higher species diversity than mixtures with fewer species.

Keywords: forage, grass, legume, mixture, diversity

Introduction

Increasing plant diversity has been observed to have a positive effect on productivity and stability of grasslands in many cases (Hector *et al.*, 1999; Isbell *et al.*, 2015). Positive effects can be mediated by niche complementarity, facilitation and sampling effect (Hooper *et al.*, 2005). Grass mixtures have also been observed to compete better against weeds (Connolly *et al.*, 2018). High latitudes, short growing seasons, and long and difficult winter conditions limit the selection of plant species and cultivars, and lead to a need to renew grassland, usually every 3-4 years. The aim of this study was to quantify the effect of increasing plant diversity on DM yield and D-value. Another goal was to measure how species composition changed in the plots during the first two years.

Materials and methods

To compare the performance of different grass species mixtures, a plot trial (nested design) was established in 2017 in Kuopio (Maaninka), Finland (63°09'N, 27°20'E; sandy loam with 3-5.9% organic matter). Mixture type (L=Grass-legume mixture; G=Grass-only mixtures) was investigated as a main plot. Sub plots (L1-L5 and G1-G4) were designed separately for both main plots. Plots were sown using barley as a cover crop at 194 kg seed ha^{-1} and 16-27 kg seed ha^{-1} for grasses, depending on the mixture. Mixture compositions are described in detail in Table 1. The seeds of clovers and sand lucerne were inoculated. G-plots were harvested three times, and L-plots twice, per growing season at the optimal stage for silage (with digestibility targets between 680 and 700 g kg^{-1} DM for G-mixtures and 650-680 for L-mixtures depending on the proportion of legumes in the mixture). G mixtures received 100+90+50 kg N ha^{-1} yr^{-1}, and L mixtures 50+50 kg N ha^{-1} yr^{-1}. Botanical composition was measured as a dry mass fraction by separating species by hand. Weeds were controlled during the experiment with Basagran® SG (4 July in 2018 and 2019) from L-mixtures. Statistical analyses were performed using ANOVA (Mixed procedure of SAS 9.4).

Table 1. Species composition (% of total number of germinating seeds) of the seed mixtures. Seeding rate (kg seed ha^{-1}) in parentheses next to mixture names.

		Phleum pratense L.	*Festuca pratensis* Huds.	*Festuca arundinacea* Schreb.	*Lolium perenne* L.	*Trifolium pratense* L.	*Trifolium repens* L.	*Trifolium hybridum* L.	*Medicago* × varia Martyn
Legume mixtures	L1 (20)	60[b]	30[d]			10[h]			
	L2 (18)	50[b]	25[d]			10[h]	5[i]	10[j]	
	L3 (16)	25[b]	25[d]			25[h]	25[i]		
	L4 (22)	50[b]	25[d]			5[h]	2.5[i]	5[j]	12.5[k]
	L5 (19)	40[b]	40[d]	11[f]	11[g]	10[h]	5[i]	10[j]	
Grass mixtures	G1 (27)	70[a]	30[d]						
	G2 (27)	70[a]	15[d]	15[f]					
	G3 (24)	70[a,b,c]	30[de]						
	G4 (26)	70[a]	10[d]	10[f]	10[g]				

[a] cv. 'Rubinia', [b] cv. 'Tuure', [c] cv. 'Nuutti', [d] cv. 'Valtteri', [e] cv. 'Inkeri', [f] cv. 'Karolina', [g] cv. 'Riikka', [h] cv. 'SW Yngve', [i] cv. 'SW Hebe', [j] cv. 'Frida', [k] cv. 'Lavo'

Results and discussion

G-mixtures produced higher (P<0.05) total DM yields (9,400-9,700 and 7,200-8,000 kg DM ha^{-1} in 2018 and 2019, respectively) and D-values (661-709 and 677-723 g kg DM^{-1}) than L-mixtures (7,400-8,500 and 5,700-7,100 kg DM ha^{-1}, D-value 647-672 and 648-705 g kg DM^{-1} respectively in 2018 and 2019) in both experiment years (Figure 1). The differences can be explained by both mixture effects and separated management strategies of the main plots. The lower D-value of L-mixtures can be compensated to some extent by greater DM intake potential and increased performance (Huhtanen *et al.*, 2007).

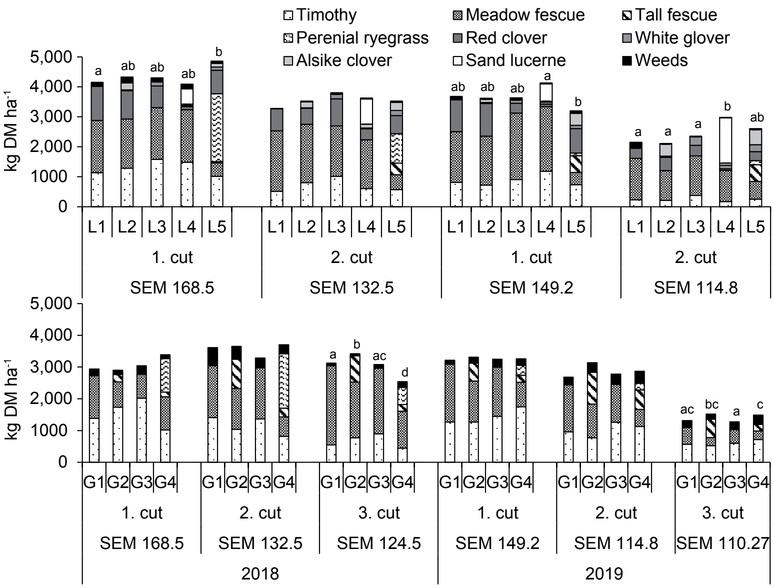

Figure 1. Biomass dry matter (DM) yield and species composition of different leys. G1-G4 are different grass-only mixtures and L1-L5 grass-legume mixtures (see Table 1 for details). Standard errors of the mean (SEM) are presented for DM yield of each cut, n=4; Columns with same letter in each cut are not significantly different at P<0.05.

The effect of species diversity on the DM yield and D-value was greater in L-mixtures than in G-mixtures. A higher plant diversity increased the total annual DM yield of L-mixtures in both years (P<0.05, Figure 1), but for G-mixtures no significant differences were observed in total yields (P>0.05). In 2018, a difference was observed only between L1 (7,400 kg DM ha^{-1}) and L5 (8,500 kg DM ha^{-1}; P<0.05). These differences can be explained by the high percentage of perennial ryegrass (46% in the first cut, 28% in the second) and its aggressive growth in relation with associated grass species, which increased the DM yield in the first year. In 2019, the total DM yield of L4 was significantly higher (7,120 kg DM ha^{-1}) than that of the other L-mixtures (average 5,840 kg DM ha^{-1}). The 2019 growing season was exceptionally dry and probably favoured the growth of deep-rooted and drought-tolerant sand lucerne and tall fescue. However, D-values decreased when either tall fescue (in 2018 and 2019) or sand lucerne (in 2019) was present (Figure 1). Perennial ryegrass is known to lack the winter hardiness of the other grass species tested here (Helgadóttir *et al.*, 2018, Østrem *et al.*, 2015), and in 2019 it suffered significant winter damage from which it could not recover. Experiments with a larger variation in growing conditions would allow a better distinction of complementarity and sampling effects, which might improve yields more at field scale than observed here at plot scale. Furthermore, because species were not grown as monocultures, it was not possible to identify whether transgressive overyielding occurred.

Conclusions

The results indicate that (1) including deep-rooted species in grass mixtures can have a positive impact on yields in dry years, (2) weak winter hardiness of perennial ryegrass decreased its yield potential in seed mixtures, and (3) the period for achieving optimal forage digestibility (D-value) can be shorter in mixtures containing tall fescue and sand lucerne than in other mixtures.

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References

Connolly J., Sebastiá M-T., Kirwan L., … and Lüscher A. (2018) Weed suppression greatly increased by plant diversity in intensively managed grasslands: A continental scale experiment. *Journal of Applied Ecology*, 55(2), 852-862.

Hector A., Schmid B., Beierkuhnlein C., … and Lawton J.H. (1999) Plant diversity and productivity experiments in European grasslands. *Science,* 286, 1123-1127.

Helgadóttir Á., Aavola R., Isolahti M., Marum P., Persson C., Aleliūnas A., … and Rognli O.A. (2018) Adaptability and phenotypic stability of *Lolium perenne* L. cultivars of diverse origin grown at the margin of the species distribution. *Journal of Agronomy and Crop Science*, 204(5), 493-504.

Hooper D.U. *et al.* (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, 75(1), 3-35.

Huhtanen P., Rinne M. and Nousiainen J. (2007) Evaluation of the factors affecting silage intake of dairy cows: A revision of the relative silage dry-matter intake index. *Animal,* 1, 758-770.

Østrem L., Helgadottir A., Niemeläinen O., Seppänen M., Greve Pedersen M., Halling M. and Rognli O.A. (2015) Environmental impact on winter survival and production in non-native grasses in the Nordic countries. In: *Proceedings of the 25th NJF Congress, Nordic View to Sustainable Rural Development,* Riga, Latvia, pp. 16-18.

Cultivating novel and diverse forage plant communities could enhance livestock farming

Lee M.A.
Royal Botanic Gardens Kew, Natural Capital and Plant Health, Jodrell Laboratory, United Kingdom

Abstract

Many farmers grow a limited number of forage species to feed their livestock. This may not be optimal depending on the nutritional requirements of their animals and local conditions. Other species may have superior nutritional and ecological traits, particularly when set against the backdrop of climate change. A meta-analysis was carried out to assess the nutritional values of forage plants grown around the world. Nutritional values ranged extensively, such as fibre (23-90 g kg^{-1}), protein (2-36 g kg^{-1}), minerals (2-22 g kg^{-1}) and dry matter digestibility (31-97 g kg^{-1}). On average, grasses and tree foliage contained the most fibre, whilst herbaceous legumes contained the most protein and minerals. There were individual species in each functional group that were highly nutritious depending on the growing conditions. Care must be taken when selecting novel forage plant species that are appropriate for a given farming system and location. However, using this dataset as an initial screen, there are several promising species which warrant further investigation. The Royal Botanic Gardens (RBG) Kew's rich collections (databases, seeds and living plants) could be utilised to identify novel forage species, and mixtures of species, for future breeding programmes and field trials.

Keywords: digestibility, diversity, ecosystem services, forage, nutrition, protein

Introduction

European farming is largely dependent on a limited number of forage plant species to feed livestock. In the UK, the 'Recommended Grass and Clover List' contains information on just 7 species; perennial ryegrass, Italian ryegrass, timothy, white clover, red clover, lucerne and cocksfoot (including hybrids and polyploids) (BGS, 2019). Reliance on a small pool of forage species leaves some farmers at risk from factors such as climate change, market forces, pests and diseases. Cultivating other forage plant species may reduce these risks and offer several opportunities. Novel forage species may provide nutritional or productivity benefits, respond positively to drought or flooding and enhance other services, for example providing habitat for wildlife or storing carbon. To date, there is a paucity of data summarizing the nutritive values of forage plants grown around the world. A meta-analysis was carried out to extend data coverage and to investigate variation between species, locations and functional groups.

Materials and methods

Data were obtained from peer-reviewed journal articles. These articles were identified by systematically searching the Web of Science (WoS). Articles were included in the database if the measurements were related to a specific forage plant species or hybrid that had been grown in field conditions at a defined location (site) and harvested for nutritional analysis at a stated time. The database contains 1,255 geo-located records with 3,774 measurements of nutritive values for 136 forage plant species or hybrid cultivars grown in 30 countries. Metrics recorded included acid detergent fibre, acid detergent lignin, mineral ash, crude protein (CP), dry matter, neutral detergent fibre (NDF), dry matter digestibility (DMD), organic matter digestibility (OMD) and productivity/yields.

Sites were allocated to a bioclimatic zone as defined by the Köppen-Geiger climate classification system (Kottek *et al.* 2006). Other covariate data were also recorded including soil type, pH, fertiliser frequency and intensity, grazing density, altitude, temperature and rainfall. Variation between functional groups and bioclimatic zones for each nutritive metric was assessed using ANOVA tests in combination with Tukey's HSD tests.

Results and discussion

This dataset demonstrates that as much as 97 g kg^{-1} of a forage plant may be digestible, compared with 31 g kg^{-1} for the least digestible plants (defined by DMD). Fibre (NDF) ranged by 23-90 g kg^{-1}, protein by 2-36 g kg^{-1}, lignin by 8-21 g kg^{-1} and minerals (ash) by 2-22 g kg^{-1}. Large variation in the nutritive values of forage plants indicates the capacity that plants have for providing animals with nutrition and highlights the potential of novel plant species.

Across the grasses growing in temperate climates the maximum CP values that were recorded were from commonly cultivated perennial ryegrass (34 g kg^{-1}) and Italian ryegrass (28 g kg^{-1}, Figure 1) but other species including *Alopecurus pratensis* (24 g kg^{-1}), *Nassella clarazii* (22 g kg^{-1}), *Cynodon dactylon* (21 g kg^{-1}), *Avena strigosa* (20 g kg^{-1}) and *Holcus lanatus* (19 g kg^{-1}) also showed high nutritive values. DMD or OMD values were not available for many of these species, however, *Avena strigosa* was recorded with a high DMD (83 g kg^{-1}).

Herbaceous legumes generally contained lower levels of NDF and higher levels of CP and minerals than grasses (P<0.05). Legumes can improve soil fertility and biodiversity. There were high maximum CP values for commonly cultivated species, such as lucerne (32 g kg^{-1}), white (32 g kg^{-1}) and red clover (28 g kg^{-1}), but other species such as *Macroptilium atropurpureum* (28 g kg^{-1}), *Lablab purpureus* (25 g kg^{-1}), *Lotus corniculatus* (25 g kg^{-1}), *Chamaecrista rotundifolia* (24 g kg^{-1}), *Vicia sativa* (24 g kg^{-1}), *Trifolium ambiguum* (22 g kg^{-1}), and *Melilotus officinalis* (20 g kg^{-1}) also displayed high CP values.

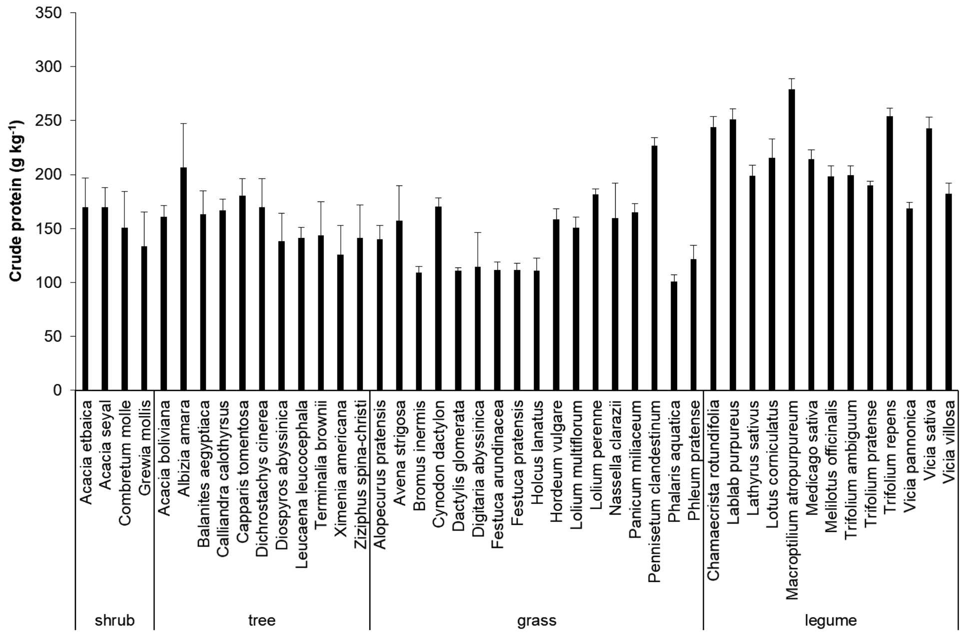

Figure 1. A subset of mean (+ standard error) crude protein (CP) contents of plants that have been grown for livestock forage in a Köppen-Geiger (KG) temperate region (only mean values above 100 g kg^{-1} are shown). Plants grown in KG tropical, tundra and equatorial regions are excluded.

Trees and shrubs can deliver forage alongside other services, including carbon storage and flood defence, and there has been recent interest in the benefits of silvopastoral livestock systems (Santos *et al.* 2016). Little is known about the use of European trees for forage. Tree and shrub foliage was generally higher in terms of lignin and NDF contents than the other functional groups (P<0.05). Some species did have high CP values such as *Albizia amara* (25 g kg^{-1}), *Capparis tomentosa* (20 g kg^{-1}) and *Leucaena leucocephala* (14 g kg^{-1}), the latter also displayed high digestibility (90 g kg^{-1}). Trees which can provide forage and a sellable crop, as is the case for *Inga edulis* in South America, may also be appealing.

Conclusions

Novel forage species may improve livestock nutrition and reduce risk exposure, such as those associated with climate change. Cultivation of drought-tolerant plants, for example, may ensure that livestock are fed when water is scarce. Increasing the diversity of forage plant communities may be beneficial because they have different nutritional profiles, mature at different times of the year, grow in different conditions and can support other valuable services (e.g. flood defence, support wildlife and store carbon). Care must be taken in species selection, however, and RBG Kew's unique collections could be used to identify novel forage species for future trials.

References

British Grassland Society (BGS). (2019) Recommended Grass and Clover Lists for England and Wales. Available at: http://www.britishgrassland.com/page/recommended-grass-and-clover-lists-0

Kottek M., Grieser J., Beck C., Rudolf B. and Rubel F. (2006) World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift* 15, 259-263.

Santos D. de C., Guimarães Júnior R., Vilela L., Pulrolnik K., Bufon V.B. and França A.F. de S. (2016) Forage dry mass accumulation and structural characteristics of Piatã grass in silvopastoral systems in the Brazilian savannah. *Agriculture, Ecosystems & Environment,* 233, 16-24.

Resistance of multiple diploid and tetraploid perennial ryegrass varieties to drought

Lee M.A.[1], Howard-Andrews V.[2] and Chester M.[1]
[1]Natural Capital and Plant Health, Royal Botanic Gardens Kew, United Kingdom; [2]School of Biological Sciences, University of Southampton, United Kingdom

Abstract

Forage plants are valuable because they support the livestock industry. Selective breeding, including polyploidization, where genome size is increased by whole genome duplication, has been shown to change the productivity and stress tolerance of new varieties. We conducted a growth chamber experiment to investigate the responses of *Lolium perenne* to climate change with four diploid and four autotetraploid varieties. We simulated projected spring and summer temperatures for the South-West of England in 2080, applying three projected drought scenarios. Drought caused a reduction in productivity, but there was substantial variation, with the optimal variety changing depending on drought severity. In the final harvest, we measured greater biomass for the tetraploids under the severe drought, whereas diploids had greater biomass under the current rainfall and likely drought scenarios. Larger stomata with faster conductance were observed in tetraploids, however, varietal differences show that there are other important factors at play. Future growing conditions will need to be considered in the breeding of *L. perenne* varieties so that they are tolerant to reductions in summer rainfall.

Keywords: climate change, drought tolerance, forage, productivity, traits

Introduction

Selective breeding, including polyploidization, has sought to increase forage yields, nutritive values and seed set. It has been suggested that polyploids may offer advantages under drought conditions (Hollister, 2015). This may be important in the future because summer rainfall has been projected to decline across many regions, linked strongly to anthropogenic greenhouse gas emissions. This may lead to a decline in forage productivity and nutritive values, and different varieties may be better adapted to future growing conditions (Lee *et al.*, 2017). We sought to test the performance of eight different varieties of the common forage grass, *L. perenne*, to drought severity under three rainfall scenarios and under temperature and humidity values predicted for 2080. We hypothesised that there would be considerable variation in drought tolerance between varieties but that tetraploids would be more resistant than diploids. We also hypothesised that stomatal size would determine drought tolerance.

Materials and methods

We simulated projected spring and summer conditions in a growth chamber using two-hourly mean monthly temperature and humidity values for the South-West of England, uplifted by the most likely scenario for 2080 (+3.9 °C). We planted 480 individuals (3 treatments × 8 varieties × 20 replicates) in groups of 10, two weeks after they had been cultivated from seed. Seedlings were watered following the current rainfall regime for two months using April 2080 and then May 2080 climate settings. After two months three rainfall treatments were applied: (1) current rainfall, (2) mean (-23%) projected decline for 2080 (hereafter: likely drought) and (3) maximum (-49%) projected decline for 2080 (hereafter: severe drought) and we switched to our summer 2080 climate setting. This is in line with our current understanding of an anticipated summer, but not spring, drought for SW England in 2080 (DEFRA, 2009). We carried out three harvests, removing a third of the individuals after approximately 3, 4 and 5 months. We measured fresh biomass, dry biomass and relative water content at each time point. We also concurrently grew seedlings in ambient conditions in a glasshouse (RBG Kew) and measured stomatal size and rates of stomatal conductance for all varieties. These data were analysed by ANOVA and post-hoc comparisons were done using Tukey's HSD tests.

Results and discussion

Mean fresh biomass (-39%), dry biomass (-27%) and water content (-9%) declined under the likely drought scenario. In addition, fresh biomass (-58%), dry biomass (-43%) and water content (-22%) declined to a greater extent under the severe drought scenario (Figure 1). Different varieties performed better than others across the treatments, with Variety8 producing the most dry biomass under current rainfall (82% more than the lowest) and Variety6 the most dry biomass under the severe drought (58% more than the lowest). Variety2 and Variety6 produced the greatest biomass under the likely drought scenario.

There were no significant differences in productivity between ploidy levels for the first two harvests (Figure 2). However, in harvest 3, the diploids produced more fresh and dry biomass under the current rainfall scenario (+25 and +28%, respectively) and likely drought scenario (+13 and +9%, respectively), whilst the tetraploids produced 13% more dry biomass with a 9% lower water content under severe drought. Although it is interesting that the tetraploids were generally more tolerant to severe drought, under the other scenarios the diploids generally produced more biomass, even under the most likely drought scenario.

Mean stomatal size varied 1.5-fold between varieties and stomatal conductance varied 1.4-fold between varieties (Table 1). On average, tetraploid stomata were 24% larger than diploids and the mean rate of conductance was 10% faster. There was no direct relationship between productivity and stomatal size or conductivity, indicating that either stomata size is not the sole driver modulating resistance to drought or that the sample size was too low.

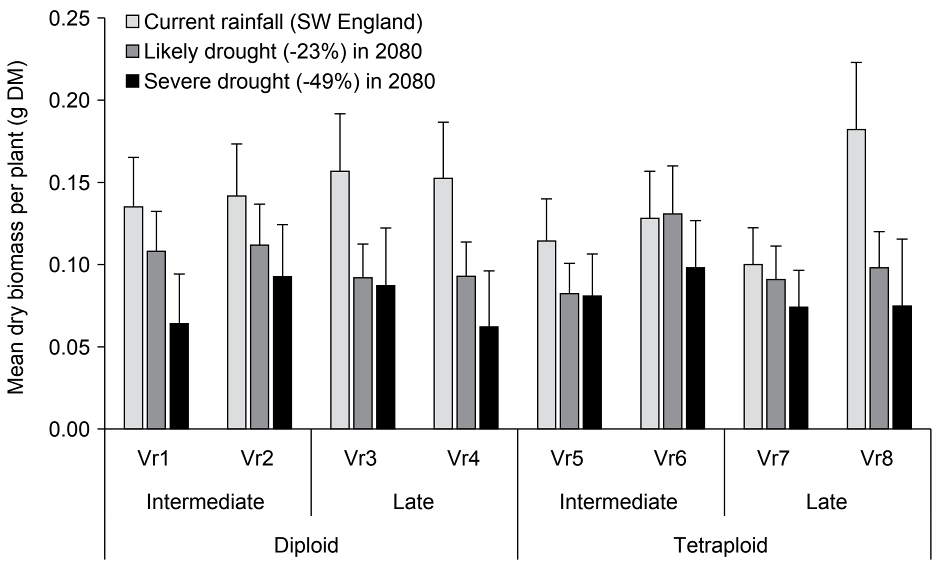

Figure 1. Mean dry biomass produced by each variety (Vr) averaged across all three harvests.

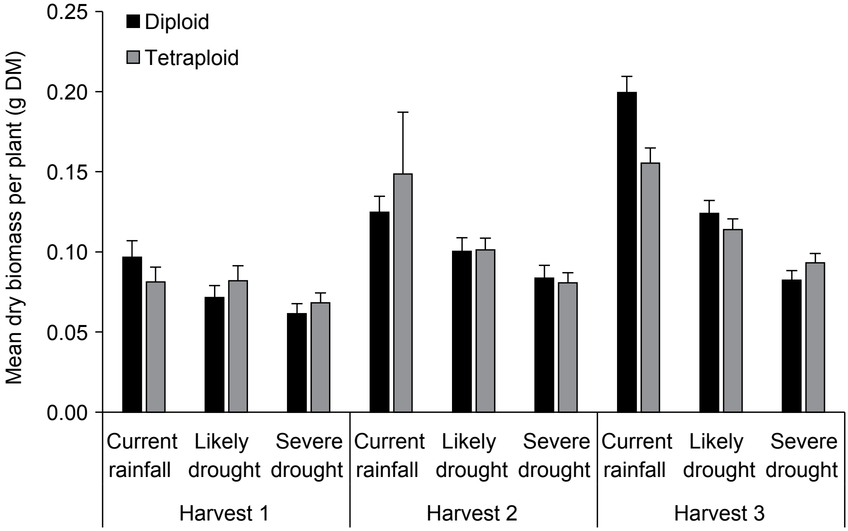

Figure 2. Mean dry biomass produced by diploids and tetraploids for all three harvests.

Table 1. Mean stomatal size (μm) and conductance (mmol m^{-2} s^{-1}) for each variety (Vr).

	Stomatal size		Stomatal conductance	
	Mean	**SE**	**Mean**	**SE**
Vr1	26.52	0.29	46.62	11.61
Vr2	29.59	0.26	41.63	4.85
Vr3	33.29	0.85	36.13	5.04
Vr4	29.86	0.43	36.25	3.02
Vr5	35.67	0.39	48.79	8.81
Vr6	40.77	0.54	41.30	7.52
Vr7	34.20	0.43	38.78	4.74
Vr8	37.60	0.26	46.73	9.29

Conclusions

The substantial variation in drought tolerance between varieties indicates that optimal variety selection will be dependent on the specific water availability. Many regions are likely to experience more frequent summer droughts in the future so regional climatic differences are likely to be important for forage grass productivity. Diploid varieties performed better under the most likely climate change scenario and tetraploids accrued a higher biomass under the severe drought scenario for 2080. Drought tolerance traits, as well as ploidy level *per se*, should therefore be considered in the breeding and selection of future *L. perenne* varieties.

Acknowledgements

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References

DEFRA (2009) Adapting to climate change: UK climate projections 2009.

Hollister J.D. (2015) Polyploidy: adaptation to the genomic environment. *New Phytologist* 205, 1034-1039.

Lee M.A., Davis A.P., Chagunda M.G.G. and Manning P. (2017) Forage quality declines with rising temperatures, with implications for livestock production and methane emissions. *Biogeosciences* 14, 1403-1417.

Resource use efficiency of grasses, legumes and their mixture for green biorefinery supply

Manevski K., Jørgensen U., Chen J. and Lærke P.E.
Department of Agroecology/Centre for Circular Bioeconomy, Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark

Abstract

Perennial grass-based systems aimed at providing biomass supply for future biorefineries are characterized by higher overall resource-use efficiency and an improved environment, relative to annual cereal-based systems. The biomass yield potentials also depend on plant physiology, i.e. how many harvests the crop can withstand and how much biomass and protein are obtained. The current study aims to understand the complex interaction between species, harvest height and frequency, and nitrogen (N) application rate. Field experiments started in 2019 in Denmark to test the effect of harvest frequency (two, four and six weeks) and height of cut (7-9 and 12-14 cm), as well as N fertiliser input (zero, 300 and 500 kg N ha^{-1} yr^{-1}) on biomass and protein yield of grasses (perennial ryegrass and tall fescue), legumes (alfalfa and red clover) and their mixture. The results of the establishment year indicate that red clover and the highly fertilised grasses harvested at 7-9 cm height and at medium frequency produce more biomass than the other tested treatments. Complementary results from a previous study at the same site show high biomass and protein yields and low N losses from highly fertilised grasses and unfertilised grass-legume mixtures harvested 3-4 times annually.

Keywords: harvest frequency, height, nitrogen, re-growth, radiation interception

Introduction

Understanding the productivity and environmental impacts of perennial systems targeting green biorefineries for production of feed protein and other bio-based products are crucial for system optimisation. Results from previous studies that investigated the effects of harvest frequency and height of cut on the biomass yields of grasses and legumes have been contradictory (e.g. review of Gastal and Lemaire, 2015). In the present study, it is hypothesised that aboveground biomass yield, its crude protein content and extractability, for perennial grasses and legumes can be increased by frequent harvests at higher stubble height than conventional harvest management, given sufficient nitrogen (N) supply, due to the harvest of younger leaves that contain more N and the rapid re-growth. A more constant (neither too low nor too high) leaf area achieved at higher stubble height is expected to sustain or even increase biomass and protein production of the perennial grasses and legumes.

Materials and methods

A field experiment started in summer 2019 at Foulumgård experimental station in Denmark (56°30'N, 9°35'E) on a loamy sand, free-draining soil. The climate is temperate and wet and the agricultural systems are mostly rainfed. Perennial systems were established, i.e. sown on 15 May on 1.5×12 m plots in a randomised complete block design with four replicates. The systems comprised fertilised ryegrass and tall fescue, unfertilised legumes alfalfa (lucerne) and red clover, and fertilised mixture of 40% perennial ryegrass, 40% tall fescue, 10% alfalfa, and 10% red clover. The description of the treatments is given in Table 1. Application of phosphorus and potassium, and treatment of pests and diseases were managed according to current recommendations. As the first year was for establishment, the first harvest occurred about three months after establishment and the amount of N fertiliser added was halved. Within each plot a net area of 1.5×10 m was cut with a Haldrup harvester (Haldrup F-55, Germany) for biomass dry

Table 1. The perennial systems and the main factors investigated in the study. As 2019 was the establishment year, harvesting started in early August and total fertiliser amount was halved. Nitrogen (N) fertiliser was applied: a basic dose after sowing and further amounts split according to harvest interval.

	System	Fertiliser (kg N ha^{-1} y^{-1})	Harvest interval (weeks)	Harvest height (cm)
G1	Perennial ryegrass (*Lolium perenne*) var. Betty	300/500	2 or 4 or 6 (corresponding to four, three and two harvests in the establishment year)	7-9 or 12-14
G2	Tall fescue (*Festuca arundinacea*) var. Swaj	300/500		
L1	Alfalfa (*Medicago sativa*) var. SW Nexus	0		
L2	Red clover (*Trifolium pratense*) var. Taifun	0		
L3	Grass-legume mixture	300		

matter determination. Canopy reflectance (visible to near infrared) was also measured throughout the season with a GreenSeeker RT200C sensor mounted on a tractor.

Results and discussion

At the end of the first (establishment) year, the canopy reflectance measurements showed acceptable variation within plot, pointing to a good establishment of each treatment (data not shown). Differences in canopy reflectance could be seen between treatments. Previous studies showed that, despite reduced light interception due to 3-4 harvests during the season, grasses and grass-legume mixtures intercepted significantly more radiation compared to grain and maize crops on an annual basis, ultimately yielding significantly more biomass (Manevski *et al.*, 2017) and protein content (Solati *et al.*, 2018) without severe N losses by leaching (Manevski *et al.*, 2018).

Aboveground biomass (dry matter) yields showed differences in relation to plant and treatment, although the presence of weeds typical in the first harvest of the establishment year probably slightly affected the results. There was overall higher productivity at the lower height at harvesting than at the higher height and, for many of the plants, lower harvesting frequencies (two to three times) resulted in more biomass annually (Figure 1). Red clover and the grass-legume mixture were the most productive systems, although the pure grasses will probably increase their productivity in the years after their establishment

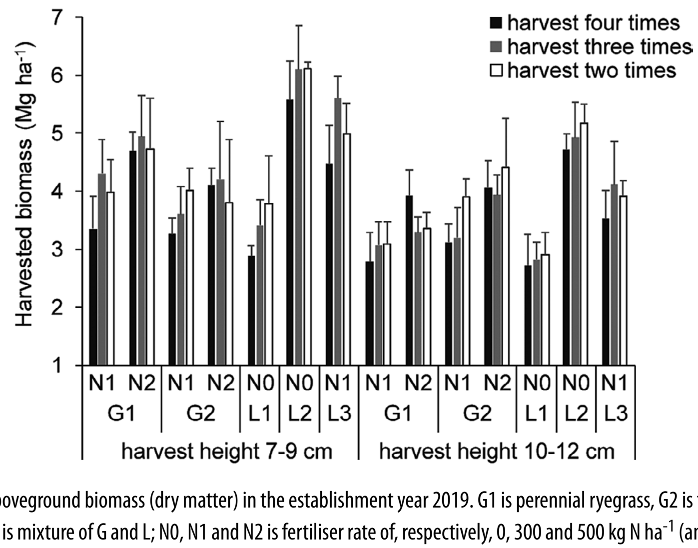

Figure 1. Harvested aboveground biomass (dry matter) in the establishment year 2019. G1 is perennial ryegrass, G2 is tall fescue, L1 is alfalfa, L2 is red clover and L3 is mixture of G and L; N0, N1 and N2 is fertiliser rate of, respectively, 0, 300 and 500 kg N ha^{-1} (amounts were halved for the establishment year). Sowing was in early May and first harvest was in early August. Error bars are standard deviation of the mean (n=4).

(Manevski *et al.*, 2017), whereas other systems such as alfalfa harvested at low height might not sustain their productivity. Hodgkinson (1973) showed that alfalfa harvested frequently at high stubble height (15 cm) produced more biomass compared to less frequent harvests. The improved biomass production may be explained by shaded leaves and low net carbon exchange rates in the dense alfalfa canopy, while more frequent harvests resulted in increased exposure of the canopy to radiation. These results indicate that frequent harvesting may help to keep an optimal leaf area through enabling the lower leaves to maintain their carbon exchange above their light compensation point (respirational losses smaller than photosynthetic gains). The data from the following years of the experiment will clarify the findings in relation to the hypothesis.

Conclusions

The results of the establishment year show a clear treatment response of the perennial systems and the next years will pinpoint which combination of harvest frequency and height and fertiliser amount is suitable for which plant species in relation to production and protein extractability.

Acknowledgements

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References

Gastal F. and Lemaire G. (2015) Defoliation, shoot plasticity, sward structure and herbage utilization in pasture: review of the underlying ecophysiological processes. *Agriculture* 5, 1146-1171.

Hodgkinson K.C. (1973) Establishment and growth of shoots following low and high cutting of lucerne in relation to the pattern of nutrient uptake. *Australian Journal of Agricultural Research* 24, 497-510.

Manevski K., Lærke P.E., Jiao X., Santhome S. and Jørgensen U. (2017) Biomass productivity and radiation utilisation of innovative cropping systems for biorefinery. *Agricultural and Forest Meteorology* 233, 250-264.

Manevski K., Lærke P.E., Olesen J.E. and Jørgensen U. (2018) Nitrogen balances of innovative cropping systems for feedstock production to future biorefineries. *Science of the Total Environment* 633, 372-390.

Solati Z., Manevski K., Jørgensen U., Labouriau R., Shahbazi S. and Lærke P.E. (2018) Crude protein yield and theoretical extractable true protein of potential biorefinery feedstocks. *Industrial Crops and Products* 115, 214-226.

Spring triticale as a raw material of whole-crop silage

Manni K., Lötjönen T. and Huuskonen A.
Natural Resources Institute Finland, 31600 Jokioinen, Finland

Abstract

Spring triticale (*X Triticosecale*) is a new crop in Finland. The objective of this experiment was to compare triticale with barley and wheat as a raw material of whole-crop silage. Yield, nutritive values and fractions of stem, leaves and ears were studied. Four triticale varieties (Bikini, Nagano, Nilex, Somtri), three barley varieties (Armas, Kaarle, Trekker) and one wheat variety (Helmi) were used in a plot experiment conducted in 2018 at Luke Ruukki Research Station. The experimental design was a randomised complete block with four replicates. Crops were harvested at the early dough stage. Feed quality was analysed with NIR from dried samples. Triticale cv. Somtri had the highest dry matter (DM) yield (10622 kg DM^{-1} ha) of all crop varieties. Ear proportions varied from 456 to 672 g kg^{-1} DM in all crop varieties and from 456 to 641 g kg^{-1} DM in triticale varieties. Triticale variety had the highest (Nilex) and the lowest (Bikini) starch concentrations in ear of all tested varieties. Digestibility of triticale varieties did not differ from others. It was concluded that triticale is a potential raw material for whole-crop silage. It can produce high yields and feed digestibility does not differ from the other cereal crops.

Keywords: forage, feed, dry matter yield, triticale, digestibility

Introduction

Although different grasses and red clover are the most commonly used forage plants in Nordic countries, using whole-crop silages in cattle feeding may increase substantially in the future. Small grain cereal-based whole-crop silages provide an opportunity to improve efficiency of forage production for ruminants under Nordic conditions (Rustas, 2009). Whole-crop silages have a potential to lower costs, particularly as a result of high dry matter (DM) yield at a single harvest. Whole-crop silage can be used for both dairy and beef cattle by mixing with grass silage or as a sole forage, depending on the quality of feeds and nutrient requirements of cattle (Huuskonen and Joki-Tokola, 2010; Keady, 2005). The low digestibility of whole-crop silages may limit their use in feeding but, on the other hand, higher DM intake may compensate for this (Keady, 2005; Sinclair *et al.*, 2003). Cereal species and varieties can vary widely in feed quality. In Finland, barley (*Hordeum vulgare*) is the dominant small-grain species utilised for whole-crop production, but oats (*Avena sativa*) and wheat (*Triticum aestivum*) are also used. Spring triticale (*X Triticosecale*) is a relatively new crop in Finland. Interest in its cultivation has recently increased in Nordic countries because of climate change and prolongation of the growing season (Peltonen-Sainio *et al.*, 2009). The objective of this experiment was to compare spring triticale with barley and wheat as a raw material of whole-crop silage. Yield, nutritive values and fractions of stem, leaves and ears were studied. It was hypothesized that triticale varieties can produce higher whole-crop yields than the other crops. It was also hypothesized that triticale varieties do not differ nutritionally from other crops.

Materials and methods

The experiment was conducted in 2018 at Luke Ruukki Research Station (64°44'N, 25°15'E). Soil type was sandy loam. Four spring triticale varieties (Bikini, Nagano, Nilex, Somtri), three spring barley varieties (Armas, Kaarle, Trekker) and one spring wheat variety (Helmi) were used in a plot experiment. Artificial NPK fertiliser was applied during sowing with 90 kg N ha^{-1}, 9 kg P ha^{-1} and 54 kg K ha^{-1}. The experimental design was a randomized complete block with four replicates. The experiment was sown in the beginning of June. Seeding rate was 500 seeds m^{-2} for all varieties, except for Helmi, which had a seeding rate of 650 seeds m^{-2}. Crops were harvested at the early dough stage using a Haldrup-plot harvester. Crude protein content and digestible organic matter in DM (D-Value) were analysed with NIR (FOSS NIRSystems 6500 spectrometer, Denmark) from dried samples. Starch was analysed according to Salo and Salmi (1968). Stem, leaf and ear fractions were analysed on a DM basis. The digestibility of fractions was based on pepsin-cellulase solubility (Nousiainen *et al.*, 2003). The data were subjected to analysis of variance using the SAS GLM procedure (version 9.4, SAS Institute Inc., Cary, NC, USA). The differences were considered statistically significant when $P<0.05$, which was used when presenting the results.

Results and discussion

The DM yield of triticale varieties varied from Bikini at 7,043 kg DM ha^{-1} to Somtri at 10,622 kg DM ha^{-1} (Table 1). Somtri also had higher ($P<0.05$) DM yield compared with barley and wheat varieties, except that barley cv. Trekker did not differ from Somtri. Numerical results for one year and one soil type suggest that triticale varieties except Bikini can produce slightly higher whole-crop silage yields than barley and wheat. Crude protein concentration was rather low in all crop varieties and varied from 85 to 106 g kg^{-1} DM. However, it is typical for whole-crop silages that crude protein concentration is lower than for moderately digestible grass silage (Huuskonen and Joki-Tokola, 2010). Ear proportions varied from 456 (Bikini) to 672 (Armas) g^{-1} kg DM between all crop varieties and from 456 to 641 (Nagano) g^{-1} kg DM in triticale varieties. Bikini had the lowest ($P<0.05$) ear starch concentration 365 g^{-1} kg DM of all crop varieties. There were differences in cellulase solubility of fractions, particularly in stems and ears. Cellulase solubility in stem was highest ($P<0.05$) in Bikini and Trekker, and lowest ($P<0.05$) in Kaarle and Armas. In ears, Kaarle and Armas had the highest cellulase solubility ($P<0.05$) and Bikini the lowest ($P<0.05$). However, triticale varieties did not differ from others in D-value. The only difference in D-values was that Trekker had higher D-value than Kaarle ($P<0.05$). Although all cereals were harvested at the soft dough stage, the differences in starch concentration, cellulase solubility of ear and stem and proportion of ear indicated that triticale Bikini was harvested at too early developmental stage.

Table 1. Dry matter yield, crude protein and digestible organic matter in the dry matter, proportion of ear, starch concentration in ear and pepsin-cellulase solubility in the stem and ear of spring varieties of triticale, barley and wheat.

	Triticale				Barley			Wheat	SEM[1]	*P*-value[2]
Varieties	Bikini	Nagano	Nilex	Somtri	Armas	Kaarle	Trekker	Helmi		
Yield, kg dry matter (DM) ha^{-1}	7,043^{c}	9,483^{ab}	9,240^{ab}	10,622^{a}	7,901^{bc}	7,838^{bc}	9,092^{ab}	7,650^{bc}	431.4	<0.001
In the DM, g kg^{-1}										
Crude protein	100^{ab}	90^{bc}	99^{ab}	85^{c}	89^{bc}	87^{c}	94^{bc}	106^{a}	2.3	<0.001
Digestible organic matter (OM)	613^{ab}	602^{ab}	613^{ab}	592^{ab}	598^{ab}	580^{b}	625^{a}	603^{ab}	7.4	0.011
Proportion of ear, g kg^{-1} DM[3]	456^{e}	641^{abc}	597^{c}	519^{d}	672^{a}	662^{ab}	610^{bc}	623^{abc}	10.3	<0.001
Starch concentration in ear, g kg^{-1} DM	365^{e}	522^{ab}	538^{a}	468^{d}	483^{cd}	505^{bc}	487^{cd}	499^{bc}	4.8	<0.001
Pepsin-cellulase solubility, g kg^{-1} OM										
In the stem	645^{a}	539^{c}	580^{b}	580^{b}	456^{d}	455^{d}	621^{a}	512^{c}	6.7	<0.001
In the ear	915^{d}	947^{b}	949^{b}	936^{c}	968^{a}	974^{a}	953^{b}	950^{b}	2.1	<0.001

[1] Standard error of the mean.

[2] Means in the same row with different superscript letters are significantly different ($P<0.05$).

[3] Reported SEM is calculated for 4 replicates. For cv. Trekker the number of replicates was 3 and so SEM must be multiplied by 1.2.

Conclusions

It was concluded that triticale is a potential raw material for whole-crop silage. It can produce high yields and its feed digestibility does not differ from the other tested crops.

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References

Huuskonen A. and Joki-Tokola E. (2010) Performance of growing dairy bulls offered diets based on silages made of whole-crop barley, whole-crop wheat, hairy vetch and grass. *Agricultural and Food Science* 19, 116-126.

Keady T.W.J. (2005) Ensiled maize and whole crop wheat forages for beef and dairy cattle: effects on animal performance. In: Park R.S. and Stronge M.D. (eds.) *Silage production and utilization*. Proceedings of the XIV International Silage Conference, July 2005, Belfast, N. Ireland. Wageningen, the Netherlands: Wageningen Academic Publishers, pp. 65-82.

Nousiainen J., Rinne M., Hellämäki M. and Huhtanen P. (2003) Prediction of the digestibility of the primary growth of grass silages harvested at different stages of maturity from chemical composition and pepsin-cellulase solubility. *Animal Feed Science and Technology* 103, 97-111.

Peltonen-Sainio P., Jauhiainen L., Hakala K. and Ojanen H. (2009) Climate change and prolongation of growing season: changes in regional potential for field crop production in Finland. *Agricultural and Food Science* 18, 171-190.

Rustas B.-O. (2009) *Whole-crop cereals for growing cattle. Effects of maturity stage and chopping on intake and utilisation.* Doctoral Thesis. Swedish University of Agricultural Sciences, Skara, Sweden.

Salo M-L. and Salmi M. (1968) Determination of starch by the amyloglucosidase method. *Journal of the Scientific Agricultural Society of Finland* 40, 38-45.

Sinclair L.A., Wilkinson R.G. and Ferguson D.M.R. (2003) Effects of crop maturity and cutting height on the nutritive value of fermented whole crop wheat and milk production in dairy cows. *Livestock Production Science* 81, 257-269.

An evaluation of the efficacy of nitrogen fertiliser type and rate on herbage production

Murray Á.[1,2], Gilliland T.J.[2] and McCarthy B.[1]

[1] *Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland;* [2] *Institute of Global Food Security, Queens University Belfast, N. Ireland, United Kingdom*

Abstract

This study compared nitrogen (N) fertiliser types and application rates on herbage output, under rotational grazing with dairy cows. The 3×2 factorial design compared calcium ammonium nitrate (CAN), urea and urea + N-(n-butyl) thiophosphoric triamide (urea + NBPT) at two application rates (150 kg ha^{-1} and 250 kg ha^{-1} yr^{-1}). The study was replicated at two locations (Moorepark and Clonakilty) with 4 replicates of each treatment at each location giving a total of 48 plots. Plots were grazed when the control treatment (CAN-250) reached a pre-grazing herbage mass of 1,500 kg of DM ha^{-1} and fertiliser was applied after each grazing event. Plots were grazed from March to October. Prior to grazing, plot yields were obtained by harvesting 5×1.2 m strips of herbage and samples taken for DM determination. All three fertiliser types gave similar annual and pre-grazing yields and so presented no evidence of differences in efficacy or N losses as ammonia (NH_3) and nitrous oxide (N_2O), even at the higher application rate. While weather conditions may have had a moderating effect, the study indicates an opportunity to use a lower emission risk urea + NBPT product without incurring herbage production losses.

Keywords: CAN, urea, NBPT-urea, grazing, ryegrass, herbage production

Introduction

Agriculture is under increasing pressure to reduce greenhouse gas (GHG) emissions as 14.5% of global anthropogenic emissions are from livestock (Gerber *et al.*, 2013), of which milk contributes 20% (www.fao.org/3/i3437e). Inorganic nitrogen (N) fertiliser is a major contributor through ammonia (NH_3) and nitrous oxide (N_2O) losses (Forrestal *et al.*, 2016a). The EU has set a target to reduce GHG emissions by 40% by 2030 compared to 1990 levels (European Council, 2014). Ammonia emissions from agriculture account for 98% of Ireland's total NH_3 emissions (Forrestal *et al.*, 2016b). In Ireland calcium ammonium nitrate (CAN) accounts for 84% of the straight N market, in contrast to other temperate regions where urea accounts for a greater proportion of the N market. This is due to questions regarding the efficacy of urea use as its susceptibility to N loss via NH_3 volatilization (Forrestal *et al.*, 2017). Therefore, there has been a growing interest in the use of N stabilizers, such as urease inhibitors to reduce NH_3 emissions. Nitrogen stabilizers are compounds that prolong the period of time the N component of the fertiliser remains in the urea form (Watson *et al.*, 2009). Ureases inhibitors (e.g. N-(n-butyl) thiophosphoric triamide) reduce NH_3 volatilization from urea by inhibiting the enzyme urease which catalyses urea hydrolysis (Forrestal *et al.*, 2016a). Forrestal *et al.* (2017) reported that there was no difference in herbage production between CAN, urea and urea + NBPT under a cutting regime and found that urea + NBPT reduced NH_3 losses compared to urea by 79%. Therefore, the objective of this study was to investigate the effect of N fertiliser type (CAN, Urea, Urea + NBPT) and rate (150 and 250 kg N ha^{-1} yr^{-1}) on perennial ryegrass (*Lolium perenne* L.) production under grazing.

Materials and methods

The experiment was undertaken at two sites, Teagasc Moorepark, Cork (52.16° N, 8.24° W) and Clonakilty Agricultural College, Cork (51°63 N, 08°85 E). The design was 3×2 factorial configuration, comparing CAN, urea and urea + NBPT at two rates (150 kg ha^{-1} and 250 kg ha^{-1}) with 4 replicates. The study was conducted during 2019 using 24 plots (8×6 m) at each site. Both sites were maintained under the same management and grazed with lactating dairy cows at the beginning of March, mid-April and then on a three-week cycle thereafter. The aim was to complete ten rounds of grazing during the grazing season, where the CAN-250 treatment was the control. Fertiliser application for the year commenced six weeks prior to first grazing, where the fertiliser was spread by hand. The 150 kg N ha^{-1} treatment received a rate 60% of the 250 kg N ha^{-1} treatment rate at each application. Fertiliser was spread after each grazing event. The next graze date decision was dictated by the pre-grazing herbage mass of the control plot. When the control treatment plot was at pre-grazing herbage mass of 1,500 kg of DM ha^{-1}, all plots within each site were grazed simultaneously. The number of cows allocated to graze the plots was based on the available herbage, with the aim of having a desired post-grazing sward height of 4 cm. Pre-grazing herbage mass was measured prior to grazing by harvesting 1 strip (5×1.2 m) from each plot to a height of 4 cm using an Etesia mower (Etesia UK Ltd., Warwick, UK). The harvested forage was weighed and a 100 g subsample dried at 60 °C for 48 hours to determine dry matter. Pre- and post-grazing sward height was also measured on each plot. Sward density (kg of DM cm^{-1}) was calculated by pre-grazing herbage mass/(pre-cut height – post-cut height). Sward density was used to calculate herbage removed: kg of DM ha^{-1} removed = (pre-grazing sward height – post-grazing sward height) × sward density. Data were analysed using PROC MIXED in SAS (SAS 9.4). Terms included in the model were site, fertiliser type, fertiliser rate, rotation and interactions.

Results and discussion

There were no significant site differences or differences in pre-grazing herbage mass or herbage production between the three fertiliser types, over the growing season (Table 1).

This agrees with Forrestal *et al.* (2016b) who reported no pre-grazing herbage mass differences between CAN and urea + NBPT. Although not significant, there was a progressive decline in herbage production from CAN to urea + NBPT (-539 kg DM ha^{-1}) to urea (-236 kg DM ha^{-1}). This might indicate small increased N losses from CAN to urea + NBPT to urea was occurring, and may merit further study. There were, however, no significant yield differences between the three fertiliser types at any individual rotation and no clear trends that could indicate an underlying effect (Figure 1). Furthermore, as expected, the higher fertiliser rate significantly ($P<0.01$) increased the pre-grazing herbage mass, by delivering a mean of 1.22 kg DM ha^{-1} at each rotation for each additional 1 kg N ha^{-1} yr^{-1} of N applied. Likewise, herbage production provided 9.86 kg DM ha^{-1} yr^{-1} for each additional 1 kg N ha^{-1} yr^{-1} of N applied. As the returns for each kg N applied at 150 kg N ha^{-1} yr^{-1} was 1.0 kg DM per rotation and 9.67 kg DM ha^{-1} yr^{-1} in total production herbage production, the efficiency of N use remained constant for all three fertiliser types when application rates were increased. It is notable, however, that there was no benefit from using urea + NBPT over urea in this one-year study. This was likely a consequence of weather conditions (Rawluk *et al*, 2001) as rainfall averaged 108% above the 10-year average for summer period (June 142%). So, conditions were consistently moister than normal, presenting a lowered risk of volatilization in the unprotected urea.

Table 1. Effect of nitrogen fertiliser type and rate on herbage production.[1,2]

	CAN	Urea + NBPT	Urea	SE	TRT	250 kg N ha^{-1}	150 kg N ha^{-1}	SE	TRT
Pre-grazing yield (kg DM ha^{-1})	1,609	1,543	1,532	441.1	NS	1,622	1,500	33.1	*
Herbage production (kg DM ha^{-1})	15,429	14,890	14,654	319.2	NS	15,484	14,498	269.3	**

[1] All data are means of two sites; N type data are means of two N rates, N rates data are means of three N types.

[2] CAN = calcium ammonium nitrate; Urea + NBPT = Urea + N-(n-butyl) thiophosphoric triamide; SE = standard error; TRT = treatment; NS = not significant; * = $P<0.05$; ** = $P<0.01$.

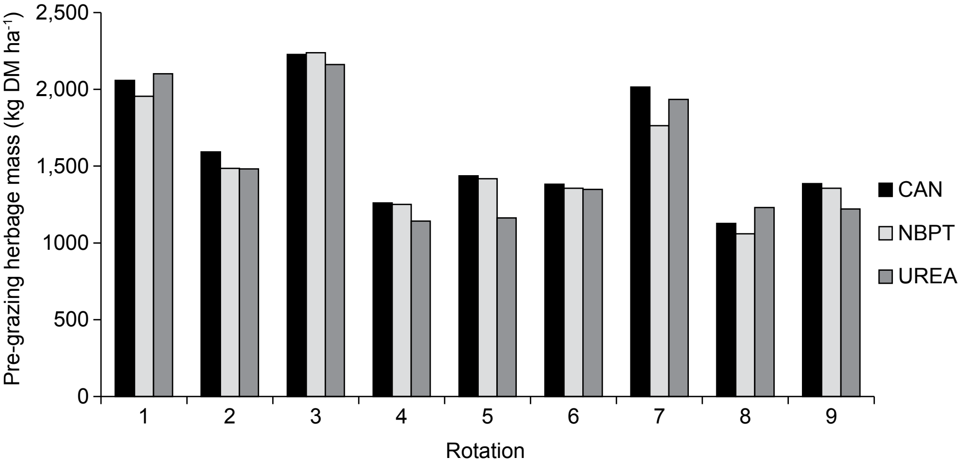

Figure 1. Effect of nitrogen fertiliser type and rate on pre-grazing herbage mass by rotation for the means of both sites (2019).

Conclusions

There was no evidence of differences in efficacy or N loss between the three fertiliser types, as all supported similar herbage production and pre-grazing yields. This consistency between CAN and both urea formulations was maintained even at the higher application rates. While these responses may, to some degree, reflect the 2019 weather, a further two years field work will be carried out with varying weather conditions to fully investigate the potential to reduce N emissions (Forrestal *et al.*, 2016a) without sacrificing herbage yield.

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References

Forrestal P.J., Harty M., Carolan R., Lanigan G., Watson C., Laughlin R., McNeill G., Chambers B. and Richards K.G. (2016a) Ammonia emissions from urea, stabilized urea and calcium ammonium nitrate: insights into loss abatement in temperate grassland. *Soil Use and Management* 32, 92-100.

Forrestal P.J., Wall D., Carloan R., Harty M.A., Roche L., Krol D.J., Watson C.J., Lanigan G. and Richards, K.G. (2016b) Effects of urease and nitrification inhibitors on yields and emissions in grassland and spring barley. *Proceedings 793. International Fertiliser Society. International Fertiliser Society,* pp. 1-23.

Forrestal P.J., Harty M., Carolan R., Lanigan G., Watson C., Laughlin G., Wall D., Hennessy D. and Richards K.G. (2017) Can the agronomic performance of urea equal calcium ammonium nitrate across nitrogen rates in temperate grassland? *Soil Use and Management* 33, 243-251.

Gerber P.J., Hristov A.N., Henderson B., Makkar H., Oh J., Lee C., Meinen R., Montes F., Ott T., Firkins J., Rotz A., Dell C., Adesogan A.T., Yang W.Z., Tricarico J.M., Kebreab E., Waghorn G., Dijkstra J. and Oosting S. (2013) Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: a review. *Animal* 7(2), 220-234.

Rawluk C., Grant C. and Racz G. (2001). Ammonia volatilization from soils fertilized with urea and varying rates of urease inhibitor NBPT. *Canadian Journal of Soil Science*, 81(2), 239-246.

Watson C.J., Laughlin R.J. and McGeough K.L. (2009) Modification of nitrogen fertilisers using inhibitors: opportunities and potentials for improving nitrogen use efficiency. *Proceedings 658. International Fertiliser Society,* pp. 1-40.

Fertilisation effect of recycled nutrients on organic feed barley and grass-clover mixture

Nurmi E.[1], Kurki P.[2] and Kivelä J.[3]
[1]*Natural Resources Institute Finland (Luke), Helsinki, Finland;* [2]*Natural Resources Institute Finland (Luke), Mikkeli, Finland;* [3]*Ecolan Oy, Tampere, Finland*

Abstract

Efficient nutrient recycling is an important means to decrease the use of mineral fertilisers in agriculture. The fertilisation effect of recycled nutrients was studied on organic feed barley and grass-clover in Mikkeli, Finland. The soil was medium fine sand with pH of 6.5 and moderate fertility. Two barley trials were fertilised with meat-bone-meal based Agra8-4-8 and manure-based biogas plant digestate and two grass-clover trials with meat-bone-meal based Agra8-4-2 and blood-meal based Agra13-0-0, Agra13-0-2 with boron and potassium sulphate. The barley yields averaged 2,950, 2,750 and 2,500 kg ha^{-1} with Agra, digestate and non-fertilised, respectively. In 2018, the dry matter (DM) yield of the first cut averaged 6,000 kg ha^{-1} in all plots. In the second cut, N and micronutrient deficiency was recorded. The yield of the second cut was 2,050 kg DM ha^{-1}. In 2019 the average first cut was 6,200 kg DM ha^{-1} and second yields 2,100 kg DM ha^{-1}. Both growing seasons suffered from lack of water during the summer. Nitrogen and minerals of recycled fertilisers especially enhanced DM production and mineral content of grass species in the mixture. The availability of micronutrients was crucial for forage production. S fertilisation enhanced soil S content.

Keywords: nutrient recycling, recycled fertilisers, clover, grass, barley, organic

Introduction

Efficient nutrient recycling is an important means to decrease the use of mineral fertilisers in agriculture. Annually in Finland 21 million tons of biomass (e.g. 17.3 million tons manure and over 2 million tons industrial and communal by-products) are being produced (Marttinen *et al.*, 2018). Meat and bone meal and blood meal-based nutrient-rich fertilisers are by-products of slaughterhouses. These by-products contain essential nutrients which can be processed to meet the requirements of plant nutrition.

Despite being required in very small amounts, micronutrient deficiencies in crops are widespread due to the decreased use of animal manures, intensive cropping practices and naturally low reserves of micronutrients in soils (Fageria *et al.*, 2002). The fertilisation effect of recycled nutrients on organic feed barley and grass-clover yields was studied in 2018-2019 with focus on micronutrients.

Materials and methods

The field experiments took place at Natural Resources Institute in Mikkeli, Finland. The field plot has been in organic production for over 20 years. The experimental design was a randomized block with four replicates. In all trials soil samples were taken before the application of fertilisers in spring and after the harvest in autumn. Plant samples were analysed in the laboratory of Natural Resources Institute in Jokioinen, Finland. Grass and clover samples were fractioned separately in order to estimate their portion in the mixture.

The soil was medium fine sand with a pH of 6.5. Contents of P, K, Ca and Mg were at a sufficient level. The fertilisers in barley trials were meat- and bone-meal-based Agra 8-4-8-3.5 (NPKS) and manure-based digestate from the biogas plant (N 3.2, P 0.51 and K 2.8 kg t^{-1}, respectively). The total nitrogen application rate was 40 kg N ha^{-1}. The pre-crop for barley was grass-clover which was ploughed into the soil in the spring before establishment. In 2018, two feed barley experiments were sown (at 250 kg ha^{-1}) on 31 May and 7 June, respectively. Both trials were harvested after 81 days.

In 2018, the grass-clover trial was carried out with the second harvest year sward. Fertilisers were meat- and bone-meal and blood-meal-based Agra and potassium sulphate (K 42-S 18). Grass-clover was fertilised with 80 kg N ha^{-1} in the spring (Table 1). Potassium sulphate was applied in the spring at 42 kg S ha^{-1} and in the summer 36 kg S ha^{-1} both with and without Agra13.

In 2019, the grass-clover trial was carried out with the third harvest year sward. The location differed from the previous year's trial and the soil was medium fine sand with pH of 6.1. Contents of P and Ca were low, K and Mg at a sufficient level. Half of the N fertilisation was applied before the first cut and the other half before the second cut. Potassium sulphate was applied at the same time, 39 kg S ha^{-1} for each cut.

Results and discussion

In 2018, feed barley yields averaged 2,950, 2,750 and 2,500 kg ha^{-1} with Agra, digestate and non-fertilised, respectively. Agra plots differed from non-fertilised plots (P<0.05). The hectolitre weight of fertilised barley was 63.1 kg and thousand seed weight 36.9 g whereas for non-fertilised barley the values were 62.6 kg and 36.2 g, respectively. The protein content averaged 13.3 and 12.9% with the digestate and the other fertilisers, respectively.

In the first cut on 25 June 2018, all grass-clover plots produced on average 6,000 kg ha^{-1} DM. In the second cut on 13 August, yields were affected by drought, nitrogen and micronutrient (S, Mn, B, Cu, Zn) deficiency. Agra13 produced the best second cut at 2,560 kg DM ha^{-1} of which 1,550 kg was grass. The DM yield of non-fertilised grass was lower (P<0.05) at 1,400 kg ha^{-1} including grass DM of 440 kg ha^{-1}. Agra13 produced 160 kg N ha^{-1} in two harvests while non-fertilised plots produced 120 kg N ha^{-1}. Potassium sulphate raised the soil sulphur from tolerable to good.

In 2019 in the first cut on 1 July, the differences were greater than the year before. The average first cut was 6,200 kg ha^{-1} DM. The lowest yield with non-fertilised plots averaged 5,850 kg ha^{-1} DM and the highest yield with the new micronutrient fertiliser, Agra 13-0-2 with boron 6,670 kg ha^{-1} DM, respectively (P<0.05). The second cut was harvested on 11 September. Yields varied from 1,880 to 2,270 kg ha^{-1} DM in non-fertilised and Agra 13-0-2 with B plots, respectively. In both years, standard deviations were similar for all treatments in the first cut but the second cut showed greater variation due to varying clover content.

In 2019, soil sulphur content increased from sufficient to good (14.4 → 17.7 mg l^{-1} soil) both with Agra 13-0-2 with boron and Agra13-0-0-2 in comparison with the non-fertilised grass-clover, respectively. Similarly, sulphur content increased in plant samples (P<0.05) from 1.37 to 1.59 and from 2.12 to 2.53 g kg^{-1} DM in the first and the second cut, respectively.

Table 1. Applied fertilisers with nutrients in grass-clover experiments.

Year	Applied amount, kg ha^{-1}	Fertiliser	N	P	K	S	B
2018	1000	Ecolan Agra 8-4-2	80	40	20	15	
2018	615	Ecolan Agra 13-0-0	80			12	
2018	435	Ecolan potassium sulphate			183	78	
2019	540	Ecolan Agra 13-0-2 with boron	70		11	17	0.24
2019	540	Ecolan Agra 13-0-0	70			12	
2019	435	Ecolan potassium sulphate			183	78	

Agra 13-0-2 with boron fertilisation increased both soil and grass boron content. In comparison with non-fertilised plots, boron content in grass increased from 0.0 to 7.5 and from 4.8 to 10.0 mg kg^{-1} DM in the first and the second cut, respectively. Boron content in clover varied from 27 to 32 mg kg^{-1} DM for all treatments.

Conclusions

Meat- and bone-meal-based fertiliser produced highest barley yields but there were no significant differences in the crop quality between fertilised and non-fertilised plots. Medium fine sand produced good first grass-clover yields despite the early summer drought in 2018 but water scarcity and nutrient deficiencies weakened the second crop. Both growing seasons suffered from lack of water especially during July though overall rainfall was higher in 2019. The clover grew well without additional nitrogen but in the mixture the growth of grass was enhanced by nitrogen fertilisers. The condition of the soil and the availability of micronutrients were crucial for crop production. The fertilisation affected the mineral content of the grass. Application of potassium sulphate improved soil S content.

Acknowledgements

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References

Fageria N.K., Baligar V.C. and Clark R.B. (2002) Micronutrients in crop production. *Advances in Agronomy* 77, 185-268.

Marttinen S. *et al.* (2018) *Towards a breakthrough in nutrient recycling: State-of-the-art and recommendations for developing policy instruments in Finland*. Natural resources and bioeconomy studies 26/2018. Natural Resources Institute Finland. Helsinki, Finland.

Potential new forage legumes for Northern Sweden

Parsons D.
Swedish University of Agricultural Sciences, Agricultural Research for Northern Sweden, Skogmarksgrand, Umeå 90596, Sweden

Abstract

The perennial legume species that are commonly included in leys and pastures in Northern Sweden typically have unreliable persistence, due to disease sensitivity. The objective of the research was to identify new or under-used legume species that could contribute to developing more persistent leys. Thirty accessions were collected locally, sourced from gene banks, or purchased. Accessions were propagated in a glasshouse and individual plants transplanted onto weed matting at Umeå, in summer 2018. In 2019 the plants were assessed for winter survival, plant vigour, and nutritive value. Early results suggest that species of *Medicago* and *Lotus* are promising in terms of both persistence and production.

Keywords: forage quality, legume, persistence, plant vigour

Introduction

In Sweden, red clover (*Trifolium pratense* L.) remains the most commonly sown forage legume, however its sensitivity to soil-borne diseases such as *Fusarium* root rot result in poor persistence after the second year (Wallenhammar *et al.*, 2008). There are other legumes which have been tried in the past but not commonly grown today. For example, Alsike clover (*Trifolium hybridum* L.) has a lower potential yield than red clover, and was previously more widely used in short term leys (3-4 years) (Peeters *et al.*, 2006). The objective of the research was to identify new or under-used legume species that could contribute to developing more persistent leys.

Materials and methods

We selected legume species based on their appearance in Northern Sweden as native or naturalized species, or their presence in countries with a similar climate. Thirty accessions were collected locally, sourced from gene banks, or purchased (in the case of established cultivars). Genera included *Galega* (G), *Lotus* (L), *Lupinus* (Lu), *Medicago* (M), *Trifolium* (T), and *Vicia* (V). Due to limited availability of seed, accessions were propagated in a glasshouse in spring 2018 and three blocks of ten individual plants were transplanted onto weed matting at Röbäcksdalen field research station, Umeå (63.806°N, 20.240°E), in June 2018. In 2019 the plants were assessed for winter survival, plant vigour, and nutritive value. Winter survival was assessed by counting the number of surviving plants per accession in each block. Plant vigour was assessed twice (11 June and 23 August 2019; only the second is presented in this paper) by assigning a value of 9 to the most vigorous accession and assigning a value of 1-9 to all other accessions in relation to the most vigorous plants. Samples for forage quality analysis were collected twice (11 June and 23 August 2019) by harvesting at least 3 plants per experimental unit to a stubble height of 5 cm. Samples were dried at 60 °C until they reached a constant weight and ground to pass through a 1-mm sieve. *In vitro* true digestibility (IVTD48) was determined according to ANKOM procedures described by Valentine *et al.* (2018) using the Daisy II 200/220 incubator (ANKOM Technology, Fairport, NY). Samples were incubated in F57 ANKOM digestion bags for 48 h at 39 °C. Digestibility values for two harvests and only one block are presented. Other forage quality data including crude protein, ash, acid detergent fibre, neutral detergent fibre, and neutral detergent fibre digestibility are not presented here. Results were compared with the control cultivar, SW Torun (*T. pratense* L), the most widely cultivated forage legume.

Results and discussion

The accessions were quite different in winter survival (Figure 1). Many of the accessions had better winter survival than the control cultivar (*T. pratense* cv. SW Torun). The alsike clovers (*T. hybridum* L.) generally had high winter survival, as did the tufted vetch (*V. cracca* L.) accessions. Of the *Medicago* accessions, *M. varia* cv. Karlu had high winter survival. Also, of note is the much higher survival of cv. Bull than other birdsfoot trefoil (*L. corniculatus* L.) cultivars.

There were clear differences in plant vigour between accessions (Figure 2). Although the vetch accessions had high winter survival they had very low plant vigour. The alsike clover accessions were similar in

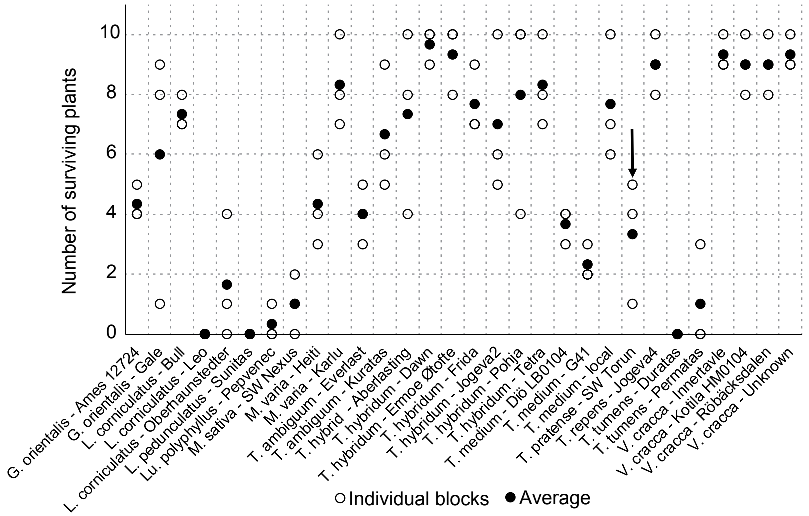

Figure 1. Winter survival, assessed on 11 June 2019. For each accession the individual block values and average are plotted. The arrow denotes the control cultivar – *T. pratense*, SW Torun. B = block. Total number of plants = 10 per block.

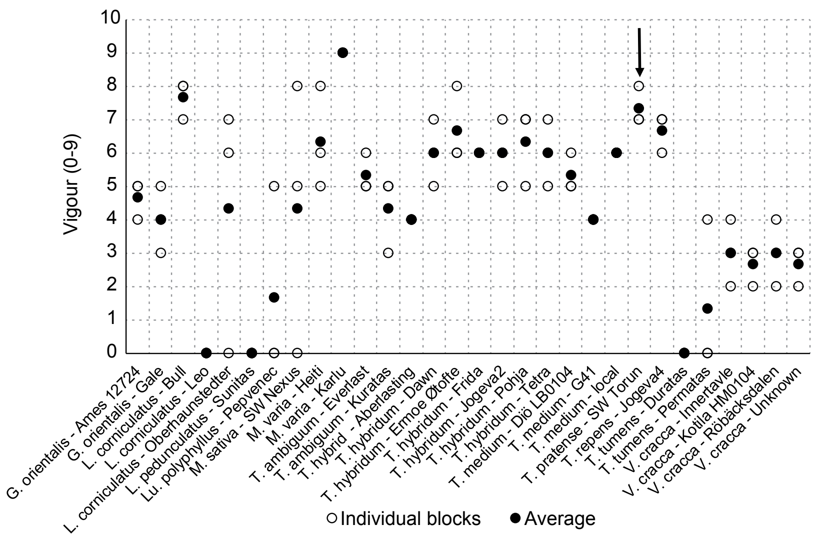

Figure 2. Plant vigour (1-9) assessed 23 August 2019. A score of zero means that no plants were alive. For each accession the individual block values and average are plotted. The arrow denotes the control cultivar – *T. pratense*, SW Torun. B=block. Total number of plants = 10 per block.

performance to each other, with lower average vigour than the control cultivar. Only *M. varia* cv. Karlu and *L. corniculatus* L. cv. Bull, had greater plant vigour than the control cultivar.

Most measured IVTD values ranged from 800 g kg^{-1} to 950 g kg^{-1} on a dry matter basis (Figure 3). The most vigorous accession (*M. varia* cv. Karlu) had similar IVTD to *T. pratense* cv. Torun for harvest 1, but slightly lower IVTD for harvest 2.

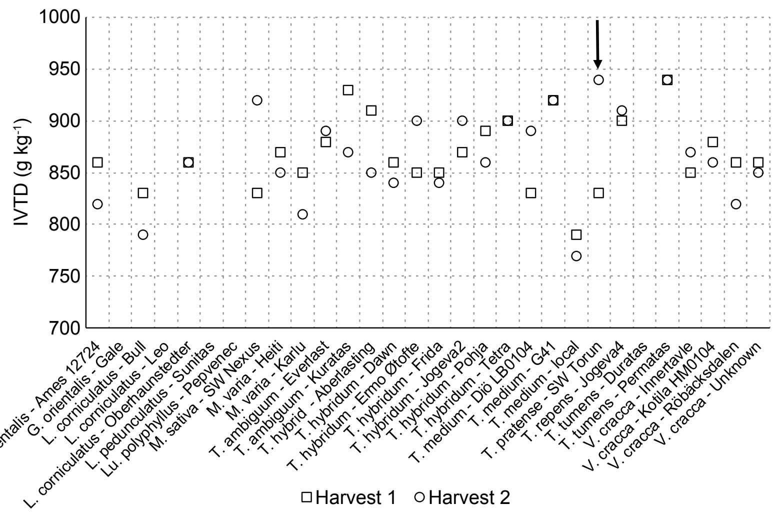

Figure 3. *In vitro* true digestibility determined from harvest 1 (26 June 2019), and harvest 2 (23 August 2019). The arrow denotes the control cultivar – *T. pratense*, SW Torun. B = block. Total number of plants = 10 per block.

Conclusions

The initial assessment of 30 forage legume accessions in Northern Sweden suggests that there are several promising established cultivars, including *M. varia* cv. Karlu and *L. corniculatus* L. cv. Bull, which combine good winter survival and vigour with a level of digestibility comparable to that of red clover. The accessions will be further assessed in coming years.

Acknowledgements

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References

Peeters A., Parente G. and Le Gall A. (2006) Temperate legumes: key-species for sustainable temperate mixtures. *Grassland Science in Europe* 11, 205-221.

Valentine M.E., Karavilanli E., Cherney J.H. and Cherney D.J. (2018) Comparison of *in vitro* long digestion methods and digestion rates for diverse forages. *Crop Science* 58, 1-14.

Wallenhammar A.-C., Nilsdotter-Linde N., Jansson J., Stoltz E. and L-Baeckström G. (2008) Influence of root rot on the sustainability of grass/legume leys in Sweden. *Grassland Science in Europe* 13, 341-343.

Impact of fertiliser nitrogen type and harvest timing on grass yield and quality for silage

Patterson J.D.[1], Allen M.[1] and Gilliland T.J.[2]

[1]Agri-Food and Biosciences Institute (AFBI), Hillsborough, BT26 6DR, Northern Ireland, United Kingdom; [2]Institute of Global Food Security, Queens University Belfast, BT7 1NN, Northern Ireland, United Kingdom

Abstract

By seeking to improve grass silage quality by cutting earlier, excessive nitrogen (N) levels at harvest can cause adverse conditions for ensiling. Modified N fertilisers (e.g. nBTPT-amended urea) can reduce NH_4 losses from surface-applied urea and increase yield and N uptake compared with unamended urea. This study compared fertiliser N type and cut timing for herbage composition and yield in a three-cut silage season. In 2018 and 2019, 3 fertilisers (calcium ammonium nitrate (CAN), stabilised urea (SU) and no fertiliser (Nzero)) were compared at 6 harvest timings (2, 3, 4, 5, 6, 7 weeks post application of 120, 100 and 100 kg N ha^{-1} for 1st, 2nd, 3rd cut silage). Herbage yield and quality were assessed at every harvest, while herbage harvested after seven weeks regrowth was ensiled. Compared with CAN, SU produced similar DM yields at all harvests (5,189.6; 5,395.8 kg DM ha^{-1}, P>0.05) with similar crude protein (17.98; 17.92 g kg^{-1} DM, P>0.05), but significantly lower nitrate concentration at the 3rd silage cut, at regrowth week 7 (1,836; 1,104.3 mg kg^{-1} DM, P<0.001).

Keywords: fertiliser N type, herbage yield, quality, nitrate levels, silage harvests

Introduction

The procedures for successful silage making are well understood but not all the risks of poor ensiling are easily controlled, such as when excessive nitrate levels remain in crops cut at a physiologically young stage in high-quality, multi-cut systems. Moreover, since 91% of all NH_4 emissions are agricultural (DAERA, 2017), modified N fertilisers, such as nBTPT-amended urea can reduce NH_4 losses from surface-applied urea (Watson *et al.*, 2008) and increase yield and N uptake compared with unamended urea (Watson *et al.*, 2009). As grass should be ensiled with an N content below 1000 mg N kg^{-1} DM (J Archer, AFBI, pers. comm.), greater uptake could cause higher N levels at ensiling. In this study nitrogen was applied as calcium ammonium nitrate (CAN) or stabilised urea (SU) in 3 silage cuts over 2 years, to measure the impact of N type and seasonality on the profile and efficiency of N uptake and dilution, in perennial ryegrass (*Lolium perenne* L.).

Materials and methods

The study lasted for 21 weeks each year at AFBI Hillsborough, (54°27' N, 6°04' W). It comprised 4 replicates of 18 treatments in a 3×6 factorial design, of 3 N regimes (CAN, SU and no fertiliser (Nzero)) and 6 destructive cut dates (2, 3, 4, 5, 6, 7 weeks post-fertiliser application (WEEK)). SU was a KαN product (38N:0P:0K:17.5S) with urease inhibitor nBTPT plus sulphur. CAN (calcium ammonium nitrate) was SulfaCAN (26.6N:0P:0K:12.5S). These were applied at 120, 100 and 100 kg N ha^{-1} for 1st, 2nd, 3rd 'Silage Cuts', respectively, with soil indices of 2 for P and K. On 10 April 2018, all 216 plots were fertilised for '1st Cut Silage'. 12 plots were cut each 'WEEK' (4 reps × 3 N types × 6 weeks) and at week 7 all remaining 144 plots were cut off (not recorded) and received '2nd Cut Silage' fertiliser with 12 plots cut in each of the following 2-7 weeks (by weeks 9-14). The remaining 72 plots were cut off, given '3rd Cut Silage' N and cut in the next 2-7 weeks (by weeks 16-21). Fertiliser was applied on 26 March 2019 and the above repeated using a new set of plots. Herbage was cut to 4 cm using an Agria mower for DM yield and analysed by NIRS (Near Infrared Reflectance Spectroscopy, as by Park *et al.*,

1998) for dry matter (DM) content; crude protein (CP); acid detergent fibre (ADF); ash; water soluble carbohydrates (WSC); dry matter digestibility (DMD); digestible organic in the dry matter (D-value); and metabolizable energy (ME). Nitrate concentration was chemically analysed using the Metrohm system (J Archer, pers. comm). The 'Week No 7' herbage was chopped and placed in mini pipe silos for 100 days prior to quality analysis. Analysis of Variance (VSNi, 2017) was used to assess the fixed effects of week, cut, fertiliser treatment and their interactions, blocking on year and the replicated experiment within year and adjusting for the covariate total rainfall.

Results and discussion

At week 7 of the 1st, 2nd and 3rd 'Silage Cuts', the yields of Nzero swards were ~54 and 56.2% of the SU and CAN-fertilised swards, respectively, and due to less developed growth had a significantly higher ME but lower CP. CAN and SU produced similar yield increases when compared in each sequential 2-7 weekly harvest and Table 1 summarises the overall responses. The average fertiliser yield responses of 21.3 and 23.2 kg DM kgN^{-1} for CAN and SU, respectively, were similar to previous findings (e.g. Frame *et al.*, 1989), and ensilability measures (DM, WSC, CP, ME) were unaffected by fertiliser type. This was further corroborated by chemical analysis of silages made from Week No 7. For example, CP levels in CAN and SU silages (172.3; 173.6 g kg^{-1} DM, respectively) were not significantly different. Figure 1A shows that nitrate levels in herbage fell steadily in the 7-week growth periods, but CAN and SU herbages were always higher ($P<0.01$) in nitrate than Nzero, which averaged around 150.1 mg N kg^{-1} DM.

Nitrate levels differed significantly between 1st, 2nd and 3rd Silage Cuts (523.8; 1,110.4 and 1,562.4 mg kg^{-1} DM, respectively, $P<0.001$). Figure 1B shows that the fast-growing 1st Silage Cut was consistently below the 1000 mg N kg^{-1} DM threshold, that the 2nd Silage Cut only achieved this after 5-7 weeks growth and all 3rd Silage Cuts exceeded this threshold, though not significantly by week 7. Also, there was a significant week × cut × fertiliser interaction ($P<0.01$) where 3rd Silage Cut nitrate levels dropped lower with SU than CAN (significant by week 7: 1,836 vs 1,104.3 mg kg^{-1} DM, $P<0.001$). These main-cut trends were similar to that reported by Binnie *et al.* (1997) and the interaction suggests that using stabilised urea for late season silage may help to lower nitrate concentrations (Figure 1C), at least with low soil mineralization, as shown by the Nzero control. For the other quality parameters, DM content was similar in 1st and 3rd Silage Cuts (173.2; 177.8 g kg^{-1}, respectively) as was water soluble carbohydrate (WSC) (27.1; 27.0 g kg^{-1} DM, respectively). Ash content was significantly higher ($P<0.001$) in the 3rd than 1st Silage Cut (110.0; 86.2 g kg^{-1} DM, respectively) as was CP (165.0; 124.7 g kg^{-1} DM, respectively). Fibre levels (ADF) were significantly higher ($P<0.001$) in 1st than in 2nd and 3rd Silage Cuts, (337.5; 303.0; 272.3 g kg^{-1} DM, respectively) but D-value was similar for both 1st and 3rd Silage Cuts (72.9; 73.8

Table 1. Effect of fertiliser N type on herbage yield (kg DM ha^{-1}), mean ME and mean CP content in sequential silage harvests (data are means of 1st, 2nd, 3rd cuts in 2018 plus 2019).[1,2]

Timeline	CAN yield		SU yield		Nzero yield	
Week No 2	1,020.5	ab	1,011.7	ab	784.5	a
Week No 3	1,687.6	c	1,711.6	c	1,028.3	ab
Week No 4	2,628.4	d	2,620.0	d	1,561.0	bc
Week No 5	3,605.4	f	3,472.4	ef	1,896.5	c
Week No 6	4,543.7	g	4,368.9	g	2,701.5	d
Week No 7	5,189.6	h	5,395.8	h	2,916.4	de
Mean ME[1] (MJ kg^{-1} DM)	10.99	a	10.99	a	11.13	b
Mean CP[1] (g kg^{-1} DM)	17.98	a	17.92	a	11.96	b

[1] Analysed for all three cuts and all weeks. Different letters denote least significant difference $P<0.05$.

[2] CAN = calcium ammonium nitrate; SU = stabilised urea; Nzero = no fertiliser; ME = metabolizable energy; CP = crude protein; DM = dry matter.

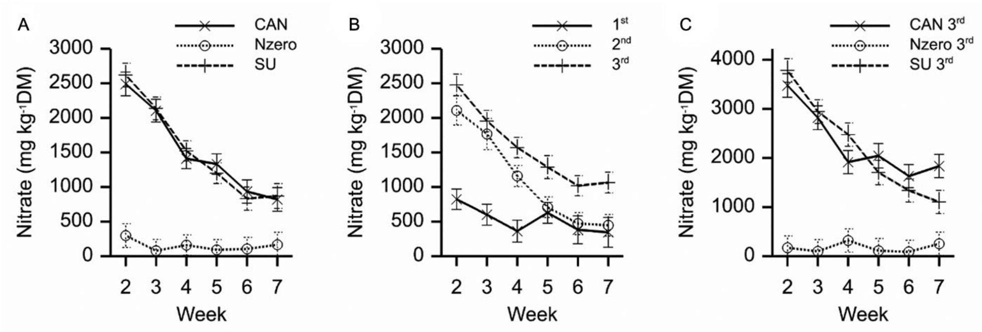

Figure 1. Weekly herbage nitrate concentrations (mean of 2018 and 2019) as affected by: fertiliser type, for all cuts (A); silage cut (B); and fertiliser type at 3rd cut (C).

D-value, respectively). Higher protein and ash with lower fibre are typical characteristics of late season silage and combined with high nitrate resulted in poorer quality herbage for ensiling at the 3rd Silage Cut.

Conclusions

Stabilized urea produced similar yields to CAN, with the same amounts of P, K and S applied. This confirms that comparable production levels and potentially reduced volatile N losses, are achievable, similar to Harty *et al.* (2017). Although the risk of poor quality silage is higher at the 3rd cut, the use of SU in late season may result in lower herbage N concentrations, compared with CAN, particularly where soil mineralisation is low. SU may, therefore, be the better N fertiliser choice when making later cut silages, though the magnitude of the benefit is such, that care in timing of harvesting and ensiling practices will remain vitally important. The yield response of unamended urea has always been more variable than with CAN (Frame and Laidlaw, 2014), but these findings, with expected economic benefits, will provide assurance when considering the use of stabilised urea fertiliser in multi-cut silage production.

Acknowledgements

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References

Binnie R.C., Kilpatrick D.J., and Chestnut D.M.B. (1997) Effect of altering length of regrowth interval in early, mid, late season on productivity of grass swards. *Journal of Agricultural Science*, *Cambridge* 128, 303-309.

DAERA (2017) *Making Ammonia Visible*. Produced by the Expert Working Group on Sustainable Agricultural Land Management for N. Ireland, 101 pp. https://www.daerani.gov.uk/sites/default/files/publications/daera/AmmoniaAnnex-ExpertWorkingGroupfinal.pdf

Frame J., Harkess, R.D. and Talbot, M (1989) The effect of cutting frequency and fertiliser nitrogen rate on herbage productivity from perennial ryegrass. *Research and Development in Agriculture* 6, 99-105.

Frame J. and Laidlaw A.S. (2014) *Improved Grassland Management*, New edition: Crowood Press Ltd., 353 pp.

Harty M.A., Forrestal P.J., Carolan R., Watson C.J., Hennessy D., Lanigan G.J., Wall D.P. and Richards K.G. (2017) Temperate grassland yields and nitrogen uptake are influenced by fertilizer nitrogen source. *Agronomy Journal* 109, 1-9.

Park, R.S., Agnew, R.E., Gordon, F.J. and Steen, R.W.J. (1998). The use of near infrared reflectance spectroscopy (NIRS) on undried samples of grass silage to predict chemical composition and digestibility parameters. *Animal Feed Science and Techology* 72(1-2), 155-167.

VSN International (2017) Genstat for Windows 19th edition. VSN International, Hemel Hempstead, UK.

Watson C.J., Akhonzada N.A., Hamilton J.T.G. and Matthews D.I. (2008) Rate and mode of application of the urease inhibitor N-(n-butyl) thiophosphoric triamide on ammonia volatilization from surface-applied urea. *Soil Use Management* 24, 246-253.

Watson C.J., Laughlin R.J. and McGeough K.L. (2009) Modification of nitrogen fertilisers using inhibitors: opportunities and potentials for improving nitrogen use efficiency. *Proceedings 658, The International Fertiliser Society*, 40 pp.

Effect of growing degree days and soil moisture on forage quality

Peratoner G.[1], Niedrist G.[2], Figl U.[1], Della Chiesa S.[2], Vitalone L.[1] and Matteazzi A.[1]
[1]*Laimburg Research Centre, Vadena/Pfatten, 39040 Ora/Auer (BZ), Italy;* [2]*EURAC research, Drususallee/Viale Druso 1, 39100 Bozen/Bolzano, Italy*

Abstract

Forage quality is strongly affected by the phenological development of grassland species. This can be described by means of growing degree days (GDD), which are usually computed using air temperature at 2 m above ground. The effects of soil water availability on forage quality are less clear. In a three-year study at two experimental sites on permanent meadows (South Tyrol, NE Italy), forage quality was estimated by means of sequential samplings for 7 weeks starting with stem elongation. Meteorological data, including air temperature at 2 m above ground, as well as soil temperature and soil moisture at 5 cm depth, were recorded daily at each experimental site. GDD between one week before stem elongation and harvest, based on air and soil temperatures, as well as several indices for soil water availability, were computed. All variables were tested for inclusion in stepwise-forward built statistical predictive models to estimate forage quality. There were very little differences in accuracy by using air or soil temperatures to compute GDD. The indices describing soil water availability affected only crude protein and Net Energy for Lactation and resulted in a minor improvement of the prediction accuracy. In comparison to the progress of the phenological development, the effect of water availability is of minor relevance.

Keywords: permanent meadows, potential forage quality, growing degree days, soil water availability

Introduction

The phenological development of grassland species is known to greatly affect their forage quality (Peratoner *et al.*, 2016). These changes can be described by means of growing degree days (GDD), which are often computed using measurements of air temperature 2 m above ground. Less clear are the effects of soil water availability on forage quality. This paper explores the suitability of using GDD based on soil temperatures instead of air temperatures and investigates the effect of water availability on crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and net energy for lactation (NEL).

Materials and methods

The experiment took place in South Tyrol (NE Italy) at two experimental sites (S1 and S2) on mountain permanent meadows (S1: 46°24'04' N; 11°27'15' E; 1420 m a.s.l., SE exposed, 4% slope; S2: 46°23'49' N; 11°27'15' E; 1493 m a.s.l., S exposed, 32% slope) from 2015 to 2017. Within an area of 50 m^2, the meadows were sequentially sampled at a cutting height of 5 cm in four replicates, using a metal frame of 0.5×0.5 m and electric scissors. Sampling started at the achievement of the stem elongation stage (15 cm sward height) and ended six weeks later. Data are missing for the 1st sampling date of S1 and S2 in 2015, the 2nd of S2 in 2015 and the 6th and 7th of S1 in 2017. The botanical composition of each sample was visually classified into one of four classes (rich in grasses/balanced/rich in forbs/rich in legumes) according to Peratoner *et al.* (2016). The forage samples were dried at 60 °C until reaching a stable weight. CP, NDF and ADF were estimated by means of near infrared spectrometry (NIRS; NIRSystem 5000, FOSS, USA) according to Romano *et al.* (2016). NEL was estimated according to RAP (1999). Air temperatures at 2 m above ground were obtained from a nearby weather station (46°25'14' N; 11°25'37' E; 1470 m a.s.l., NE exposed, 14% slope). Soil temperature and soil water content (SWC) (CS655, Campbell Scientific) at 5 cm soil depth were recorded daily by a meteorological station at each experimental site. Two variables describing soil water availability were estimated: soil water deficit index (SWDI) according to Martínez-Fernández *et al.* (2015) and soil water potential (SWP) derived from

SWC time series adopting pedotransfer functions according to van Genuchten (1980). Reference values for field capacity and wilting point (0.15 and 0.065 m^3 m^{-3} for SWC respectively and -30 and -1,500 kPa for SWP) were estimated, accounting for soil texture according to Allen *et al.* (1998) and to Martínez-Fernández *et al.* (2015). To compute all indices using SWP, the natural logarithm of the absolute values (pF) was used. GDD between one week before stem elongation and harvest, as well as the mean value of SWDI and SWP within the same period were computed. In a second set of indices ($SWDI_{thr}$ and SWP_{thr}), the values between field capacity and wilting point were used as they were, whilst those beyond field capacity and wilting point were set equal to field capacity and wilting point respectively. A further index (DNSWP) was computed as the ratio between the number of days in which SWP was higher than the reference value for field capacity and the number of observation days. Statistical predictive models were stepwise-forward developed by means of mixed models, starting from a baseline model accounting for (1) site, year and their interaction as random terms, (2) the serial correlation of the measurement taken at the same sampling event (site × year × sampling week) over the observation period and (3) the plant stand type. In a first step, the effect of GDD based on air or soil temperatures (GDD_{air} and GDD_{soil} respectively), using a base temperature of 0 °C for CP, NDF and NEL, and of 4.5 °C for ADF. Only the best performing variable was included, due to collinearity effects. In further steps, the inclusion of the indices for water availability and the interaction with GDD was evaluated. The improvement of the prediction accuracy over all steps was investigated by means of three-fold cross-validation according to Romano *et al.* (2016). If necessary, data transformation was applied to achieve homoscedasticity and normality of the residuals.

Results and discussion

The inclusion of GDD in step 1 of each model resulted in a strong improvement of the prediction accuracy for CP, ADF and NEL, whereas the improvement was relatively small for NDF (Figure 1A). For CP, NDF and ADF, GDD_{air} was found to provide a slight accuracy improvement in comparison with GDD_{soil}, whilst the opposite was true for NEL. However, differences in accuracy (R^2) between models including either GDD_{air} or GDD_{soil} for the same quality parameter ranged from low (0.039 for NDF and 0.015 for NEL) to almost negligible values (0.009 for CP and ADF). Hence, no relevant improvement can be expected by using GDD based on soil temperatures. Only CP and NEL were affected by soil water availability (Figure 1A and 1B). The inclusion of DSNWP and its interaction with GDD_{air} for CP and that of SWC for NEL caused only a slight accuracy increase of the model (R^2 difference: 0.043 and 0.022 respectively). This confirms that the forage quality is less affected by soil water availability than the forage

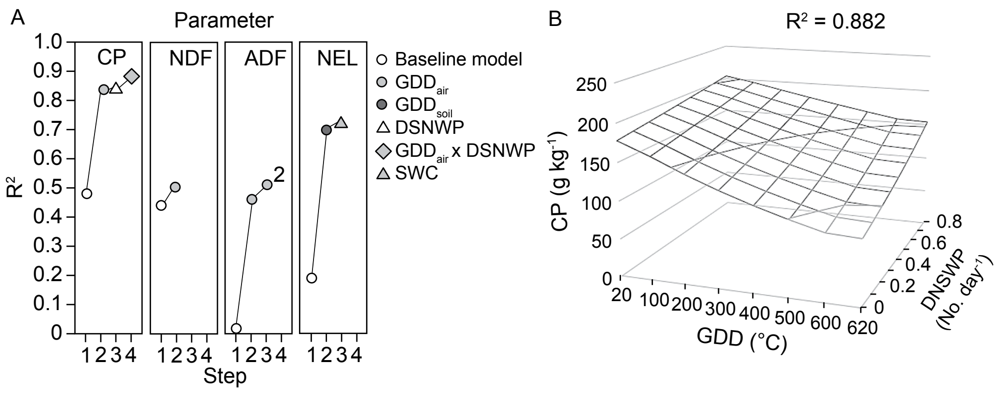

Figure 1. (A) Changes of the prediction accuracy by stepwise forward model selection for crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and net energy for lactation (NEL). Analysis of CP after logarithmic transformation (base 10). [2] indicates the quadratic term of the polynomial. (B) Effect of GDD_{air} with base temperature 0 °C and DNSWP on CP. Back-transformed predicted values are shown.

yield, and that decreasing water availability results in increasing nitrogen concentration (Grant *et al.* 2014). The apparent decrease of CP by advancing phenology was more pronounced at higher soil water availability than under conditions of water limitation. This may be related to a shift of the leaf-to-stem-ratio towards higher values under drought conditions because of a reduced production of stems under dry conditions (Dumont *et al.* 2015). The models for CP including other water availability indices instead of DSNWP (SDWI and SWP) had nearly negligible losses of accuracy in comparison with the model with DSNWP (R^2 difference: -0.004 and -0.011, respectively). This is explained by the strong correlation of SDWI and SWP with DSNWP ($R_{Pearson}$=-0.917, P<0.001 and $R_{Pearson}$=0.984, P<0.001 respectively). $SWDI_{thr}$ and SWP_{thr} performed worse than their counterparts (a R^2 difference of 0.090 in comparison with SDWI, and of 0.026 in comparison with SWP; only SWP_{thr} was significant).

Conclusions

Soil temperatures at 5 cm depth were found to be similarly suited to air temperatures at 2 m above ground for computing GDD to predict forage quality. In comparison with the effect of GDD, water availability was found to play a minor role on forage quality, for CP only, and did not affect NDF, ADF and NEL. The different methods to describe water availability did result in minor differences of prediction accuracy.

References

Allen G., Pereira L.S., Raes D. and Smith M. (1998) *Crop evapotranspiration: guidelines for computing crop water requirements*. FAO Irrigation and Drainage Paper, 56. FAO, Rome, I.

Dumont B., Andueza D., Niderkorn V., Lüscher A., Porqueddu C. and Picon-Cochard C. (2015) A meta-analysis of climate change effects on forage quality in grasslands: specificities of mountain and Mediterranean areas. *Grass and Forage Science* 70, 239-254.

Grant K., Kreyling J., Dienstbach L.F.H., Beierkuhnlein C. and Jentsch A. (2014) Water stress due to increased intra-annual precipitation variability reduced forage yield but raised forage quality of a temperate grassland. *Agriculture, Ecosystems and Environment* 186, 11-22.

Martinez-Fernandez J., González-Zamora A., Sánchez N. and Gumuzzio A. (2015) A soil water based index as a suitable agricultural drought indicator. *Journal of Hydrology* 522 (Supplement C), 265-273.

Peratoner G., Romano G., Piepho H.-P., Bodner A., Schaumberger A., Resch R. and Pötsch E.M. (2016) Suitability of different methods to describe the botanical composition for predicting forage quality of permanent meadows at the first cut. *Grassland Science in Europe* 21, 311-313.

RAP (1999) Fütterungsempfehlungen und Nährwerttabellen für Wiederkäuer. Landwirtschaftliche Lehrmittelzentrale, Zollikofen, CH, 327 pp.

Romano G., Piepho H.-P., Schaumberger A., Bodner A. and Peratoner G. (2016) Statistical models to estimate the potential forage quality of permanent meadows at the first cut. *Grassland Science in Europe* 21, 305-307.

Van Genuchten M.T. (1980) A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. *Soil Science Society of America Journal* 44, 892-898.

Effect of sward height of rotationally grazed perennial ryegrass (*Lolium perenne* L.) on light interception

Shewmaker G.[1] and Hooper L.[2]

[1]*Extension Forage Specialist, University of Idaho, Kimberly Research & Extension Center, 3806 N 3600 E, Kimberly, ID 83341, USA;* [2]*Livestock producer and former graduate student, TLC Angus, 1854 S 750 E, Bliss, ID 83314, USA*

Abstract

The objective was to measure photosynthetically active radiation (PAR) intercepted and relate it to sward height throughout the grazing season on rotationally grazed perennial ryegrass (*Lolium perenne* L.) pastures. A rotationally grazed irrigated pasture was sampled weekly from April to September over two years and measured for PAR, sward mass and canopy height. The sward canopy closed in a logarithmic function of height (PAR = 493 ln(height) – 317) in irrigated perennial ryegrass during April-September and across 2 years. With a 5 cm canopy, 476 μmol m^{-2} s^{-1} PAR was intercepted and with a 15 cm canopy, 1,018 μmol m^{-2} s^{-1} PAR was intercepted. This study indicates that increasing the canopy height from 5 to 15 cm increases the PAR intercepted by 114%. Grazing closer than 7 cm drastically reduces PAR interception, which means that regrowth energy must come from a higher proportion of stored energy in the crown. Grazing closer than 7 cm also drastically removes more herbage mass and thus the stored energy. In contrast, above 20 cm of canopy the rate increase of PAR interception per cm of canopy height is low. This study reaffirms the concept of leaving enough photosynthetic capacity in grazed plants to quickly restore net photosynthesis.

Keywords: sward height, PAR, perennial ryegrass, *Lolium*

Methods

An irrigated perennial ryegrass (*Lolium perenne* L.) paddock rotationally grazed at a stocking rate varying from 15 to 50 beef cow/calf pairs for a grazing period of 5 days and rested for 20 to 30 days, was sampled in 2006 and 2007. The soils were sprinkler-irrigated silt loam (coarse-silty, mixed, mesic, Durixerollic Calciorthids) near Wendell, Idaho, USA (42°48' N 114°45' W, 1,040 m above sea level). The average precipitation and daily air temperature from April to September 2006 was 31 cm and 18 °C, respectively. The 2007 measurements were 17 cm and 19 °C, respectively.

Sward height (cm), density (visual rating), herbage mass (kg DM ha^{-1}), and PAR (μmol m^{-2} s^{-1}) were determined once per week in three replications within three canopy densities during the growing season. Canopy density was visually rated using photo guides of low, medium, and high canopy cover. Only the medium canopy density is presented in this paper. Herbage was clipped to ground level just above the soil, sorted by species, oven dried, and weighed to develop regression equations to predict herbage mass. Sward height was measured with a prediction stick (ruler) at the height where an estimated 90% of the herbage mass occurred.

A Sunfleck PAR Ceptometer Model SF-80 (Decagon Devices, Inc. Pullman, WA) was used to measure photosynthetically active radiation (PAR) at the 400-700 nm wavebands. This 88.5 cm probe containing 80 independent sensors, reads the sunlight PAR radiation. These independent sensors are scanned by a microprocessor to give an average PAR reading in μmol m^{-2} s^{-1} (Decagon). Light interception was calculated as the difference between a PAR reading above the canopy and close to ground level under the canopy. All samples were taken between 10:00 a.m. to 1:00 p.m. on sunny to partly cloudy days. An Excel™ spreadsheet was used to calculate and graph natural logarithmic regression statistics.

Results and discussion

The sward canopy closed in a logarithmic function of height (PAR = 493 ln(height) – 317) in irrigated perennial ryegrass during April-September and across 2 years (Figure 1). With a 5-cm canopy, 476 μmol m^{-2} s^{-1} PAR were intercepted and with a 15-cm canopy, 1018 μmol m^{-2} s^{-1} PAR were intercepted. This study indicates that increasing the canopy height from 5 to 15 cm increased the PAR intercepted by 114%. Grazing closer than 7 cm drastically reduces PAR interception, which means that regrowth energy must come from a higher proportion of stored energy in the crown. Grazing closer than 7 cm also drastically removes more herbage mass and thus the stored energy. In contrast, above 20 cm of canopy the rate increase of PAR interception per cm of canopy height is low.

Our results compare similarly to Pedreira *et al.* (2000) with studies on bermudagrass (*Cynodon dactylon* L.) pastures that were grazed to heights of 8 cm capturing 22% of the radiation while pastures with heights of 24 cm intercepted 78% of the radiation. Akmal and Janssens (2004) found maximum interception took place at a critical leaf area index of 3 in perennial ryegrass, but they did not report canopy height. The capturing of PAR is dependent on the path of the sunbeam through the sward, stand density and foliage characteristics (Welles and Norman, 1991; Rohrig *et al.*, 1999).

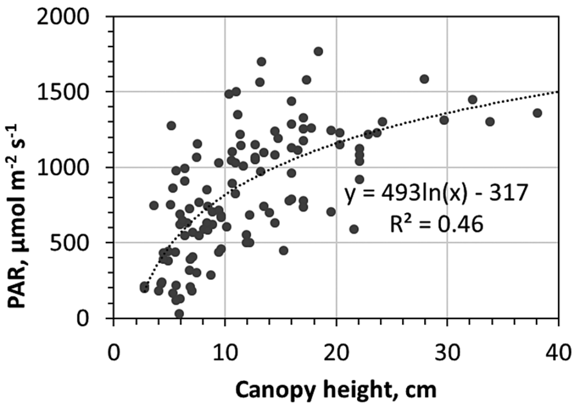

Figure 1. Photosynthetically active radiation (PAR) intercepted by rotationally grazed perennial ryegrass (*Lolium perenne* L.) canopies as a function of canopy or sward height across 2 growing seasons.

Conclusions

The sward canopy closes in a logarithmic function of height or mass in irrigated medium-density stands. This study indicates that doubling the canopy height from 12.5 to 25 cm will increase the PAR intercepted by 33%.

References

Akmal M. and Janssens M.J.J. (2004) Productivity and light use efficiency of perennial ryegrass with contrasting water and nitrogen supplies. *Field Crops Research* 88, 143-155.

Decagon (2005) How the LP80 Measures Leaf Area Index. Application Note. AN20LA-10. Decagon Devices, Inc. Pullman, WA, USA.

Pedreira C.G.S., Sollenberger L.E. and Mislevy P., (2000) Botanical composition, light interception, and carbohydrate reserve status of grazed 'Florakirk' bermudagrass. *Agronomy Journal* 92, 194-199.

Rohrig M., Stutzel H., and Alt C. (1999) A three-dimensional approach to modeling light interception in heterogeneous canopies. *Agronomy Journal* 91, 1024-1032.

Sunfleck PAR Ceptometer Operator's Manual. Decagon Devices, Inc. Pullman, WA, USA.

Welles J.M. and Norman J.M. (1991) Instrument for indirect measurement of canopy architecture. *Agronomy Journal* 83, 818-825.

The influence of different grass-legume mixtures on the productivity of first-year spring cutting

Sidlauskaite G. and Kadziuliene Z.
Lithuanian Research Centre for Agriculture and Forestry, Lithuania

Abstract

Lithuania is located in a nemoral climate zone. However, observations indicate that heatwaves and droughts, which can adversely affect plant development, are becoming more common. In multifunctional grasslands spring harvest is important because the highest quality yield of biomass is accumulated and can be used for winter forage for livestock. The study identified the formation rates of the first year of grassland use after first cutting on which depends the further growth of grassland. This experiment used new, less-investigated, Lithuanian varieties of four grasses and four legumes plants. Mixtures with one, three, four, six and eight grassland species were sown in experimental areas. There was a positive relationship between species richness and yield. Sward yields of eight-species communities were 1.4 times higher than in monocultures. Dry matter yields were significantly increased by diversity of multi-species. Non-nitrogen fertilised perennial ryegrass and *Festulolium* grasses developed more slowly and their yield was lower due to lack of nutrient supply. Lucerne developed the best growth qualities and the yields of all mixtures with lucerne were higher than others with different species mixtures. Comparison of mixtures containing other legumes showed that spring yields were improved in mixtures with red clover and sainfoin more than with white clover.

Keywords: grasses, legumes, multi-species, diversity

Introduction

Climate fluctuations, rising temperatures, changing precipitation and other extreme climatic events all contribute to changes in plant diversity. In intensively managed grasslands, even modest increases in species richness can result in strong yield benefits when species are selected for complementary traits (Brophy *et al.*, 2017). The higher species diversity provides stability to grasslands and enhances the environmental function of grasslands. Cultivating mixtures of different plants can be a sustainable way to increase agricultural productivity, but it is very important to optimise functional diversity by combining different species characteristics that are well adapted to local growing conditions (Goslee *et al.*, 2013). The mixture composition affects the total herbage in various harvests (Elgersma and Søegaard, 2016), but differences may already be noticeable in the first cut, and this may also affect the further development of the swards. The composition of grass mixtures was chosen taking into account soil conditions, biological properties and the use of grasses. *Festulolium* is a hybrid or a hybrid derivative between species of fescue (*Festuca* L.) and ryegrass (*Lolium* L.) designed for their combined complementary characters. Spring growth of *Festulolium* in particular is earlier than for perennial ryegrass (*L. perenne*) commonly cultivated in northern Europe for fodder production. Therefore, the first cut of *Festulolium* can normally be done earlier. The aim of the present study was to identify the influence of different grass-legume mixtures on the productivity of spring cutting of the first-year sward use.

Materials and methods

The grassland field experiments were conducted at the Lithuanian Research Centre for Agriculture and Forestry, in Akademija (55°23'N, 23°49' E) Central Lithuania. The year of sowing was 2018 and 2019 was the first year of sward use. All treatments were fertilised in spring 2018 with the same rate of mineral fertiliser (N-P-K at 5-20.5-36 kg ha^{-1}). The main agrochemical characteristics of the top soil layer (0-25 cm) measured at the beginning of the experiments in 2018 were: neutral soil (pH 7.0), relatively high organic matter content (3.06%) with high plant available P (220 mg kg^{-1}) and K (173 mg kg^{-1}). The 12 different mixtures sown consisted of grasses *Lolium perenne* L. cv. Elena DS (*L.p.*), *Festuca pratensis* L. cv. Raskila (*F.p.*), ×*Festulolium* L. cv. Vėtra (*xF.*), and *Phleum pratense* L. cv. Dubingiai (*P.p.*): and legumes *Trifolium repens* L. cv. Dotnuviai (*T.r.*), *Trifolium pratense* L. cv. Sadūnai (*T.p.*), *Onobrychis viciifolia* L. cv. Meduviai (*O.v.*), and *Medicago sativa* L. cv. Malvina (*M.s.*) (Table 1). The legume/grass ratio in the sown mixtures was 40:60. The experiment had a randomized block design with four replicates. The net plot size was 1.5×10 m. The first harvested yield of the swards was taken before flowering stage of grasses, 8 weeks after start of growth. To determine the biomass dry matter (DM), portions of 1-1.5 kg fresh herbage were oven dried at 105 °C to constant weight for each harvest sample. For assessment of species composition additional samples (0.5-1.0 kg) were taken from each plot and sorted by individual species. An analysis of variance (ANOVA) was conducted to analyse effects of the treatments. Significant differences between the experimental treatments were determined using Fisher's test at the 0.05 probability level.

Results and discussion

Dry matter yield was significantly influenced by grass sward composition (Table 2). Monocultures evolved at a slower rate, with the average yield of monocultures being 15% lower when compared to mixtures. Grass mixtures with lucerne developed the best (mixture 7, 8, 11, 12), and the average biomass yield of these mixtures was 945 kg ha^{-1} higher, compared to the average of other mixtures. Mixtures with lucerne increased the yield by 20%. The less-investigated species sainfoin in mixtures showed better growth properties than white clover, but red clover was more productive in mixtures. No significant differences were observed between the growth rates of perennial ryegrass and *Festulolium*.

Previous studies have reported that different mixtures could affect the total biomass productivity of swards and each component differently (Elgersma and Søegaard, 2016), but have also shown that diversity of mixtures has positive influence (Brophy *et al.*, 2017). The results of first spring cutting could be different from annual yield of the grassland. Further studies are needed to characterise the contribution of individual plant species to the grassland.

Table 1. Experimental design.

Mixture	FG	SP-r	G	G2	G3	G4	L1	L2	L3	L4
1	1	1	1							
2	1	1		1						
3	2	3	0.6				0.2	0.2		
4	2	3		0.6			0.2	0.2		
5	2	4	0.3	0.3			0.2	0.2		
6	2	6	0.15	0.15	0.15	0.15	0.2	0.2		
7	2	4	0.3	0.3			0.2			0.2
8	2	6	0.15	0.15	0.15	0.15	0.2			0.2
9	2	4	0.3	0.3			0.2		0.2	
10	2	6	0.15	0.15	0.15	0.15	0.2		0.2	
11	2	6	0.3	0.3			0.1	0.1	0.1	0.1
12	2	8	0.15	0.15	0.15	0.15	0.1	0.1	0.1	0.1

Each proportion is based on the seeding rate in monoculture for a species. FG = Functional group – combination of forage species that comprise grasses and legumes; SP-r = species richness. Grasses: G1 = *Lolium perenne* L.; G2 = *Festulolium* L.; G3 = *Festuca pratensis* L.; G4 = *Phleum pratense* L.; Legumes: L1 = *Trifolium repens* L.; L2 = *Trifolium pratense* L.; L3 = *Onobrychis viciifolia* L.; L4 = *Medicago sativa* L.

Table 2. The effect of grass mixtures on the productivity and botanical composition of the sward in the 1st cut.[1]

Mixture	DM yield, kg ha^{-1}		Botanical composition of species, %								
			G1	G2	G3	G4	L1	L2	L4	L3	Forbs
1	3,667	abc	99.9								0.1
2	3,638	ab		96.4							3.6
3	3,921	abcd	76.3				1.4	19.9			2.3
4	4,396	bcde		76.7			0.4	21.9			1.0
5	4,332	cdefg	9.3	69.5			0.9	18.0			2.3
6	3,409	a	6.6	47.4	13.4	8.9	1.6	18.8			3.4
7	4,581	defg	8.4	63.1			0.9		26.2		1.3
8	5,261	g	4.2	41.1	13.9	6.6	1.4		32.0		0.7
9	3,556	a	13.0	69.7			3.0			8.1	6.2
10	4,049	abcd	13.3	46.6	18.3	6.9	4.7			9.0	1.3
11	4,499	defg	10.0	53.0			0.6	18.1	13.3	4.6	0.5
12	4,966	efg	11.6	34.3	21.0	7.3	1.2	14.9	8.0	0.5	1.0
LSD 0.05	733.55										

[1] Grasses: G1 = *Lolium perenne* L.; G2 = *Festulolium* L.; G3 = *Festuca pratensis* L.; G4 = *Phleum pratense* L.; Legumes: L1 = *Trifolium repens* L.; L2 = *Trifolium pratense* L.; L3 = *Onobrychis viciifolia* L.; L4 = *Medicago sativa* L.; LSD = least significant difference; DM = dry matter.

Conclusions

For all mixtures, and in almost all cases, higher yields were found in mixtures with legume plants and they showed better growth properties than monocultures in the spring herbage cut. Lucerne develops the best and the yield of all mixtures with lucerne was the highest. In almost all cases, monocultures had the lowest average biomass yield.

References

Brophy C., Finn J. A., Lüscher A., Suter M., Kirwan L., Sebastia M.T., Helgadottir A., Baadshaug O. H., Belanger G., Black A., Collins R. P., Cop J., Dalmannsdottir S., Delgado I., Elgersma A., Fothergill M., Frankow-Lindberg B.E., Ghesquiere A., Golinska B., Golinski P., Grieu P., Gustavsson A.M., Hoglind M., Huguenin-Elie O., Jørgensen M., Kadziuliene Z., Kurki P., Llurba, Lunnan T., Porqueddu C., Thumm U. and Connolly J. (2017) Major shifts in species' relative abundance in grassland mixtures alongside positive effects of species diversity in yield: a continental-scale experiment. *Journal of Ecology* 105, 1210-1222.

Elgersma A. and Søegaard K. (2016) Effects of species diversity on seasonal variation in herbage yield and nutritive value of seven binary grass-legume mixtures and pure grass under cutting. *European Journal of Agronomy* 78, 73-83.

Goslee S.C., Veith T.L., Skinner H. and Comas L.H. (2013) Optimizing ecosystem function by manipulating pasture community composition. *Basic and Applied Ecology* 14, 630-641.

Seed maturation and harvesting time of lucerne (*Medicago sativa* L.)

Slepetys J. and Slepetiene A.
Institute of Agriculture, LAMMC, 58344, Akademija, Kedainiai distr., Lithuania

Abstract

Lucerne seed yield is unstable and highly dependent on weather conditions during the flowering and ripening stages. The objectives of this study were to investigate the course of seed maturation, identify a straightforward technique for the determination of harvesting time and validate the optimum harvesting time of desiccated lucerne seed crops. During a five-year period, testing of seed maturation dynamics of lucerne was started 35-42 days after the onset of flowering of the stand and was continued once a week for 2-3 months. It was determined that to establish the earliest seed harvesting time, it is necessary to analyse a composite seed sample collected from different places of the stand. Harvesting time may be determined more precisely by using the growing degree days (GDD) rather than the actual number of days from the onset of stand flowering. The earliest time for lucerne desiccation is when 80% of pods in the stand are brown and yellowish brown (speckled), approximately within the 9th-10th week from the onset of flowering, when the GDD is 1,100 °C. The optimum duration of harvesting is three weeks (within 70-91 days from onset of flowering).

Keywords: *Medicago sativa*, seed maturity, harvesting time

Introduction

In Lithuania, lucerne (*Medicago sativa* L.) is less widespread than red clover and white clover. Yet, the area under this perennial legume tends to increase, as lucerne is very suitable for organic production. Stands of lucerne are able to flower over a long time during rainy weather. The seed maturation is uneven. If seed harvesting is delayed or done prematurely, the seed losses are high (Barnes *et al.,* 2007). There is limited experimental evidence on lucerne seed maturation and harvesting time. The stems of lucerne are tall with many leaves, and therefore most researchers have recommended direct combine harvesting, preceded by a stand desiccation (Greenhill, 1974, Frame *et al.*, 1998, Barnes *et al.*, 2007). In general, it is rather difficult to determine the harvesting time as the stand matures unevenly and the mature pods tend to dehisce (Kirkby, 2011). Thus, maturation and harvesting of lucerne seed depend on the weather conditions and the biological peculiarities of the species. The objectives of this research were: (1) to identify a straightforward technique for the determination of harvesting time; (2) to investigate the process of the seed maturation; and (3) to validate the optimum harvesting time for desiccated lucerne seed crops.

Materials and methods

Field experiments were carried out on a loam soil (*Epicalcari – Endohypogleic Cambisol*) in the central part of Lithuania (55°23' N, 23° 51' E). The soil contained on average 2.63% humus, 104 mg kg^{-1} of P, 125 mg kg^{-1} of K, and pH was 7.0. Lucerne did not receive any fertilisation. During a five-year study, the maturation dynamics of lucerne seed was tested 8 weeks after the onset of flowering when 10% of clusters were in bloom. The tests were continued for 2-3 months on 1 m^2 plots with four replications. Once a week, or twice during dry weather, the seed stands were desiccated with Reglone and after 3-7 days were threshed with a laboratory thresher. In the subplots, at each occasion prior to desiccation, 3-4 plant samples were collected from a 0.25 m^2 area for estimating seed maturity. The pods were detached and divided into the following groups: (1) green, underdeveloped; (2) yellowish green or speckled, semi-mature; (3) brown, completely mature. The pod number of each group was expressed as percentage.

In order to establish the course of seed maturation, in the subplots 100-150 of newly flowering inflorescences (clusters) were marked with red thread throughout the period of 14 days after the onset of stand flowering, and later on every week until the end flowering. Each time, 10-15 inflorescences were detached 21, 28, 35, 42, 49, 56 days after marking. The seeds present in the pods were dried, hulled, and tested for quality (germination, content of hard seed, 1000 seed weight). With a view to establishing the effect of desiccation, the above-mentioned groups of inflorescences were marked with red thread. Ten to twenty marked desiccated inflorescences as well as the same number of not desiccated inflorescences from each group were left for maturation. The marked inflorescences were detached after 3-7 days, threshed and later analysed for quality. The results were processed using ANOVA.

Results and discussion

Seed growth and development from the stage of setting to maturity are best demonstrated by the 1000 seed weight and variation of germination. The most valuable clusters of lucerne flowered in June or in the first half of July. Our data indicate that one can expect high quality lucerne seed yield from the clusters flowering no later than within the third 10-day period of July. One thousand seed weight of the clusters flowering later than end of July was 1.37 g and the seed number in clusters was 1.9 times lower, and the seed germination did not meet the standard requirements (Table 1). Clusters of lucerne that were in bloom at the onset of the stand flowering, and during the mass flowering, produced ripened the seeds after 6-7 weeks. Lucerne seed needs 856 °C growing degree days (GDD) to ensure full maturity.

Lucerne seed crops are usually harvested by direct combining after spraying with a crop desiccant. To avoid loss of seed by shedding, correct timing of harvesting is necessary, i.e. when 65-75% of the seed pods are dark brown (Frame, 2005). When desiccation was carried out in each week during the experimental period, clusters with different ripeness, ranging from flowering to shattered seeds, were found in the stand. Desiccation of the youngest clusters of lucerne (3-4 weeks from the onset of flowering) was detrimental. One thousand seed weight of the not desiccated plants was significantly higher and the seed germination was also higher. The seeds from the yellowish-green or speckled desiccated pods did not differ in quality from the seeds of the not desiccated pods of the same maturity. As a result, when determining the harvesting time, the clusters of this group may be attributed to the group of ripened seeds. It is important to know the earliest feasible harvesting time of lucerne, as in this case it is possible to better adjust to rainy weather. The earliest harvesting (desiccation) time was established taking into account the following conditions: (1) the seed yield must be the highest or increasing within the limits of error; (2) the seed germination must meet the standard requirements. Using the Fodder Plant Seeds Regulations for Lithuania, certified seed requires a minimum germination of 80% and permissible maximum hard seed content of 40% by number of pure seeds in the sample. If rainy weather prevents the seed germination from reaching the standard requirements, the earliest possible harvesting time is determined according to the highest germinable seed yield. The earliest lucerne desiccation time can be better established according to the number of brown (mature) and semi-mature (part of pods are yellowish green) clusters

Table 1. Seed quality of lucerne at different flowering time of clusters (not-desiccated plants, averaged over five years).

Flowering time of clusters (month, ten-day period)	Germination, %	Hard seed, %	1000 seed weight, g	Number of seed in cluster
June III	74	43	1.92	6.2
July I	82	47	1.94	8.3
July II	79	42	1.73	8.1
July III	72	48	1.58	6.3
August I	36	46	1.37	3.3
LSD 0.05	10	12	0.25	2.6

rather than only by the number of brown clusters (Table 2). For the determination of harvesting time, GDD (V 8%) from the onset of flowering is a more accurate indicator than the number of days (V 13%). The mean data show that the earliest desiccation time for lucerne stands is when brown and yellowish green (speckled) clusters make up 81%. This occurs around the 9^{th}-10^{th} week from the onset of flowering, when the GDD value is 1,110 °C. The optimum period of harvesting is three weeks.

Table 2. Mean data for the earliest possible harvesting time of lucerne seed crop (desiccated plants, averaged over five years).

Indices	Mean	Variation, %
Days from onset of flowering	67±4.5	13
Growing degree days from onset of flowering, °C	1,110±45	8
Mature pods, %	66±4.3	15
Mature and semi mature pods, %	81±3.9	9
Seed germination, %	80±6.0	
1000 seed weight, g	2.03±0.1	
Seed yield, g m^{-2}	8.0±2.5	
Harvest time, weeks from onset of flowering	10-13	

Conclusions

The GDD from the onset of lucerne flowering is a more accurate indicator for tentative determination of the earliest time for desiccation, rather than the actual number of growing days. The earliest time for desiccation of a lucerne seed crop is when 80% of pods in the stand are brown and yellowish brown (speckled). This is approximately within the 9^{th}-10^{th} week from the onset of flowering, when the GDD reaches 1,100 °C. The optimum period of harvesting is three weeks (within 70-91 days from the onset of flowering). When the area under lucerne is small and pod ripeness analyses are not made, it is best to desiccate lucerne within the 11^{th} week from the onset of flowering. The onset of flowering is considered to occur when 10% of the inflorescences are in bloom.

References

Barnes R.F., Nelson C.J., Moore K.J. and Collins M. (2007) *Forages*, vol. II, 6^{th} edition, Blackwell, Ames, USA, 791 pp.

Frame J. (2005) *Medicago sativa* L. In: *Forage Legumes for Temperate Grasslands.* Science Publishers, Inc., Enfield, USA, pp. 101-115.

Frame J., Charlton J.F.L. and Laidlaw A.S. (1998) *Temperate Forage Legumes.* CAB International, Wallingford, UK, 327 pp.

Greenhill W. (1974) Lucerne seed harvesting by desiccation, *Tasmanian Agronomy Journal*, 45, 253-254.

Kirkby L. (2011) *Agronomic support manual for lucerne seed production*. Publication No10/106, Griffith, NSW, pp.1-32.

Phosphorus fertilisation enhances biomass yield as well as nitrogen yield and herbage nutritional status in a long-term grassland experiment

Suter M. and Huguenin-Elie O.
Agroscope, Forage Production and Grassland Systems, Reckenholzstrasse 191, 8046 Zürich, Switzerland

Abstract

We investigated effects of phosphorus (P) fertilisation on yield and forage nutrient content measured for 28 years in a long-term grassland experiment. Mountainous grassland plots were set up at constant nitrogen (N) fertilisation of 25 kg ha^{-1} yr^{-1} and at two levels of P: no P (P0) and 31 kg P ha^{-1} yr^{-1} (P31). Potassium was applied in non-limiting amounts. Here, we report biomass yield, N yield, and the nutritional status of herbage from the first cut per year, on average across years. Biomass yield was 3.74 and 4.38 Mg ha^{-1} yr^{-1} at P0 and P31, respectively, indicating a yield loss of 15% due to the lack of P (P<0.001). Despite this yield difference, N content did not differ between P treatments and was 18.2 g N kg^{-1} DM on average. As a result, N yield was significantly enhanced by adequate P supply (P<0.001), and was 66.5 and 79.2 kg ha^{-1} yr^{-1} at P0 and P31, respectively. The phosphorus nutrition index (PNI) indicated P limited growth at P0 (PNI=0.6), while it confirmed adequate P supply at P31 (PNI=1.1). We conclude that adequate P fertilisation not only enhanced biomass yield but also allowed plants to exploit N sources better, leading to more N yield for a given amount of soil N.

Keywords: long-term grassland experiment, P fertilisation, phosphorus nutrition index, N yield, nitrogen nutrition index, nutrient limitation

Introduction

Adequate nutrient supply is essential for plant growth. While grasslands are generally nitrogen (N) limited, phosphorus (P) limitation can also lead to strong yield losses (Fay *et al.*, 2015). Interestingly, adequate supply of one nutrient can also stimulate the uptake of another nutrient. For example, it has been demonstrated that increased availability of N in the soil can also enhance uptake of other soil resources such as P (Hoekstra *et al.*, 2015; Husse *et al.*, 2017). While interactions between N availability and other soil resources (including water) have been studied repeatedly (e.g. Farrior *et al.*, 2013), the relation between P supply and the uptake of N is less clear. It must be assumed that increased fertilisation of P at constant rates of N leads to an enhanced N limitation relative to P (Güsewell, 2004). Under such conditions, plants might increase their root biomass to exploit soil N resources better, leading to an indirect positive effect on N yield. Here, we evaluated how increased P fertilisation affected biomass yield and the P nutrition status of herbage in a long-term grassland experiment, and how P fertilisation indirectly affected N yield and the N nutrition status.

Materials and methods

A grassland fertilisation experiment was established in 1990 in the Swiss mountains at 1,200 m a.s.l., and was measured for 28 years. Prior to experimentation, the species-rich, permanent grassland was used for hay production for decades. It contained more than 30 plant species per 10 m^2 and was dominated by *Trisetum flavescens* (L.) PB. and *Dactylis glomerata* L. Sixteen plots of 10 m^2 were set up, and a P treatment was established in that one half of the plots were not fertilised with P (P0) while the other half were fertilised with 31 kg P ha^{-1} yr^{-1} (P31) as triple superphosphate. All plots received N fertiliser (NH_4NO_3) at a rate of 25 kg N ha^{-1} yr^{-1}, while potassium was applied in non-limiting amounts. Plots were arranged in a randomized complete block design. They were generally mown three times per year and biomass yield per plot was sampled. Dry matter (DM; after drying biomass to constant weight) was analysed for its total P and N content, which allowed calculating N yield by multiplying DM with its N content. To determine the nutritional status of plant biomass, the phosphorus nutrition index (PNI) was calculated following Duru and Ducrocq (1997):

$$PNI = P\% / 0.15 + 0.065 \times N\%)$$

with P% and N% being the measured P and N content in bulk DM. In addition, the nitrogen nutrition index (NNI) was calculated following Lemaire and Gastal (1997):

$$NNI = N\% / 4.8 \times DM^{-0.32}$$

An index value ≥1 indicates adequate or surplus provision of the respective nutrient, a value <0.8 indicates that plant growth is limited. Here, we analysed biomass yield, P and N content, N yield, and the PNI and NNI as affected by P fertilisation. All presented data are from the first harvest of the year, which was taken at the beginning of June and made up 56% of the annual biomass yield. For analyses, all data were averaged across the 28 years. Differences between the P treatments were analysed with *t* tests.

Results and discussion

On average across years, biomass yield was 3.74 and 4.38 Mg ha^{-1} yr^{-1} at P0 and P31, respectively, indicating a yield loss of 15% due to the lack of P (P<0.001, Figure 1A). Absence of P fertilisation strongly reduced the P content in plant biomass, P contents being 1.5 (P0) and 3.0 g P kg^{-1} DM (P31) (P<0.001, Figure 1B). Concordantly, the PNI clearly indicated P limited growth at P0, while it confirmed adequate P supply at P31 (Figure 1C).

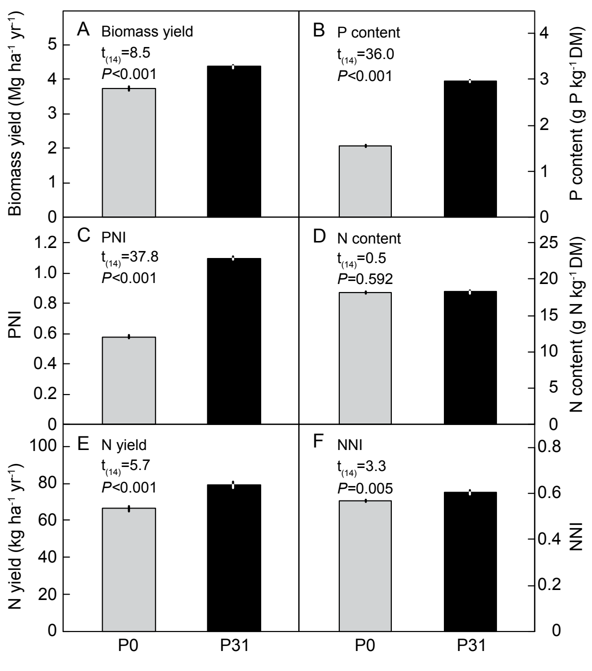

Figure 1. Effects of P fertilisation (P0: no P, P31: 31 kg P ha^{-1} yr^{-1}) on biomass yield (A), P content (B), the phosphorus nutrition index (PNI) (C), N content (D), N yield (E), and the nitrogen nutrition index (NNI) (F) in a long-term grassland experiment. Data were averaged across 28 years. The inference refers to the difference between the P treatments.

Despite the difference in biomass yield, the N content did not differ between P treatments and was 18.2 g N kg^{-1} DM on average (P=0.592, Figure 1D). As a result, N yield was significantly enhanced by 19% at increased P fertilisation (P<0.001), and was 66.5 and 79.2 kg ha^{-1} yr^{-1} at P0 and P31, respectively (Figure 1E). This means that N yield per unit of applied N, i.e. N efficiency, was higher at P31 than at P0. Evaluating the NNI showed that all communities grew N limited (Figure 1F). The NNI was fairly similar at P0 (0.57) and P31 (0.60), yet slightly enhanced at P31 (P=0.005). A recent study by Perotti *et al.* (2020) indicates that the relatively high proportions of forbs in the communities did not cause the NNI to be underestimated. We argue that increased P availability allowed plants to grow better, which enhanced their relative demand for other nutrients such as N. In addition, plants may have produced more roots under adequate P supply in the long-term, allowing the plant community to better exploit soil and/or fertiliser N resources and thereby reduce N deficiency relative to P. A combination of both factors might have caused increased N yields at P31. Notably, the P fertilisation effect on N yield was not related to legume proportion. On average across both treatments, the proportion of grass, legume, and forb species was 55, 10 and 35%, respectively, without substantial difference between treatments.

Conclusions

Alleviating P limitation can modify the exploitation on N resources in the soil. P fertilisation not only enhanced biomass yield but also allowed the grassland community to take up more N in the long-term, leading to a higher N yield for a given amount of N fertilisation.

References

Duru M. and Ducrocq H. (1997) A nitrogen and phosphorus herbage nutrient index as a tool for assessing the effect of N and P supply on the dry matter yield of permanent pastures. *Nutrient Cycling in Agroecosystems* 47, 59-69.

Farrior C.E., Tilman D., Dybzinski R., Reich P.B., Levin S.A. and Pacala S.W. (2013) Resource limitation in a competitive context determines complex plant responses to experimental resource additions. *Ecology* 94, 2505-2517.

Fay P.A., Prober S.M., Harpole W.S., Knops J.M.H., Bakker J.D., Borer E.T., and Yang, L.H. (2015) Grassland productivity limited by multiple nutrients. *Nature Plants* 1, 15080.

Güsewell S. (2004) N:P ratios in terrestrial plants: variation and functional significance. *New Phytologist* 164, 243-266.

Hoekstra N.J., Suter M., Finn J.A., Husse S. and Lüscher A. (2015) Do belowground vertical niche differences between deep- and shallow-rooted species enhance resource uptake and drought resistance in grassland mixtures? *Plant and Soil* 394, 21-34.

Husse S., Lüscher A., Buchmann N., Hoekstra N.J. and Huguenin-Elie O. (2017) Effects of mixing forage species contrasting in vertical and temporal nutrient capture on nutrient yields and fertilizer recovery in productive grasslands. *Plant and Soil* 420, 505-521.

Lemaire G. and Gastal F. (1997) N uptake and distribution in plant canopies. In: Lemaire G. (ed) *Diagnosis of the Nitrogen Status in Crops*. Springer, Berlin, Germany, pp. 3-43.

Perotti E., Huguenin-Elie O., Meisser M., Dubois S., Probo M. and Mariotte P. (2020) Impacts of forb abundance on plant nutrition indexes along the growing season in intensively managed permanent grasslands. *Grassland Science in Europe*, this volume.

Productivity of Alaska brome and smooth brome in pure stand and in mixture with lucerne

Tamm S., Bender A., Aavola R., Meripõld H., Pechter P. and Sooväli P.
Estonian Crop Research Institute, J. Aamisepa 1, Jõgeva 48309, Estonia

Abstract

Alaska brome (AB) (cv. Hakari) and smooth brome (SB) (cv. Lehis) were tested in pure stands and in mixtures with lucerne (Lu) (cv. Jõgeva 118 and Karlu) in a field trial during 2017-2019. Seeding rates used were 30 kg ha^{-1} for AB and 38 kg ha^{-1} for SB. In the mixture with Lu the seeding rates were 20 kg ha^{-1} for grasses and 12 kg ha^{-1} for Lu. Pure stands of AB and SB received 200 kg ha^{-1} N in three applications. A three-cut system was used during harvest. Dry matter yield (DMY) and its quality were determined. Occurrence of plant diseases (*Puccinia striiformis*, *Drechslera siccans* and *Ustilago bullata*) was also estimated. SB matured earlier than AB. AB had a faster regrowth than SB. AB proved to be a better companion for Lu than SB. The largest difference in yield distribution occurred across years and was influenced mostly by weather conditions. The mixtures differed significantly in nutritive value. Pure stand of SB exceeded the recommended concentration of NDF (550 g kg^{-1} DM) in the first cuts in both years. Our preliminary results show that AB is less tolerant to different plant diseases than previously assumed.

Keywords: Alaska brome, smooth brome, yield, forage quality, diseases

Introduction

Alaska brome (AB) (*Bromus sitchensis* Trin. in Bong.) is a newly introduced species in Estonia. It has been investigated and cultivated here only recently (Tamm *et al.*, 2018, 2019). AB originates from the Pacific coast of North America. It is considered a drought-resistant and productive species as well as being fast in establishment and regrowth (https://www.forageseeds.com/). AB is suitable as a companion for alfalfa (Hakari, 2018). Smooth brome (SB) (*Bromus inermis* Leysser) has been cultivated for a long period in Estonia. It is characterized by cold resistance, persistence over five years and is well-adapted to dry site conditions. Lucerne (Lu) (*Medicago sativa* L.) is one of the most important forage legumes in Estonia because of its high nutritive value and yield. It is also productive on drought-prone soils. The objective of the study was to compare production abilities and forage quality of two *Bromus* species in pure stands and in mixtures with two Lu cultivars in the growth conditions of Estonia.

Materials and methods

The trial was carried out during 2017-2019 at the Estonian Crop Research Institute at Jõgeva, in north-eastern Europe (58°45'N, 26°24'E, average annual temperature of 5.3 °C and precipitation of 670 mm) on clay loam (40-50% of clay) classified as Calcaric Cambic Phaeozem (Loamic) soil (IUSS, 2015). The characteristics of the soil horizon were as follows: pH_{KCl} 5.8, P 191, K 220 and Ca 1501 mg kg^{-1}. The trial was arranged in a randomized complete block design with four replicates. AB Hakari, SB Lehis and Lu cultivars Karlu and Jõgeva 118 (the most widespread Lu cultivars in Estonia) were included in the trial. Seeding rates used in the study were 30 kg ha^{-1} for AB and 38 kg ha^{-1} for SB. In the mixture with Lu the seeding rates were 20 kg ha^{-1} of grasses and 12 kg ha^{-1} of Lu. Pure stands of AB and SB received 200 kg ha^{-1} N in three applications (80-60-60 kg ha^{-1}). The sward was harvested three times a year: first cut at the stage of early heading, then 45 days later and the last cut at the end of the growing season (end of September). The plots were harvested with a Hege 212 forage harvester. The proportion of Lu and grasses was determined from a sample of one kg of fresh biomass at harvest time. Crude protein content (CP) and neutral detergent fibre (NDF) were determined. Occurrence of plant diseases was determined before harvests by visual examination on a 1-9 scale. Weather conditions for both years were severe for plant growth: air temperatures were above long-term average (1982-2019) with insufficient precipitation (Table 1). Statistical significance of differences among the DMYs and their quality characteristics was computed using package Agrobase 20™. All quality characteristics of the DMY were averaged over four replicates.

Results and discussion

There was a yield depression caused by a long drought period in June, July and first ten days of August 2018 (Table 1). The unfavourable (high air temperature, insufficient rainfall) weather conditions impeded plant growth and resulted in extremely low dry matter yield (DMY) (Table 2). Pure stands of SB yielded significantly more DM in the first cut, and in the entire season, than AB. Its mixtures yielded significantly less than pure stands in 2018, whereas the differences were mostly not significant in 2019. AB proved to be a better companion for Lu than SB. The yield distribution of AB was more uniform than in SB in both years. The aftermath of AB grew faster and its DMY in mixtures was significantly higher in the second and third cuts than that of the mixtures with SB.

The mixtures differed significantly in nutritive value (Table 3). CP content was very low in the first and second cuts in the first harvest year. It was higher in the mixed stands for the second year, which was caused by increased proportion of Lu in the yield (Table 4). NDF content should remain below 550 g kg^{-1} DM in high quality feed. Pure stands of SB exceeded that level in the first cuts in both years.

Table 1. Weather data means of 10-day periods during each of 5 months of the vegetation periods in 2018 and 2019 at Jõgeva.[1]

	May			June			July			August			September		
	10-day period			10-day period			10-day period			10-day period			10-day period		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Air temperature, °C															
2018	11.1	16.1	16.2	13.4	16.8	14.8	15.6	21.7	23.2	21.1	17.3	15.6	17.2	14.1	9.5
2019	6.1	12.0	13.3	18.8	18.0	16.7	13.9	14.7	18.8	14.0	16.4	16.6	15.7	10.2	7.5
1982-2019[1]	9.6	10.7	12.4	14.0	14.2	15.5	16.7	17.4	17.2	17.1	15.6	14.3	12.3	10.3	9.2
Precipitation, mm															
2018	10	1	4	4	8	12	11	4	0	31	29	15	1	26	45
2019	10	19	21	15	34	6	21	8	5	17	22	10	9	44	15
1982-2019[1]	12	18	19	17	34	29	22	27	27	34	26	32	21	18	22

[1] Long term average.

Table 2. Dry matter yield of Alaska brome (AB) and smooth brome (SB) in the mixtures with lucerne (Lu) cultivars and in pure stands.[1]

	Dry matter yield, kg ha^{-1}							
	2018				2019			
	cut 1	cut 2	cut 3	Total	cut 1	cut 2	cut 3	Total
SB Lehis + Lu Karlu	4,270^{b}	430^{d}	760^{b}	5,460^{b}	4,440^{b}	1,790^{b}	1,560^{b}	7,790^{b}
AB Hakari+ Lu Karlu	3,060^{d}	850^{c}	890^{b}	4,800^{c}	2,840^{c}	2,800^{a}	2,290^{a}	7,930^{b}
SB Lehis + Lu Jõgeva 118	4,070^{bc}	310^{d}	550^{b}	4,930^{bc}	2,880^{c}	1,500^{b}	1,860^{b}	6,240^{c}
AB Hakari + Lu Jõgeva 118	2,920^{d}	780^{c}	770^{b}	4,470^{c}	3,190^{c}	2,560^{a}	2,380^{a}	8,130^{b}
SB Lehis	5,580^{a}	1,560^{b}	2,460^{a}	9,590^{a}	5,290^{a}	2,910^{a}	1,610^{b}	9,810^{a}
AB Hakari	3,710^{c}	2,720^{a}	2,950^{a}	9,390^{a}	4,270^{b}	2,650^{a}	1,130^{c}	8,050^{b}

[1] Within columns, mean values followed by the same letter are not significantly different at $P \leq 0.05$.

Table 3. Crude protein (CP) and neutral detergent fibre (NDF) concentrations (g kg^{-1} DM) in Alaska brome (AB) and smooth brome (SB) in pure stands and in the mixtures with lucerne (Lu) cultivars.[1]

	cut 1, 2018		cut 2, 2018		cut 3, 2018		cut 1, 2019		cut 2, 2019		cut 3, 2019	
	CP	NDF	CP	NDF	CP	NDF	CP	NDF	CP	NDF	CP	NDF
SB Lehis + Lu Karlu	90	498	113	494	150	504	132	555	162	439	208	409
AB Hakari + Lu Karlu	100	460	100	511	160	512	153	474	176	433	218	407
SB Lehis + Lu Jõgeva 118	88	523	116	484	155	493	130	518	167	428	208	406
AB Hakari + Lu Jõgeva 118	105	452	108	509	175	488	153	475	170	435	208	397
SB Lehis	118	566	158	509	145	514	109	587	110	507	139	493
AB Hakari	148	518	115	565	135	526	123	542	101	527	148	513
LSD, $P \leq 0.05$	6.2	17.2	11	13.9	11.5	13.4	10	16	13	16	7.9	9

[1] LSD = least significant difference.

Table 4. Proportion of lucerne (Lu) and grasses (Gr) in the yield, %.

	cut 1, 2018		cut 2, 2018		cut 3, 2018		cut 1, 2019		cut 2, 2019		cut 3, 2019	
	Lu	Gr	Lu	Gr	Lu	Gr	Lu	Gr	Lu	Gr	Lu	Gr
SB Lehis + Lu Karlu	28	72	9	91	15	85	17	83	50	50	81	19
AB Hakari + Lu Karlu	29	71	17	83	28	72	46	54	68	32	89	11
SB Lehis + Lu Jõgeva 118	15	85	12	88	25	75	27	73	53	47	82	18
AB Hakari + Lu Jõgeva 118	36	64	18	82	29	71	48	52	64	36	90	10

Various levels of disease resistance have been reported for AB. In our experiment, AB was found to be susceptible to the wheat stripe rust (yellow rust), whereas SB was resistant to it. Falloon *et al.* (1988) reported that AB is susceptible to head smut (*Ustilago bullata*). The disease was also found on our stands of AB. We found that SB was more susceptible to leaf spot (*Drechslera siccans*).

Conclusions

Smooth brome matured earlier than Alaska brome. SB needs earlier harvesting to assure the good quality of feed. AB has a faster regrowth compared to SB. Due to higher nutritional value, lesser competitiveness and matching developmental rhythm, AB proved to be a better companion for lucerne than SB. The largest difference in yield distribution occurred across years and was influenced mostly by weather conditions. Our preliminary results show that AB is less tolerant to different plant diseases than previously assumed.

References

Falloon R.E., Rolston M.P. and Hume D.E. (1988) Head smut of prairie grass and mountain brome: a review. *New Zealand Journal of Agricultural Research* 31, 459-466.

Hakari (2018) https://www.kingsagriseeds.com/wp-content/uploads/2018/02/Hakari.pdf.

Hakari Alaska Brome. https://www.forageseeds.com/product/barenbrug-seeds-hakari-alaska-brome-seed/.

[IUSS] Working Group WRB (2015) World reference base for soil resources 2014, update 2015. *World Soil Resource Reports* 106. FAO, Rome, Italy.

Tamm U., Meripõld H., Tamm S. and Edesi L. (2018) The nutritive value of Alaska brome and tall fescue forage using different growing technologies. *Grassland Science in Europe* 23, 363-365.

Tamm U., Meripõld H., Tamm S. and Tamm S. (2019) Alaska luste ja roog-aruheina erinevad viljelusviisid. *Agronoomia*, lk 178-183 (in Estonian).

Nutritional values of leaf and stem fractions in the second growth of timothy and meadow fescue

Termonen M., Korhonen P. and Virkajärvi P.
Natural Resources Institute Finland (Luke), Production Systems, Maaninka, Finland

Abstract

To measure the growth dynamics of timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.), a two-year field experiment was conducted in Maaninka, Finland. The aim was to follow how delaying the second cut affects the nutritional value of different biomass fractions. The leaf weight ratio (LWR), digestibility, neutral detergent fibre (NDF) and indigestible NDF were measured at each sampling time. The timothy LWR in the second cut decreased markedly when the harvest was delayed, while the meadow fescue LWR remained high and stable. In timothy, the D-value decreased rapidly especially in stems when the cutting interval was more than six weeks. Our results indicate large differences between the species in the second cut, both in the LWR and nutritive value. An earlier harvest of the second yield and higher investments on the spring and autumn yields could thus be worth considering when the aim is on high-quality feed. The optimal time for harvest can also be lengthened by including meadow fescue in seed mixtures due to the slower decrease in nutritive quality.

Keywords: forage grasses, leaf:stem ratio, silage, regrowth, nutritive value

Introduction

Timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.), the two most common forage grass species used in Finland, have different growth habits and stem formation dynamics during the course of the growing season (Virkajärvi and Järvenranta, 2001). Different growth patterns lead to different proportions of leaves and stems in the harvested biomass, which has a direct effect on the nutritional value of silage. The aim of this experiment is to describe how delaying the second cut affects the proportion of leaves in the biomass and the nutritional values of different biomass fractions in two species with different growth dynamics.

Materials and methods

The experiment was conducted in Maaninka, Finland (63°09'N, 27°20'E) in 2015-2016. The plots were established in 2014 using timothy (cv. Nuutti), meadow fescue (cv. Valtteri), and cover crop barley. The species were sown as identical randomized block experiments with 15 treatments and three replicates; eight treatments were used in this paper. The experimental design included two cutting times (T; early and normal) in the first cut which were both followed by four different cutting times (W; 5, 6, 7 and 8 weeks from the first cut) in the second cut. The plots with earlier first cut were harvested three times and fertilised using 100, 90 and 40 kg N ha^{-1} for the first, second and third harvests, respectively. The plots with the normal first cut timing were harvested two times, and both harvest yields were fertilised at 100 kg N ha^{-1}. Thus, the effect of timing in the first cut and N fertilisation in the second cut are indistinguishable when interpreting results of the second cut. The proportions of leaves (leaf weight ratio; LWR), stems (leaf sheaths included), inflorescences and weeds were quantified at each harvesting time. Each fraction was dried at 60 °C for 40-48 h to measure the dry matter (DM) content (%). The digestibility (D value) was analysed at Luke using the pepsin-cellulase method (Nousiainen *et al.*, 2003; Huhtanen *et al.*, 2006). The neutral detergent fibre (NDF) and indigestible NDF (iNDF) were measured at Valio Ltd by near infrared spectrometry (NIRS) technique (FOSS NIRSystems XDS Analyzer). Two or three treatment replicates were pooled into one sample if sample sizes were below those required by the NIRS analysis. The effective temperature sum was calculated for each harvesting date using data from the Finnish Meteorological Institute. The data were investigated using the *Mixed* model of SAS software 9.4. The species were analysed separately.

Results and discussion

The average effective temperature sum was 253 °C d in the early first cut and 302 °C d in the normal first cut. The average first yield LWR was 43% for timothy and 59% for meadow fescue. The early first cut increased the second yield D-value and decreased the NDF and iNDF concentrations with the same second yield growing time after the early and normal first cut (Table 1). This can be the consequence of a higher N fertiliser application rate for the plots with normal first cut timing or the higher effective temperature sum in the second cut. In timothy the same phenomenon was seen only in the NDF concentration.

The timothy LWR in the second cut decreased markedly when the harvest was delayed, as observed also by Nissinen *et al.* (2010), while the meadow fescue LWR remained high and stable (Table 1). The average content of inflorescences in the second cut was 0.2% in meadow fescue (D value 676 g kg^{-1} DM; n=1 pooled sample) and 0.5% in timothy (D value 711±18.1 g kg^{-1} DM; n=5 pooled samples; ± standard deviation). In timothy, the D value decreased at a faster rate in stems than in leaves whereas the D values of the meadow fescue leaves and stems were overall very high. The low D values in timothy stems are most likely due to formation of elongated vegetative tillers; these have a true stem that can comprise over half of the DM yield in the second cut in timothy (Virkajärvi *et al.*, 2012). In contrast to Nissinen *et al.* (2010), in our study the D value of timothy stems was considerably lower than the D value of timothy

Table 1. The leaf weight ratio (LWR), digestibility (D) value, neutral detergent fibre (NDF) and indisgestible NDF (iNDF) of stems and leaves in the second cut of timothy and meadow fescue after the early (ear.) or normal (nor.) first cut and 5, 6, 7 or 8 weeks (w) from the first cut.[1]

		Timothy							Meadow fescue						
	TS	LWR	D value		NDF		iNDF		LWR	D value		NDF		iNDF	
	°C d	%	g kg^{-1} DM		g kg^{-1} DM		g kg^{-1} DM		%	g kg^{-1} DM		g kg^{-1} DM		g kg^{-1} DM	
			stem	leaf	stem	leaf	stem	leaf		stem	leaf	stem	leaf	stem	leaf
ear.	459	59	662	706	588	533	80	46	86	750	736	532	497	57	42
nor.	487	57	652	708	595	533	89	57	86	756	727	532	510	53	50
SEM		0.8	6.3	2.1	6.3	3.7	4.2	2.5	0.7	3.6	3.3	2.9	4.5	2.0	2.0
5w	350	71c	680b	716b	614b	512a	78	43ab	86	746	742b	531	479a	53	32a
6w	429	62b	668b	708ab	597b	542b	77	39a	87	749	729a	540	503b	55	41b
7w	515	50a	638a	702a	590ab	543b	90	51b	86	756	731a	529	514b	55	49c
8w	599	48a	641a	701a	565a	535b	92	76c	84	761	724a	527	518b	56	61d
SEM		1.1	7.7	3.0	10.9	5.1	7.3	3.1	1.0	4.6	3.8	4.0	5.3	2.8	2.4
n		48	47	48	39	47	39	47	48	47	48	33	47	33	48
T		*	0					***			***		**	0	***
W		***	***	**	**	***		***		*	***	0	***		***
Y					*	0		0	*		0	*			
TW				*	0	***		***	0	0					
TY		***		*		*				***					
WY		**				*		0				*			
TWY		*						*				*			

[1] TS = effective temperature sum (base 5 °C). SEM = standard error of the mean; when n is varied, the highest SEM is presented. n = number of observations. Statistical significances: T = timing of the first cut (early or normal), W = growing weeks (5, 6, 7 or 8), Y = year (2015, 2016). *** = P<0.001, ** = P<0.01, * = P<0.05, o = P<0.10. Values marked with a different letter differ significantly (Tukey-Kramer's test; P<0.05).

leaves. The D values were generally high in this study compared to typical D values measured for the second cut yields.

Unexpectedly, the NDF concentration decreased in the timothy stems while it increased in the leaves of both species during the second yield observation period. The behaviour of the timothy stem fraction is in contrast with Bélanger and McQueen (1997) who observed an increase in the stem NDF with decreasing the LWR in the first cut. The amount of iNDF almost doubled during the observed period in the leaves of both species while it remained stable in the stems. The NDF and iNDF concentrations were generally lower in the meadow fescue than in the timothy.

Conclusions

Comparisons between growth processes of timothy and meadow fescue have rarely been made, and due to the experimental design it was not possible to statistically compare the two species in this experiment. However, our results indicate that large differences between the species can be expected in the second cut, both in the LWR and nutritive value. The reason behind the differences found is most likely the atypical stem formation of timothy compared with that of other species commonly used for grass silage production. In general, the nutritive value of timothy stems in the second cut was low, especially when the cutting interval was more than six weeks. An earlier harvest of the second cut and higher investments on the spring and autumn yields on the timothy-dominated swards could thus be worth considering when the aim is for high-quality feed. The optimal time for harvest can also be lengthened by including meadow fescue in seed mixtures due to its slower decrease in nutritive quality.

References

Bélanger G. and McQueen R.E. (1997) Leaf and stem nutritive value of timothy cultivars differing in maturity. *Canadian Journal of Plant Science*, 77(2), 237-249.

Huhtanen P., Nousiainen J. and Rinne M. (2006) Recent developments in forage evaluation with special reference to practical applications. *Agricultural and Food Science* 15(3), 293-323.

Nissinen O., Kalliainen P. and Jauhiainen L. (2010) Development of yield and nutritive value of timothy in primary growth and regrowth in northern growing conditions. *Agricultural and Food Science* 19, 252-268.

Nousiainen J., Rinne M., Hellämäki M. and Huhtanen P. (2003) Prediction of the digestibility of the primary growth of grass silages harvested at different stages of maturity from chemical composition and pepsin-cellulase solubility. *Animal Feed Science and Technology* 103, 97-111.

Virkajärvi P. and Järvenranta K. (2001) Leaf dynamics of timothy and meadow fescue under Nordic conditions. *Grass and Forage Science*, 56(3), 294-304.

Virkajärvi P., Pakarinen K., Hyrkäs M., Seppänen M. and Bélanger G. (2012) Tiller characteristics of timothy and tall fescue in relation to herbage mass accumulation. *Crop Science* 52(2), 970-980.

Grass yield enhancement network (YEN)

Wade R.N., Evans K., Jepson M., Martyn T. and Berry P.
ADAS Spring Lodge, 172 Chester Road, Helsby, WA6 0AR, United Kingdom

Abstract

Home-grown grass-based forage usually provides the most economical, high quality livestock feed, but it is recognised that many farmers do not fully exploit this resource. Grass Yield Enhancement Network (Grass YEN) aims to increase yields of high-quality forage through supporting farm innovators and developing platforms for industry-science interactions. Grass YEN has been running for two seasons (2018 and 2019) with more than 20 UK farmers. Growers submit soil, husbandry and yield data from two silage cuts per year, which is compared against data from other growers and benchmark values, to identify areas for improvement. We developed a model to calculate site-specific potential forage yields from available natural resources (water and light) at any particular location. We estimated UK potential grass yields of 20-25 Mg of dry matter per hectare per annum, yet most growers achieve less than half of this and even the best growers in the Grass YEN only achieved 70% of their potential. Conserved grass yields are rarely measured. Therefore, to encourage growers to quantify their grass yields, we tested different methods of measuring yield. The knowledge gained through Grass YEN will be used to develop hypotheses to be tested experimentally, supporting innovations for grass yield enhancement.

Keywords: forage, yield, farmer engagement

Introduction

Home-grown forage can provide the most economical, high quality feed source for ruminant livestock in the UK, yet it is generally recognised that this feed source is not fully exploited. Part of the reason for this lack of exploitation is that forage yield in the UK is substantially less than the potential forage yields calculated for the UK. Average UK grass yields achieved on-farm of 6-10 Mg ha^{-1} dry matter (DM) are less than half of the biological potential for the UK environment, which are estimated to be over 20 Mg DM ha^{-1} (Alberda, 1977; Leafe, 1978; Sylvester-Bradley *et al.,* 2005). A key problem associated with raising forage production is a lack of quantitative measurements of quality and quantity of forage produced, particularly for conserved grass. Without measurements of yield and quality it is not possible to understand how to improve productivity. To address these questions, we have created a Grass Yield Enhancement Network (Grass YEN) to gain a better understanding of commercial grass yields, develop practical methods of measuring yield and assess how much these yields differ from site-specific potential yields.

Materials and methods

ADAS (Agricultural and Environmental Consultancy Company) together with industrial partners, established the Grass YEN in 2018 sponsoring up to 15 growers each year to enter a forage competition over two years. Each grower submitted husbandry and yield information of two cuts per year. Yield was measured by weighing the forage biomass of five 3 m lengths of swath from a minimum area of one hectare. Representative subsamples of forage were analysed for moisture content, metabolizable energy and crude protein content. ADAS developed a simple but plausible grass growth model which calculates potential forage growth using local radiation, rainfall and temperature data (provided by Iteris, Applied Informatics Consultancy Company) and soil texture and rootable soil depth to inform water availability, and then uses the maximum plausible values of resource capture and conversion parameters that can be found in the scientific literature to calculate bio-physically possible forage yields. The model, performed in R studio (version 1.1.456), first calculates daily biomass production as a product of radiation interception

(100%) and conversion of radiation to biomass (1.4 Mg forage/TJ solar radiation); then calculates the amount of daily water required to create this biomass (5.5 Mg biomass per 100 mm water). Assuming that the soil begins the season at field capacity, the amount of water available for crop uptake was calculated as 75% of plant available water to a depth of 1.5 m plus daily rainfall. If soil water is available, then biomass is accumulated; alternatively if there is no soil water available no biomass is accumulated. Zero forage growth was assumed below 6 °C air mean daily temperature. The grower's measured yield was then expressed as a percentage of the estimated potential yield. The competition also categorized entries based on grass-clover composition and age. In total, 44 forage crops (first and second cuts) were entered into Grass YEN in 2018 and 2019. The majority of Grass YEN entries were 35 pure grass fields and 9 grass-clover mixtures, predominantly planted with perennial ryegrass mixes from Germinal (Aber Magic), Sinclair Mcgill Castlehill, Oliver seeds (Sabre), Barenbrug and own mixes.

Results and discussion

The method of measuring yield was practical, but was regarded as quite time consuming and there were occasional issues with coinciding the yield measurements with silage contractors. The quality and quantity of UK forage yields show very large ranges in yields and percentage of potential yields (Table 1) providing further evidence that there are major opportunities to improve yields. In 2018, first cut yields (cut between 07/05/2018 and 05/07/2018) were higher than second cut yields (cut between 05/06/2018 and 14/09/2018), despite higher solar radiation available for second cuts (when expressed as a percentage of the potential yield). The summer in 2018 was dry and this result suggests that water availability can be a large constraint to second cut yields and that rooting depth could be key to achieving higher yields. In 2019, the summer was wetter and there was little difference between the yields of the first (cut between 03/05/2019 and 15/07/2019) and second cuts (cut between 21/06/2019 and 24/08/2019), expressed as a percentage of the potential yield. In 2019 we found that first cut forage entered in the 1-yr-old grass category achieved the highest average yields at 9.1 Mg DM ha^{-1}, compared with the <3yr-old grass category which achieved an average of 6.8 Mg DM ha^{-1} and >3-yr-old grass category which achieved an average of 7.1 Mg DM ha^{-1}. There was a large gap between potential total grass yield of >24 Mg ha^{-1} and current UK yields (Figure 1) with yield potentials often better in the west than in the east, primarily due to greater rainfall levels. Two crops (one in north-east Ireland and one on Orkney Island) achieved more than the estimated potential yield, which may have been because the roots of these crops extended beyond the assumed maximum depth of 1.5 m.

Table 1. Summary of grass YEN measurements 2018 and 2019, showing, minimum, maximum and average values.

	2018			2019		
	Min.	Max.	Av	Min.	Max.	Av.
1st cut date (DD/MM)	29/05	05/07	14/05	03/05	12/06	25/05
2nd cut date (DD/MM)	05/06	14/09	30/06	21/06	24/08	25/07
1st cut yield (Mg ha^{-1})	3.1	20.2	6.8	2.6	13.3	7.4
2nd cut yield (Mg ha^{-1})	3.1	8.1	4.8	4.4	7.4	5.5
1st cut % of potential yield (%)	33	110	64	23	102	62
2nd cut % of potential yield (%)	36	65	50	37	90	61
1st cut ME (MJ kg^{-1})	10.7	12.0	11.3	9.6	11.6	10.8
2nd cut ME (MJ kg^{-1})	NA	NA	NA	9.7	11.8	10.9
1st cut crude protein (%, N×6.25)	2.60	3.60	2.38	2.40	7.30	3.74
2nd cut crude protein (%, N×6.25)	0.70	6.70	3.40	2.10	3.90	3.03

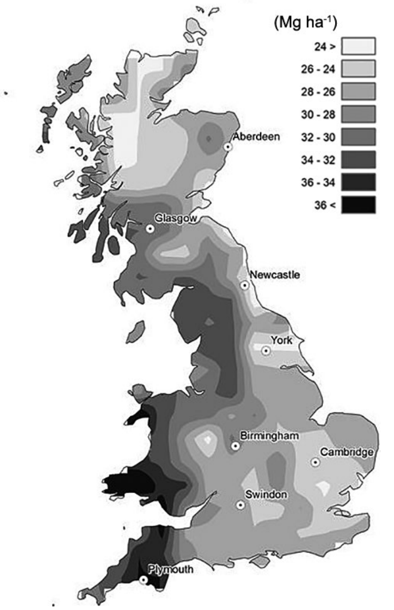

Figure 1. Map of potential yields across the UK for February to September growing period calculated from long term historic data over 20 years (from 1980 to 2010).

Conclusions

Measuring grass yields is difficult but imperative to further our understanding of yield formation. Weighing lengths of swath was achievable but more practical methods of measuring grass at a commercial scale are required. On average there is a large gap between potential forage yield and what is achieved on-farm. However, some farmers did get close to their potential yield, which indicates there are significant opportunities to increase yield by exploiting current crop management technologies. From the first two years of data we showed that growers need to check rooting depth and nutrient supply to ensure these are adequate for high yields. Grass YEN aims to identify further opportunities to increase yield when the dataset is large enough to gain significant insights. The Grass YEN demonstrated a successful approach for creating a network of farmers, industry and scientists with a common interest of increasing forage productivity.

Acknowledgements

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References

Alberda T. (1977) Possibilities of dry matter production from forage plants under different climatic conditions. In: *Proceedings of the 13th International Grassland Congress*, Leipzig, Germany, pp. 61-69.

Leafe E.L. (1978) Physiological, environmental and management factors of importance to maximum yield of the grass crop. In: Gasser J.K.R. and Wilkinson B. (eds.) *Maximizing yields of crops*. ARC Symposium Proceedings, HMSO, London, UK.

Sylvester-Bradley R., Berry P.M., Wiseman J. and Cottrill B. (2005) *Yields of UK crops and livestock: physiological and technological constraints, and expectations of progress to 2050*. DEFRA Project No. IS0210. Final Report, 21 pp.

Challenges in ley farming systems based on legumes in Sweden

Wallenhammar A.-C.[1], Edin E.[2], Omer Z.[3] and Granstedt A.[4]

[1]*Research and Development, The Rural Economy and Agricultural Society, Gamla vägen 5 G, 702 27 Örebro, Sweden;* [2]*Brunnby Gård, 725 97 Västerås, Sweden;* [3]*P.O. Box 412, 751 06 Uppsala, Sweden;* [4]*Foundation of Biodynamic Research Institute, SBFI, Skilleby, 153 91 Järna, Sweden*

Abstract

Red clover (RC; *Trifolium pratense* L.) is an important forage legume in Sweden, cultivated in mixtures with grass. RC is considered a short-lived legume due to its poor persistence, originating in root rot. The objectives were to identify strategies for sustainable legume cropping. Persistence and production capacity of white clover (WC; *Trifolium repens* L.), lucerne (LU; *Medicago sativa* L.), birdsfoot trefoil (BFT; *Lotus corniculatus* L.) and RC grown in mixed swards with timothy (TI; *Phleum pratense* L.) was compared in a field experiment during 3 years. Two or three harvests were taken with a forage harvester and total DM yields and the proportion of legumes was quantified. The prevalence of root rot was visually assessed in uprooted plants and fungal pathogens in the roots were quantified by real-time PCR. LU continued to show an extreme competitive yield and high legume content the third harvest year (2018). Disease severity index (DSI) of the visual symptoms, was significantly highest in red clover in the third harvest year at Nibble, Järna. Real-time PCR analyses showed prevalence of *Fusarium avenaceum*, *Phoma medicaginis* and *Cylindrocarpon destructans* in all tested legumes, thus indicating that birdsfoot trefoil and lucerne show tolerance to these pathogens.

Keywords: red clover, white clover, birdsfoot trefoil, lucerne, root rot, qPCR

Introduction

Production of locally produced protein crops is of great significance in order to strengthen competitiveness and improve the nutritional economy of Swedish livestock production. Red clover (RC) is the most cultivated forage legume species, with high protein content and beneficial properties for feed consumption by ruminants. RC is considered a short-lived legume due to its poor persistence, originating in root rot caused by infection of soil-borne pathogens; *Fusarium avenaceum*, *Phoma medicaginis* and *Cylindrocarpon destructans* (Almquist *et al.*, 2016; Öhberg, 2008; Rufelt, 1986). No significant differences in persistence have been shown in previous studies of RC cultivars of Scandinavian origin (Almquist, 2016Wallenhammar, 2010). The objectives were to identify and develop strategies for sustainable forage legume cropping. Yield and disease severity of root rot was compared in RC, WC, LU and BFT intercropped with TI over three years. This paper focuses on results from the third harvest year.

Materials and methods

A field experiment was established in 2015 at Nibble, Järna (N 59° 19', E 18°4'). Field plots (4.5×10 m) of RC, WC, BFT and LU were sown individually, each in a mixture with TI, in a randomized block design with four replicate blocks. Seed rates were: RC cv. SW Vicky sown at 8.0 kg ha^{-1}, WC cv. Hebe at 4.0 kg ha^{-1}, BFT cv. Oberhaunstedter at 10.0 kg ha^{-1} and LU cv. Marshall at 17 kg ha^{-1}, all in mixed plots with TI cv. Lischka at 8.0 kg ha^{-1}. The LU seed was inoculated with *Sinorhizobium meliloti* and the BFT seed was inoculated with *Rhizobium loti*. No manure or other fertilisers were added. All plots were seeded with oats (200 kg ha^{-1}) as nurse crop established prior to the forage seed. Plots were harvested three times (in 2018: 30 May; 24 July; 3 October) (Table 1), with a forage harvester at a height of 5-7 cm, except for plots of BFT, which were harvested twice (30 May and 13 August). Fresh weights were measured, and the botanical composition analysed from a 500 g subsample from each plot. Dry matter yields were calculated on a 1000 g subsamples pre-dried at 60 °C for 24 h and thereafter dried at 105 °C for at least

3 h. Ten roots of the legume plants were dug out per plot in late October, transported to the laboratory and rinsed in running tap water. Visual assessment of root rot symptoms was performed according to Rufelt (1986). The roots were cut lengthwise with a scalpel and were assessed and classified as follows: 0 = no root rot or discolouring; 1 = the root is discoloured; 2 = minor root rot in parts of the root, the remaining part can be discoloured; 3 = at least one third of the root is rotten, some of the plant shoots are dead; and 4 = at least two thirds of the root is rotten, many of the plant shoots are dead. Disease severity indices (DSI) were calculated according to Rufelt (1986). DNA was extracted from 250 mg subsamples of ten roots per plot. Details regarding DNA extraction methods, primers, probes and qPCR analyses are described in Almquist (2016). Statistical analyses were performed by analysis of variance (ANOVA). Data were analysed using Tukey´s HSD.

Results and discussion

Dry matter (DM) yield in the first harvest year and the composition of legumes, grass and weeds at Nibble are shown in Table 1. The LU-TI treatment produced the highest DM yield and was significantly higher than the other treatments. Treatments RC-TI, BFT-TI and LU-TI showed significantly higher legume content than WC-TI. The proportion of TI was lowest in LU-TI treatment, reflecting the competitive properties of LU.

Disease severity index (DSI) for external and internal injuries was significantly higher in RC than LU, BFT and WC (Table 2). The real-time PCR analyses showed presence of the three analysed pathogens *F. avenaceum*, *C. destructans* and *P. medicaginis* in all legume species (data not shown). The results indicated that LU and BFT are more tolerant to the tested pathogens. On the other hand, WC improves its sustainability by a continuous formation of new stolons and shoots.

Table 1. Dry matter yield (Mg ha^{-1}), dry matter content (DM) of red clover, white clover, birdsfoot trefoil and lucerne with companion grass and composition of species at Nibble, Järna in the third harvest year (2018).[1]

Treatments	Forage yield					Composition of species, average of 3 cuts (%)			
	1st cut (Mg DM ha^{-1})	2nd cut (Mg DM ha^{-1})	3rd cut (Mg DM ha^{-1})	Total (Mg DM ha^{-1})	DM (%)	Sown legume	Other legume	Timothy	Weed
RC-TI	1,439	669^{c}	0^{a}	2,108^{b}	30.2	44.4^{b}	2.4^{ab}	36.3^{b}	16.8^{ab}
WC-TI	1,641	477^{c}	0^{b}	2,118^{b}	33.0	14.7^{c}	0^{b}	66.0^{a}	16.1^{a}
BFT-TI	1,865	1,377^{b}		3,243^{b}	34.4	46.6^{b}	3.7^{a}	37.2^{b}	12.5^{bc}
LU-TI	1,960	1,874^{a}	1,443^{a}	5,307^{a}	26.7	71.7^{a}	2.2^{ab}	16.0^{c}	10.1^{c}
P-value	ns	<0.001	0.003	0.030	<0.001	<0.001	0.011	<0.001	<0.031

[1] Different letters indicate significant differences according to Tukey's HSD-test (P<0.05).

Table 2. External ($_E$) and internal ($_I$) disease severity index (DSI) and disease incidence (DI) of RC, WC, BFT and LU at Nibble, Järna, the third harvest year (2018).[1]

Treatments	DSI_E	DSI_I	DI_E (%)	DI_I (%)
RC-TI	89^{a}	94^{a}	100^{a}	88
WC-TI	41^{b}	30^{b}	93^{ab}	100
BFT-TI	22^{c}	44^{b}	78^{b}	100
LU-TI	45^{b}	53^{b}	100^{a}	83
P-value	<0.001	<0.001	0.022	<0.001

[1] Different letters indicate significant differences according to Tukey's HSD-test (P<0.05).

The results give a clear message regarding species selection in extremely dry and hot conditions as in 2018, and provide guidance for legume production in a climate where the temperatures exceed the normal temperature of today. LU has shown very high production capacity and the total crop surpassed WC and RC by 250% in 2018. The RC-plants were weakened by disease and also WC was severely affected by the drought and heat during 2018 and no third harvest was taken on 3 October. BFT-TI had a different harvest interval, and the total yield was 53% higher and significantly different from RC-TI and WC-TI. A successful establishment of LU with a new inoculant enabled growth during the following dry summer and this promotes LU as an interesting legume crop of high productive capacity in a sustainable Swedish forage production system (Wallenhammar *et al.*, 2019). For the grower, it is important to adapt legume species according to the production direction, site of cultivation and requirements for feed quality so that the overall forage production is sustained.

Conclusions

Swards containing LU and BFT showed significantly higher yield and high drought resistance in the third harvest year. Legume content was significantly higher for LU than RC, WC and BFT. RC suffered severely from root rot and showed significantly higher injuries than WC, BFT and LU. Molecular detection showed prevalence of the target pathogens in all of the legume species; hence, BFT and LU show a tolerance to severe disease symptoms.

Acknowledgements

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References

Almquist C. (2016). *Monitoring important soil-borne plant pathogens in Swedish crop production using real-time PCR*. Doctoral Thesis. Department of Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden. 78 pp.

Almquist C., Stoltz E., and Wallenhammar A-C., (2016) Incidence of root pathogens associated to clover root rot in Sweden. *Grassland Science in Europe* 21, 786-788.

Öhberg, H. (2008) *Studies of the persistence of red clover cultivars in Sweden.* Doctoral Thesis. Department of Agricultural Research for Northern Sweden. Swedish University of Agricultural Sciences, Umeå, Sweden. 46 pp.

Rufelt, S. (1986) *Studies on* Fusarium *root rot of red clover (*Trifolium pratense *L.) and the potential for its control*. Doctoral thesis, Department of Plant and Forest Protection. Swedish University of Agricultural Sciences, Uppsala, Sweden. 28 pp.

Wallenhammar A.-C., Stoltz E., Omer Z. and Granstedt A. (2019) Production capacity of forage legumes and persistence to root rot in organic mixed swards. *Grassland Science in Europe* 24, 99.

Theme 2.
Grasses in animal nutrition

Methane mitigating options with forages fed to ruminants

Eugène M.[1], Klumpp K.[2] and Sauvant D.[3,4]
[1]INRAE – VetAgroSup UMR 1213 Unité Mixte de Recherche sur les Herbivores, Centre de recherche Auvergne-Rhône-Alpes, Theix, 63122 Saint-Genès-Champanelle, France; [2]INRAE – VetAgro Sup, UMR 0874 UREP Unité Mixte de Recherche sur l'Ecosystème Prairial, Centre de recherche Auvergne-Rhône-Alpes, Clermont-Ferrand, France; [3]INRAE and [4]AgroParisTech, UMR 791 MoSAR, 16 rue Claude Bernard, 75231 Paris Cedex 05, France

Abstract

Enteric methane (CH_4) contributes up to 40% of livestock-related greenhouse gas (GHG) emissions. Nutritional strategies, including feed management measures, are promising measures for CH_4 and overall GHG reduction. These differ in their viability, cost, and acceptance by producers. Evidence from literature is reviewed in relation to effects of forage quality (digestibility: DOM) and forage type (grasses vs legumes, and maize). The major determinants of forage quality are the stage of growth and species mixture. With increased stage of growth, the fibre contents also increase and DOM decreases. Using legumes in ruminant systems can reduce overall GHG emissions due to decreased N fertiliser use. Dietary measures are recommended that reduce N excreted by better matching dietary protein to animal needs, shifting N excretion from urine to faeces (by tannin inclusion at low levels) and reducing the amount of fermentable organic matter excreted. Reduced CH_4 emissions from ruminants fed on forage-based diets will decrease the overall contribution of livestock and agriculture to total GHG emissions. Employing these emerging strategies will allow improved productive efficiency of ruminants in both developing and developed countries. Net CH_4 output needs to take account of enteric CH_4 emissions as well as soil C sequestration from feed (grassland) management. Multi-criteria assessment of GHG mitigations based on forages, such as life cycle analysis or process-based modelling, are required to take into account the interactions and trade off/synergy between different GHG.

Keywords: methane reduction, forage quality, forage type, fertiliser, modelling

Introduction

Livestock production is responsible of some environmental burdens and it is estimated that around 14.5% of global warming is due to livestock-related greenhouse gas (GHG) emissions (Gerber *et al.,* 2013a). Among GHG, enteric methane (CH_4) produced in the rumen of ruminants contributes up to 40% of livestock GHG (Gerber *et al.,* 2013a). Consequently, several nutritional strategies to mitigate enteric CH_4 have been studied and developed (Hristov *et al.,* 2013) and they differ in terms of their viability, cost, and acceptance by producers. To be adopted, these strategies should provide similar or increased animal performance and economic viability, while also reducing CH_4 intensity (emissions per unit of milk or meat) and also that of other sources of GHG such as N_2O from crop fertilisers/manure and CO_2 from feed production (Gerber *et al.,* 2013a; Pereira *et al.,* 2015). Among the different options, feed and feed management measures are the most promising strategies to reduce enteric CH_4; they include enhanced forage quality, feed processing and livestock precision feeding (Gerber *et al.,* 2013b). Animal performance improvement will be achieved in production systems (mainly those related to efficient forage use) associated with good management of nutrition, health and reproduction (Pereira *et al.,* 2015).

Forage quality

Increasing forage digestibility and digestible forage intake is one of the main recommended CH_4 mitigation practices (Hristov *et al.,* 2013). The effect of forage organic matter (OM) digestibility on CH_4 emissions has been studied for different systems of forage utilisation, like fresh herbage or silage, and for different forage types, such as grass, legume or maize, and for different animal categories (Phelan *et al.,* 2015; Van Gastelen *et al.,* 2019). The response in CH_4 emissions is not consistent and depends on the unit as well as the animal category considered (Van Gastelen *et al.,* 2019). When CH_4 emission (g d^{-1}) increased, forage digestibility resulted in increased dry matter intake (DMI, kg d^{-1}) and consequently increased CH_4 emission (g d^{-1}) for dairy and beef cattle but not for sheep. When intake or production was taken into account the CH_4 yield (g kg^{-1} DMI) and CH_4 intensity (g kg^{-1} milk) decreased with increased digestibility for dairy cattle, but no difference was observed for beef cattle. For sheep, CH_4 yield (g kg^{-1} DMI) decreased with increased forage digestibility (Van Gastelen *et al.,* 2019).

Several equations have been developed in recent years for different animals fed forages or on pasture (Archimède *et al.,* 2011; Ellis *et al.,* 2007; Escobar-Bahamondes *et al.,* 2017; Eugène *et al.,* 2014; Rico *et al.,* 2016; Singh *et al.,* 2012; Suzuki *et al.,* 2018; Van Lingen *et al.,* 2019; Zao *et al.,* 2016). A specific equation was developed using the 'Methafour' database that gathers results for CH_4 emission, measured using the currently applicable measurement techniques, in ruminants fed only with forages, and used for the GHG inventory methodology and in the INRA feeding system (INRA, 2018). It consists of the intra-experiment equation based on the combination of feeding level (FL, DMI% of body weight (BW)) and neutral detergent fibre (NDF) content of forage already proposed by Eugène *et al.* (2014), improved by taking into account the digested organic matter (DOM) content of the forage. The specific effect of type of forage and species is not significant and cannot be assessed because there are only a few direct comparisons in *in vivo* trials: CH_4 (g kg^{-1} DOM)=34.95 – 4.05 × FL + 0.027 × NDF – 0.010 × DOM. (n of data point s= 412, number of trials=153, root-mean-square error (RMSE)=3.1) (INRA, 2018). Where CH_4 (g kg^{-1} DOM) (34.7±9.1, min=10.9, max=69.1 g kg^{-1} DOM) is the CH_4 production per kg DOM, NDF content (528±129 g kg^{-1} dry matter (DM)) and DOM (596±80 g kg^{-1} DM).

Furthermore, from a large dataset of forage data, Sauvant *et al.* (2011) reported a positive curvilinear relationship between CH_4 yield (22.3±5.8 g kg^{-1} DMI, min=6.8; max=36.1) and DOM (612±78 g kg^{-1} DM, min=407, max=797) and a negative relationship with feeding level (DMI%BW: CH_4 (g kg^{-1}DMI) = -22.4 – 2.25 × DMI%BW + 0.137 DOM (g kg^{-1} DM) – 0.00009 DOM^2 (g kg^{-1} DM); (n=283, n trials=53, RMSE=1.6 g kg^{-1} DMI). According to this relationship, for poor forages (DOM=400 to 450 g kg^{-1} DM) the marginal response to DOM increase (delta CH_4 /delta DOM) is important (around 60 g kg^{-1} DOM). For these forages the average CH_4 production is around 40 g kg^{-1} DOM. For better forages the marginal response (delta CH_4 /delta DOM) decreases up to 0 for DOM contents of 760 g kg^{-1} DM which then corresponds to an average CH_4 production of about 36 g kg^{-1} DOM.

This is in agreement with previous findings where increased organic matter digestibility (OMD) of forage was observed, especially in dairy cattle where some concentrate supplementation was proposed. Differences between animals (bovine vs sheep) could be linked to mean retention time differences and fractional degradation rates (Poppi *et al.,* 1981; Siddons and Paradine, 1983). Poppi *et al.* (1981) reported larger mean retention time and lower fractional degradation rates in beef and dairy cattle than in sheep.

The major factor of forage quality is the stage of growth; according to the 'Methafour' database (INRA, 2018) when the stage of growth increases there is an increase in crude fibre (CF) (3.72±3.28 g kg^{-1} DM) and NDF contents (4.78±3.54 g kg^{-1} DM) for 10 days of growth, and therefore a decrease of quality. However, there is also a decline in the level of DMI/BW (-0.15±0.08 g kg^{-1} BW for 10 days). This last parameter has a dominant effect on CH_4 production, which increases per unit of DMI (0.145±0.106 g kg^{-1} DM for 10 days) but not per kg BW. The mean decrease of CH_4 in relation to DMI is of 0.51±0.06 g kg^{-1} DM.

Forage type and conservation methods

Legumes vs grass

Feeding forages, especially legumes (rich in protein), could represent an interesting strategy to provide N to the animal and decrease CH_4 emissions, thus enhancing animal productivity (growth, milk and wool production) and reducing gaseous emissions (CH_4 and ammonia) that affect climate change (Makkar, 2003; Reed, 1995). The ability of such forages to fight against the effects of gastrointestinal parasitic nematodes is also most important (Makkar, 2003). *In vitro*, it has been shown that forages containing condensed tannins or polyphenol oxidase enzymes have reduced rumen protein degradation (Makkar, 2003); ruminants seem to capture these proteins more efficiently for meat and milk. However, more evidence is required using *in vivo* production experiments. A quantitative review by meta-analysis was conducted to assess the quantitative effects of tannins on CH_4 emissions (Eugène *et al.*, 2019). Although several reviews have been published on this topic (Jayanegara *et al.*, 2012), few general equations, mostly derived from *in vitro* trials, have been published because of the diversity of methods and types of tannins. Using the Methafour database (INRA, 2018), which is composed of studies collected from the literature on the effect of forages fed to ruminants on CH_4 emissions, it was possible to significantly complete the equation:

$$CH_4\ (\text{g kg}^{-1}\ \text{DOM}) = 34.95 - 4.05\ \text{FL} + 0.027\ \text{NDF} - 0.010\ \text{DOM}$$
$$(\text{n}=412,\ \text{n trials}=153,\ \text{RMSE}=3.1\ \text{g kg}^{-1}\ \text{DOM}) \qquad (1)$$

when taking tannins content into account (TAN, g kg^{-1} DM).

$$CH_4\ (\text{g kg}^{-1}\ \text{DOM}) = 34.26 - 3.96\ \text{FL} + 0.027\ \text{NDF} - 0.008\ \text{DOM} - 1.72\ \text{Log}_{10}\ (1+\text{TAN})$$
$$(\text{n}=398,\ \text{n trials}=147,\ \text{RMSE}=3.1) \qquad (2)$$

where CH_4 (g kg^{-1} DOM) (34.7±9.1, min=10.9, max=69.1 g kg^{-1} DOM)) is the CH_4 production per kg digested organic matter, FL is the feeding level (DMI%BW), NDF the NDF content (g kg^{-1} DM) and DOM is the digested OM content (g kg^{-1} DM) and TAN is the tannin content (g kg^{-1} DM) transformed on a logarithmic basis because of its largely abnormal distribution (Sauvant *et al.*, 2018). In this dataset, only 13 experiments of 147 could be used to study the influence of tannins. The coefficients of regression of other variables remain fairly stable between the two Equations 1 and 2. Based on current scientific knowledge we propose to use the coefficient of TAN in Equation 2 to evaluate the average quantitative effect of tannins in all types of diets. However, as described above, more data are needed to fully assess the differential effects of the wide variety of tannins in all diets, concerning the structure/activity relationships of tannins (condensed or hydrolysable), the persistency of the effects.

There are some limitations for the use of forage legumes, because under grazing legumes are less persistent than grasses, they are rich in degradable protein that predisposes livestock to bloat, and they are more difficult to conserve as silage or hay (Phelan *et al.,* 2015). When compared to ruminant systems based on grass or cereals supplemented with fertiliser N, forage legume-based ruminant systems tend to have less negative environmental impact on biodiversity, less N loss to water and lower greenhouse gas emissions (Phelan *et al.*, 2015). Although these legume forages generally have lower yields and persistence, genetic modification would allow insertion of these traits into more widely cultivated forages (Broderick, 2018). As the soluble carbohydrate content of legume forages is low compared with grass forages, the use of supplements rich in starch (cereals) is required (Ruckle *et al.,* 2017). However, leaf starch content may be high in species such as red clover and they have the genetic potential to accumulate up to one third of their leaf dry mass as starch (Ruckle *et al.,* 2017).

Table 1. Effect of forage type on CH_4 emissions, expressed as l kg^{-1} dry matter intake (DMI), l kg^{-1} organic matter intake (OMI), l kg^{-1} digested OM (DOM) (Archimède *et al.,* 2011).[1]

		CH_4 (l kg^{-1} DMI)	CH_4 (l kg^{-1} OMI)	CH_4 (l kg^{-1} DOM)
Grasses	C3 type	30.0^{b}	33.1^{b}	52.1^{b}
	C4 type	33.7^{c}	38.8^{c}	57.7^{b}
Legumes	Cool	30.1^{bc}	33.7^{bc}	52.4^{b}
	Warm	25.9^{a}	27.2^{a}	40.7^{a}
SEM		1.8	1.8	2.9
P-value		0.001	0.001	0.001

[1] Superscripts: mean values within column carrying no common letters are significantly different at $P<0.05$. SEM = standard error of the mean.

Forages rich in plant secondary compounds, such as tannins, have been studied both for their nutritional effects on animal productivity (Reed, 1995) and for their antimethanogenic properties (Doreau *et al.* 2011; Jayanegara *et al.,* 2012, 2015). Condensed tannins (CTs) account for up to 20% of the dry matter in forage legumes used as ruminant feeds. Compared with temperate forages, tropical forages have a lower digestibility and differ in their chemical and structural composition (Leng, 1990). Ruminants fed tropical grass forages seem to have increased CH_4 emissions compared with those fed on tropical legume forages (Archimède *et al.,* 2011; Eugène *et al.,* 2014).

However, the mitigating effect of tannins on CH_4 is inconsistent (Beauchemin *et al.,* 2008; Makkar, 2003). The discrepancies of responses of tannins among different studies are attributed to the different tannin concentrations in the diet, chemical structures of tannins, and type of diets. Recent research has also highlighted the importance of their molecular structures (Mueller-Harvey *et al.,* 2019). An establishment of structure-activity relationship would be required to explain differences among studies and obtain consistent beneficial tannin effects (Patra and Saxena, 2011).

More interestingly, legume silage (Hristov *et al.,* 2013) or mixtures of grass plus legumes (Phelan *et al.,* 2015) and the use of legume as an intercropping culture (Hassen *et al.,* 2017) could increase the forage productivity at the system level and help preserve biodiversity, reduce N losses to water and reduce greenhouse gas emissions.

Silages

Maize silage

Although responses vary, CH_4 emissions can be reduced when maize silage replaces grass silage in the diet (Hristov *et al.,* 2013). Several studies have compared the CH_4 emission of ruminants fed maize silage as a replacement for grass silage or legume silage, and these were summarised in Van Gastelen *et al.* (2019).

Different responses in CH_4 emissions and intake have been observed between dairy, beef and sheep. There was a quadratic effect on CH_4 yield reported by Jonker *et al.* (2016) when maize silage replaced lucerne silage fed to sheep at intake level of 2% BW. Methane yield (g kg^{-1} of DMI; % of gross energy intake (GEI)) increased with up to 50% supplement inclusion in the diet, and then decreased with greater supplement inclusion, but the level did not fall below that for 100% lucerne silage. Van Gastelen *et al.* (2019) reported that in dairy cattle, increased levels of maize silage resulted in decreased CH_4 yield (g kg^{-1} DMI; MJ MJ^{-1} GEI) on average, and the relationship was quadratic in some studies (Arndt *et al.,* 2015; Hassanat *et al.,* 2013; Van Gastelen *et al.,* 2019). Several factors may be responsible for the responses observed; first of all DMI and consequently intake level (DMI%BW), feed digestibility, and retention time in the rumen. Moreover, there might be a starch concentration threshold that orientates the fermentation in the rumen towards more propionate formation (Hassanat *et al.,* 2013), but this was not evidenced in Jonker *et al.* (2016), in sheep fed increasing levels of maize silage in replacement of lucerne silage. The propionate production decreases methanogenesis (Moss *et al.,* 2000).

Similarly, when maize silage was fed to feedlot cattle in their finishing phase in replacement of barley silage, Beauchemin and McGinn (2005) indicated that CH_4 yield (g kg^{-1} DMI; MJ MJ^{-1} GEI) was decreased. Nevertheless, this was not the case during the background phase of these animals, when intake level was lower no difference was observed. Although maize silage decreases enteric CH_4 production, manure CH_4 could increase due to increased output of fermentable OM. Especially soil CO_2 emissions are much greater for maize silage compared with grass silage.

Grass silage

Some studies and literature reviews indicate that the improvement of digestibility of grass silage could lead to reduced methanogenesis (Van Gastelen *et al.,* 2019). Because of the adequate stage of maturity at which grass swards are harvested for silage, they can have lower fibre concentration, higher fibre digestibility and also higher N content. Reduced retention time in the rumen with high-quality forage-based diets (Dewhurst *et al.,* 2009) decreases methanogenesis. These strategies are most effective for dairy cattle, are effective for beef cattle to a certain extent, but seem to have minor or no effects in sheep (Van Gastelen *et al.,* 2019).

Mitigation through C sequestration of grazed grasslands

The GHG balance of growing ruminants shows a mean partition of ingested C between faeces (29%), CH_4 (4%), urine (4.5%), CO_2 (58%) and a value of 4.5% for the ultimate C balance (i.e. calorimetric studies of the 'Rumener' database; Sauvant and Giger-Reverdin (2009)). In lactating ruminants, the corresponding values are 29, 3.5, 3.5, 42 and 3% with, moreover, 21% in milk. The majority of ingested C is partitioned into CO_2 emitted into the air (around 50% for cattle), and as faecal C (around 30%, which is returned to grassland), followed by urine, CH_4, milk and C balance (Sauvant and Giger-Reverdin, 2009).

There is evidence that the GHG balance of ruminants can be improved by considering grass-fed systems and the related capacity of grassland to sequester C in soil. In general, grasslands have a higher soil organic matter content and soil C in grassland has a longer residence time than in croplands because there is less soil disturbance and a greater proportion of the input from root turnover is physically protected as chemically stabilised particulate organic matter (Six *et al.,* 2004).

In grasslands, the degree of sequestered C is influenced primarily by plant productivity and the frequency and extent of disturbance (i.e. grazing; grassland ploughing and renovation). Therefore, grazing has a direct impact on grassland productivity, plant community structure and biogeochemical cycling. In grazed grasslands, much of the primary production is ingested by animals and 50% is excreted and returned to the soil in the form of faeces (non-digestible carbon; 25 to 40% of the intake, depending directly on the digestibility of diet); the remainder is returned to the soil in the form of plant litter (ungrazed leaves and roots) or root exudates. Accordingly, effects of grazing are driven by plant tissue removal (defoliation), excretion (dung and urine deposits) and the C urine/C faecal ratio (12.2±6.0%, Sauvant and Giger-Reverdin, 2009), but also by trampling, which exerts mechanical pressure and causes physical damage to the vegetation where animals pass repeatedly. Grazing animals also promote spatial heterogeneity in C-N-P pools and fluxes via uneven patterns of defoliation and animal returns which add to a mosaic of patches of variable vegetation height and feed quality, and C storage potential (Bloor and Pottier, 2014).

For example, at low grazing intensities it seems that animal excretion favours the N cycle in the soil and the net primary productivity of vegetation cover (via a reduction in above ground standing biomass), as well as litter production and plant nutrient status. If there is much dead plant material in the sward, with shading of live leaves (e.g. very extensive low productive swards), grazing events can allow light to penetrate into the plant canopy and encourage new tillers leading all together to an increase in the storage of C (Zhou *et al.,* 2017). Conversely, if grazing is too intense or the period between successive grazing is too short, the biomass and soil cover (e.g. amount of live leaves) can be reduced in the way that light interception falls and growth and C capture is reduced as well as litter and root production. In these cases, intense grazing can lead to a reduction in the soil C storage (e.g. Derner and Schumann 2007; Zhou *et al.* 2017). There exists, therefore, a compromise between promoting animal production and promoting carbon sequestration (Soussana and Lemaire, 2014), which is the compromise between biomass production (and intensity of use), and C inputs to soil (via litter, animal wastes and roots). In the relation between C storage and herbage use (i.e. ratio between produced biomass and biomass removal by grazing), we observe an increase of both until an optimum beyond which the storage of C decreases (threshold of ~ 0.5 to 0.7) with further increase in herbage use (Klumpp *et al.,* 2020). The C sequestration potential of European grazed grasslands is, despite large uncertainties related to the effect of grazing, on average (± standard deviation) 0.21±0.6 Mg C ha^{-1} yr^{-1} (Klumpp *et al.,* 2020).

Effects of forage quality on C sequestration

In productive systems, biomass production is associated with forage quality (if there is sufficient N available). Grasslands adapted to low grazing levels are generally characterized by slow-growing plant species and lower aboveground net primary productivity and quality, a microbial community dominated by fungi, as well as greater N retention and C storage (see also Eugene *et al.,* 2014). In these latter pastures, grazing has long-term effects on litter quality and quantity, which are driven by changes in plant community composition and defoliation-tolerant species or unpalatable species (see Wardle *et al.,* 2004). Under medium to high grazing pressure, fast-growing, palatable species typical of nutrient-rich, managed grasslands show a high above-ground productivity and quality (lower C:N), promoting higher C inputs to the soil and a rapid degradation by bacteria (Cotrufo *et al.,* 2013).

Effects of plant biodiversity on C sequestration

Grazing has the capacity to change vegetation by modifying plant community composition (presence of legumes in particular) (Bagchi *et al.,* 2010; Zhou *et al.,* 2017), which play on the supply of soil with aerial and root plant biomass These in turn can affect not only grassland productivity, but also soil organic matter (SOM) decomposition. Then again, in agricultural settings, plant diversity is often associated with low biomass yield and forage quality. Recent studies have underlined that plant diversity is an important production factor independent of management intensity, as it enhances quality-adjusted yield and revenues similarly to increasing fertilisation and cutting frequency (Schaub *et al.,* 2020). Besides, it appears that grasslands with complex flora allow higher C storage (Hungate *et al.,* 2017; Lange *et al.,* 2015). This storage increases with the specific richness of the meadow and with the presence of legumes (Cong *et al.,* 2014; Rutledge *et al.,* 2017), and where is probably linked to a diversity of root systems (more or less dense and deep), as well as to an increase in the availability of N in the presence of urine, dung and legumes and thus, variations in primary productivity.

Furthermore, most grasslands are subject to marked seasonality of biomass production. Annual cycles of temperature or rainfall impose cycles of plant growth and phenology that result in cycles of biomass abundance and quality. For instance, factors that affect forage quality are leaf-to-stem ratio (such as newly emerged leaves), stage of phenology, diseases and pests. Forage digestibility declines with an increase of stem in biomass (i.e. reduction in leaf-to-stem ratio) and across growth stages (vegetative, bud, flower). For that reason, information on the nutritive value of forage quality by use of phenological stage, may help to choose suitable grazing times and stocking rates. This in turn may help to achieve higher animal performance without damage to vegetation and related decline in C sequestration potential, increase in soil N_2O and enteric CH_4 (see Van den Pol *et al.,* 2018). As for poor forage quality, the increases in CH_4 production of ruminants are often related to lower intake and digestibility (Archimède *et al.,* 2011; Rossignol *et al.,* 2014). However, effects on CH_4 yield seem to be variable, but CH_4 intensity increases with reduced forage quality. Accordingly, there are a number of trade-offs that need to be considered when intensifying grazed systems (Soussana and Lemaire, 2014, Figure 1): (1) an increase in productivity (and subsequent biomass removal) leads to a decline in the amounts of organic carbon returned to the soil; (2) maximisation of forage quality (low C/N ratio) and the related increase of digestibility (i.e. improved animal production) leads to a decline in mean residence time of soil organic C (i.e. increase of root and shoot litter decomposability); and (3) increasing net primary productivity through fertiliser supply and legumes (biological N fixation), leads to an increase of N_2O emissions (from fertiliser and urine) and CH_4 emissions from enteric fermentation due to increased forage quality.

Conclusions

Mitigating CH_4 emissions from ruminants fed on forage-based diets will decrease the contribution of livestock and agriculture to total GHG emissions. The major determinant of forage quality is the stage of growth and species mixture. With increased stage of growth, the fibre contents also increase (CF and NDF contents) and therefore forage quality (DOM) decreases. The impact of DOM on CH_4 (g kg^{-1} DMI) is curvilinear.

Forage legumes used in ruminant systems decrease GHG emissions due to a decreased N fertiliser use and fertiliser production, preservation of biodiversity and decreased parasitism in ruminants. Therefore, they are environmentally and economically beneficial for some systems, for which there is no overload of N fertilisation. Moreover, with regard to manure, dietary measures are recommended that reduce the amount of N excreted (e.g. better matching of dietary protein to animal needs), shift N excretion from urine to faeces (e.g. tannin inclusion at low levels) and reduce the amount of fermentable organic matter excreted.

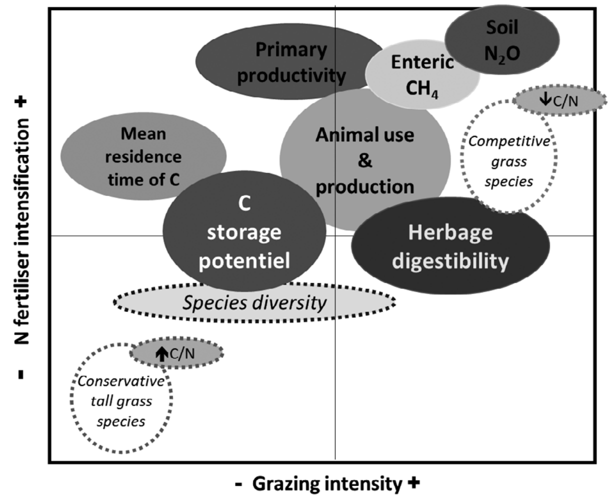

Figure 1. Estimated range and directions of effects of grassland intensification by grazing (x-axis) and fertilisation (y-axis) (adapted from Soussana and Lemaire, 2014).

Ruminants will retain their niches because of their ability to produce valuable human food from low value feedstuffs. Employing these emerging strategies will allow improved productive efficiency of ruminants in both developing and developed countries. Net CH_4 output is required to take into account the enteric CH_4 emissions and soil C sequestration from feed management (e.g. grasslands). Moreover, multicriteria assessment of GHG mitigations based on forages, such as life cycle analysis or process-based modelling, are required to take into account the interactions and trade off/synergy between GHG.

References

Archimède H., Eugène M., Magdeleine C.M., Boval M., Martin C., Morgavi D.P., Lecomte P. and Doreau M. (2011) Comparison of methane production between C3 and C4 grasses and legumes. *Animal Feed Science and Technology* 166-167, 59-64.

Arndt C., Powell J. M., Aguerre M. J. and Wattiaux M.A. (2015) Performance, digestion, nitrogen balance, and emission of manure ammonia, enteric methane, and carbon dioxide in lactating cows fed diets with varying alfalfa silage-to-corn silage ratios. *Journal of Dairy Science* 98, 418-430.

Bagchi S. and Ritchie M.E. (2010). Introduced grazers can restrict potential soil carbon sequestration through impacts on plant community composition. *Ecology Letters* 13, 959-968.

Beauchemin K.A. and McGinn S.M. (2005) Methane emissions from feedlot cattle fed barley or corn diets. *Journal of Animal Science* 83, 653-661.

Bloor J. and Pottier J. (2014) Grazing and spatial heterogeneity: implications for grassland structure and function. In: Mariotte P., Kardol P. (eds) *Grassland biodiversity and conservation in a changing world*. Nova Science Publishers, Inc., Hauppage, New York, USA, pp. 135-162.

Broderick G.A. (2018) Review: Optimizing ruminant conversion of feed protein to human food protein. *Animal* 12, 1722-1734.

Cong W.F., van Ruijven J., Mommer L., De Deyn G.B., Berendse F. and Hoffland E. (2014) Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. *Journal of Ecology* 102, 1163-1170.

Cotrufo M.F., Wallenstein M.D., Boot C.M., Denef K. and Paul E. (2013) The microbial efficiency matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology* 19(4), 988-995.

Derner J. and Schuman G. (2007) Carbon sequestration and rangelands: a synthesis of land management and precipitation effects. *Journal of Soil and Water Conservation* 62(2), 77-85.

Dewhurst R.J., Delaby L., Moloney A., Boland T. and Lewis E. (2009) Nutritive value of forage legumes used for grazing and silage. *Irish Journal of Agricultural and Food Research*, 167-187.

Escobar-Bahamondes P., Oba M. and Beauchemin K.A. (2017) An evaluation of the accuracy and precision of methane prediction equations for beef cattle fed high-forage and high-grain diets. *Animal* 11(1), 68-77.

Ellis J., Kebreab E., Odongo N., McBride B., Okine E. and France J. (2007) Prediction of methane production from dairy and beef cattle. *Journal of Dairy Science* 90(7), 3456-3466.

Eugène M., Archimède H., Giger Reverdin S., Doreau M. and Sauvant D. (2014) Effect of feeding forages on enteric methane emissions from ruminants: a meta-analysis. In: *Harnessing the Ecology and Physiology of Herbivores*. Paper presented at the (30) 2014; Joint ISNH/ISRP International Conference 2014, Canberra, Australia, pp. 223.

Eugène M., Doreau M., Archimède H., Giger-Reverdin S. and Sauvant D. (2019) Modelling by meta-analysis enteric methane emissions from ruminants fed forages supplemented or not with tannins. Presented at the 9th Workshop on Modelling Nutrient Digestion and Utilization in Farm animals (MODNUT). Itamambuca Eco Resort, Brazil.

Gerber P.J., Steinfeld H., Henderson B., Mottet A., Opio C., Dijkman J., Falcucci A. and Tempio G. (2013a) Tackling climate change through livestock – a global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Gerber P.J., Hristov A.N., Henderson B., Makkar H., Oh J., Lee C., Meinen R, Montes. F., Ott T., Firkins J., Rotz A., Dell C., Adesogan A.T., Yang W.Z., Tricarico J.M., Kebreab E., Waghorn G., Dijkstra J. and Oosting S. (2013b) Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: a review. *Animal* 7, 220-234.

Hassanat F., Gervais R., Julien C., Massé D.I., Lettat A., Chouinard P.Y., Petit H.V. and Benchaar C. (2013) Replacing alfalfa silage with corn silage in dairy cow diets: effects on enteric methane production, ruminal fermentation, digestion, N balance, and milk production. *Journal of Dairy Science* 96(7), 4553-4567.

Hassen A., Talore D.G., Tesfamariam E.H., Friend M.A. and Mpanza T.D.E. (2017) Potential use of forage-legume intercropping technologies to adapt to climate-change impacts on mixed crop-livestock systems in Africa: a review. *Regional Environmental Change* 17(6), 1713-1724.

Hristov A.N., Oh J., Firkins J.L., Dijkstra J., Kebreab E., Waghorn G., Makkar H.P.S., Adesogan A.T., Yang W., Lee C., Gerber P.J., Henderson B. and Tricarico J.M. (2013) Special topics – Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. *Journal of Animal Science* 91(11), 5045-5069.

Hungate B.A., Barbier E.B., Ando A.W., Marks S.P., Reich P.B., Van Gestel N., Tilman D., Knops J.M., Hooper D.U. and Butterfield B.J. (2017) The economic value of grassland species for carbon storage. *Science Advances* 3(4), e1601880.

INRA (2018) INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, the Netherlands, 640 pp.

Jayanegara A., Leiber F. and Kreuzer M. (2012) Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from *in vivo* and *in vitro* experiments. *Journal of Animal Physiology and Animal Nutrition* 96(3), 365-375.

Jayanegara A., Goel G., Makkar H.P.S. and Becker K. (2015) Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population *in vitro*. *Animal Feed Science and Technology* 209, 60-68.

Jonker A., Lowe K., Kittelmann S., Janssen P.H., Ledgard S. and Pacheco D. (2016). Methane emissions changed nonlinearly with graded substitution of alfalfa silage with corn silage and corn grain in the diet of sheep and relation with rumen fermentation characteristics *in vivo* and *in vitro*. *Journal of Animal Science* 94(8), 3464-3475.

Klumpp, K. and Graux, A.I. (2020) Etat des connaissances sur les pratiques réputées « stockantes » en prairies permanentes. In: Pellerin *et al.* (ed.) *Stocker du carbone dans les sols français, Quel potentiel au regard de l'objectif 4 pour 1000 et à quel coût*? Synthèse du rapport d'étude, INRA (France), 114 pp.

Laliberté E. and Tylianakis J.M. (2012) Cascading effects of long-term land-use changes on plant traits and ecosystem functioning. *Ecology* 93(1), 145-155.

Lange M., Eisenhauer N., Sierra C., Bessler H., Engels C., Griffiths R., Mellado-Vazquez J., Malik A. and Roy J. and Gleixner, G. (2015) Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications* 6, 6707.

Mueller-Harvey I., Bee G., Dohme-Meier F., Hoste H., Karonen M., Kölliker R., Lüscher A., Niderkorn V., Pellikaan W.F., Salminen J., Skøt L., Smith L.M., Thamsborg S.M., Totterdell P., Wilkinson I., Williams A.R., Azuhnwi B.N., Baert N., Brinkhaus A.G., Copani G., Desrues O., Drake C., Engström M., Fryganas C., Girard M., Huyen N.T., Kempf K., Malisch C., Mora-Ortiz M., Quijada J., Ramsay A., Ropiak H.M. and Waghorn G.C. (2019) Benefits of condensed tannins in forage legumes fed to ruminants: importance of structure, concentration, and diet composition. Crop Science 59(3), 861-885.

Moss A.R., Jouany J.-P. and Newbold J. (2000) Methane production by ruminants: its contribution to global warming. *Annales de Zootechnie, EDP Science*s 49, 231-253.

Patra A.K. and Saxena J. (2011) Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture* 91(1), 24-37.

Pereira L.G.R., Machado F.S., Campos M.M., Guimaraes R., Tomich T.R., Reis L.G. and Coombs C. (2015) Enteric methane mitigation strategies in ruminants: a review. *Revista Colombiana De Ciencias Pecuarias* 28(2), 124-143.

Phelan P., Moloney A.P., McGeough E.J., Humphreys J., Bertilsson J., O'Riordan E.G. and O'Kiely P. (2015) Forage legumes for grazing and conserving in ruminant production systems. *Critical Reviews in Plant Sciences* 34(1-3), 281-326.

Rico D., Chouinard P., Hassanat F., Benchaar C. and Gervais R. (2016) Prediction of enteric methane emissions from Holstein dairy cows fed various forage sources. *Animal* 10(2), 203-211.

Rossignol N., Andueza D., Carrère P., Cruz P., Duru M., Fiorelli J.-L., Michaud A., Plantureux S., Pottier E. and Baumont R. (2014) Assessing population maturity of three perennial grass species: Influence of phenology and tiller demography along latitudinal and altitudinal gradients. *Grass and Forage Science* 69(3), 534-548.

Ruckle M.E., Meier M.A., Frey L., Eicke S., Kolliker R., Zeeman S.C. and Studer B. (2017) Diurnal leaf starch content: an orphan trait in forage legumes. *Agronomy* 7(1), 16.

Rutledge S., Wall A., Mudge P., Troughton B., Campbell D., Pronger J., Joshi C. and Schipper L. (2017) The carbon balance of temperate grasslands part I: the impact of increased species diversity. *Agriculture, Ecosystems & Environment* 239, 310-323.

Sauvant D. and Giger-Reverdin S. (2009) Modelling of digestive interactions and methane production in ruminants. *Productions Animales* 22(5), 375-384.

Sauvant D., Giger-Reverdin S., Serment A. and Broudiscou L. (2011) Influences of diet and rumen fermentation on methane production by ruminants. *Inra Productions Animales* 24(5), 433-446.

Sauvant, D., Giger-Reverdin, S., Eugène, M. (2018) Enteric methane emissions. In: Noziere P., Sauvant D. and Delaby L. (eds.) INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 209-212.

Schaub S., Finger R., Leiber F., Probst S., Kreuzer M., Weigelt A., Buchmann, N. and Scherer-Lorenzen M. (2020) Plant diversity effects on forage quality, yield and revenues of semi-natural grasslands. *Nature Communications* 11(1), 768.

Singh S., Kushwaha B.P., Nag S.K., Mishra A.K., Singh A. and Anele U.Y. (2012) *In vitro* ruminal fermentation, protein and carbohydrate fractionation, methane production and prediction of twelve commonly used Indian green forages. *Animal Feed Science and Technology* 178(1-2), 2-11.

Six J., Bossuyt H., Degryze S. and Denef K. (2004) A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research* 79(1), 7-31.

Soussana J.-F. and Lemaire G. (2014) Coupling carbon and nitrogen cycles for environmentally sustainable intensification of grasslands and crop-livestock systems. *Agriculture, Ecosystems & Environment* 190, 9-17.

Suzuki T., Sommart K., Angthong W., Nguyen T.V., Chaokaur A., Nitipot P., Phromloungsri A., Cai Y., Sakai T. and Nishida T. (2018) Prediction of enteric methane emission from beef cattle in Southeast Asia. *Animal Science Journal* 89(9), 1287-1295.

Van den Pol A., Chabbi A., de Vliegher A., Hennessy D., Hutchings N. and Klumpp K. (2018) *Grazing for carbon: end report.* EIP-AGRI.

Van Gastelen S., Dijkstra J. and Bannink A. (2019) Are dietary strategies to mitigate enteric methane emission equally effective across dairy cattle, beef cattle, and sheep? *Journal of Dairy Science* 102(7), 6109-6130.

Van Lingen H.J., Niu M., Kebreab E., Valadares Filho S.C., Rooke J.A., Duthie C.-A., Schwarm A., Kreuzer M., Hynd P.I. and Hristov A.N. (2019) Prediction of enteric methane production, yield and intensity of beef cattle using an intercontinental database. *Agriculture, Ecosystems & Environment* 283, 106575.

Wardle D.A., Bardgett R.D., Klironomos J.N., Setälä H., Van Der Putten W.H. and Wall D.H. (2004) Ecological linkages between aboveground and belowground biota. *Science* 304 (5677), 1629-1633.

Zhao Y.G., O'Connell N.E. and Yan T. (2016) Prediction of enteric methane emissions from sheep offered fresh perennial ryegrass (*Lolium perenne*) using data measured in indirect open-circuit respiration chambers. *Journal of Animal Science* 94(6), 2425-2435.

Zhou G., Zhou X., He Y., Shao J., Hu Z., Liu R., Zhou H. and Hosseinibai S. (2017) Grazing intensity significantly affects belowground carbon and nitrogen cycling in grassland ecosystems: a meta-analysis. *Global Change Biology 23*(3), 1167-1179.

Can milk production in Sweden become more sustainable?

Krizsan S.J.[1], Chagas J.C.[1], Pang D.[2] and Cabezas-Garcia E.H.[3]
[1]Swedish University of Agricultural Sciences (SLU), Skogsmarksgränd 90183 Umeå, Sweden; [2]C-Lock manufacture GreenFeed (GF), Rapid City, SD 57703, USA; [3]Agrifood and Biosciences Institute (AFBI), Northern Ireland, United Kingdom

Abstract

Resource use efficiency and economic initiatives point toward using less concentrates in ruminant food production. The global population is growing and food production will need to increase to feed more people in the future. The Swedish government has launched a national food strategy, which aims to move Swedish food production towards self-sufficiency and greater exports. Simultaneously, strong economic development has stimulated consumers to request more high-value foods, such as meat and refined dairy products. Public opinion states that today's food production from ruminants is negative for the environment and contributes to climate change. This paper assesses some aspects of dietary ingredient composition and important feeding values that can contribute to making Swedish dairy production more sustainable. Efficient dietary methane mitigating strategies can also decrease emissions of greenhouse gases in line with European Union targets and avoid major changes in dietary consumption patterns of meat and milk from ruminants. Although feeding management seems to be the most important approach to decrease nitrogen losses, rational use of fertiliser and improved manure management practices on dairy farms should also be considered, to decrease the impact of nitrogen losses on the environment.

Keywords: agro industrial by-products, dairy production, food production, greenhouse gases, grass, resource efficiency

Introduction

The geographical location and extended north-south gradient (55-69°N) of Sweden results in considerable variation in farming conditions. In addition, variations in topography, contrasts between coastal and inland climates, and agricultural policies influence the distribution of crops. Crop production is strongly dominated by cereals (mainly barley) and grass leys. The proportion of leys increases towards the north of Sweden, where it is the dominant crop on arable land. Grass is also the major single crop in Sweden as a whole, occupying more than 1 million ha of agricultural land (close to half of all agricultural land) and producing an average annual yield (1990-2018) of almost 4 million Mg of dry matter (DM) (Jordbruksverket, 2019). The long, light days in summer allow grass plants to build up energy-rich carbohydrates from photosynthesis for almost 24 hours per day, while the relatively low temperature in early summer reduces lignification, promoting a high feeding value. Domestic milk production based on grass plays a very important role in Swedish agriculture, food production and the food industry.

Long-term fluctuations in grain prices on the world market have raised economic concerns and promoted production and feeding of high-quality forages in ruminant production systems. Despite this, intensive milk production in Sweden is still supported by high amounts of concentrate feed (Swensson *et al.*, 2017). Greater use of concentrate feed means that fertile agricultural land is devoted to animal feed production and that more pesticides are needed, giving a greater environmental footprint. A large proportion of feed resources fed in ruminant production systems could instead be used directly as human foods, or utilised with higher efficiency in poultry and pig production. Additionally, changes in biodiversity and ecosystem services following changes in the land use are rarely measured and accounted for in the food value chain seeking to meet consumer preferences and demands (Cederberg *et al.*, 2018).

The global population is growing and, although food production has increased markedly in the past 50 years, it will need to increase significantly to feed a population of more than 11 billion people by next century (FAO, 2018). Even today, not everyone has access to sufficient protein and energy from their diet. At the same time, strong economic development is influencing the global demand for food products. Swedish food consumption and food trade patterns have changed in recent decades, mainly towards increased consumption of high-value foods with a larger environmental footprint, such as meat and refined dairy products (in contrast to low-value foods such as fresh milk, flour and potatoes). These changes impose a measurable burden of food consumption and trade on the environment, e.g. through greater use of agrochemicals and greenhouse gas (GHG) emissions (Cederberg *et al.*, 2019). According to Bryngelsson *et al.* (2016), methane (CH_4) and nitrous oxide (N_2O) emissions from agriculture must be reduced to meet an emissions allowance in line with European Union (EU) targets of 300-1,300 kg CO_2-equivalents per capita and year by 2050.

In 2017, the Swedish government launched a National Food Strategy (Government Bill 2016/17:104) to increase domestic food production by 2030 through active food policy (Regeringskansliet, 2017). The strategy involves the entire food supply chain and is intended to secure increased, sustainable food production for global food security and better self-sufficiency and export. Food production from ruminants is well-suited for Swedish conditions, but will have to adapt to meet climate targets and employ innovative marketing to maintain consumer confidence in safe and low-emitting food. This paper deals with some nutritional and future aspects of Swedish dairy production systems which, if implemented, could contribute to more sustainable milk production.

Swedish dairy production systems

Today there are around 63,000 farms in Sweden, but in 1970 there were more than twice as many. However, production level has not decreased, as individual farms have expanded considerably, while also obtaining significantly more from every hectare and from every animal. For example, in 2017 one hectare of ley yielded an average of 5,110 kg DM ha^{-1}, while the corresponding figure for 2002 was 4,380 kg DM ha^{-1}. An average dairy cow today produces about 8,700 litres of milk per year, but in 1970 the corresponding figure was only around 4,000 litres (Jordbruksverket, 2019).

Food production from ruminants is characterised by low use efficiency of resources, despite grass providing the majority of total DM intake in the dairy cow diet (Table 1). Ruminants have a low feed conversion ratio and long reproduction interval, resulting in a large proportion of dietary energy being used for maintenance. Despite this, ruminants have been shown to perform better than monogastrics in comparison to human-edible efficiency of energy and protein for different livestock production systems (Gill *et al.*, 2010) (Table 1).

Table 1. Comparative efficiency of different livestock production systems in the USA (adopted from Gill *et al.*, 2010).

Product	Energy efficiency		Protein efficiency	
	Total[1]	Human-edible[2]	Total[1]	Human-edible[2]
Milk	0.25	1.07	0.21	2.08
Beef	0.07	0.65	0.08	1.19
Pigs	0.21	0.30	0.19	0.29
Poultry meat	0.19	0.28	0.31	0.62

[1] Total efficiency calculated as outputs of human-edible energy and protein divided by total energy and protein inputs.

[2] Human-edible efficiency calculated as outputs of human-edible energy and protein divided by human-edible inputs.

Depending on diet composition, even greater differences in edible feed conversion ratio (eFCR) have been observed. Pang *et al.* (2018) fed diets consisting of grass silage and concentrate in a ratio of 661:339, and reported eFCR for energy and protein between 0.92 and 4.56 MJ MJ^{-1} edible input and between 0.94 and 4.15 g g^{-1} edible input, respectively, when a by-product-based concentrate replaced a conventional grain-based concentrate in dairy cow diets. There were no effects of concentrate source on feed intake, milk production, diet digestibility or CH_4 emissions, and there were indications of better energy status in cows fed the concentrate made of by-products compared with the grain-based concentrate. Notably, the eFCR for both energy and protein was greater when the cows were fed high- rather than low-digestibility grass silage (Pang *et al.*, 2018). Guinguina A.T., Krizsan S.J., Hetta M. and Huhtanen P. (unpublished data) evaluated the effects of reducing dietary starch content by replacing cereal grain with a fibrous by-product mixture, on the performance of early-lactation dairy cows fed a grass silage-based diet. They found that feed intake, milk yield and energy status were not affected by concentrate type, and observed substantial improvements in eFCR. Ertl *et al.* (2015a) observed higher propionate content and lower acetate to propionate ratio *in vitro* for a diet supplemented with by-products compared with a control concentrate mixture. They attributed this to more easily fermentable fibre such as pectin and hemicellulose in the by-products, which was assumed to stimulate propionate formation, and to higher abundance of *Prevotella*. They also speculated that by-products which stimulate propionate formation and gluconeogenesis in dairy cows may be beneficial, particularly during early lactation, through improved energy efficiency (Ertl *et al.*, 2015a). Similarly, Guinguina A.T., Krizsan S.J., Hetta M. and Huhtanen P. (unpublished data) recorded lower CH_4 emissions from dairy cows fed concentrate in the form of by-products rather than cereal grain.

Feeding agro-industrial by-products is often suggested as a viable option to improve sustainability in terms of human-edible output in dairy production (Dann *et al.*, 2014; Ertl *et al.*, 2015b, 2016; Whelan *et al.*, 2017). Total recorded use of agro-industrial by-products in commercial feeds for farm animals was 535,989 ton in Sweden in 2014 (Jordbruksverket, 2014). Up to 80% of these by-products were fed to ruminants and most were produced domestically. In Sweden, imported rapeseed by-products comprise 20% of the total amount of agro-industrial by-products used in ruminant production systems, while at the same time there is a surplus of dried distillers' grain, which is exported to Europe (Swedish Board of Agriculture, 2014). Efficient use of non-human edible feed resources produced locally or nationally could improve the resource use efficiency of dairy food production in Sweden.

It is well-known that early-harvested grass from spring growth generally produces highly digestible silage that can support higher milk yield with less concentrate feed than grass harvested at a more mature stage (Ferris *et al.*, 2001; Randby *et al.*, 2012; Rinne *et al.*, 1999). Randby *et al.* (2012) fed early harvested grass silage with 747 g digestible organic matter kg^{-1} DM, with and without concentrate supplementation, and found that total DM intake and milk yield increased with increased concentrate supplementation. They cited metabolisable protein deficiency in early lactation as the main reason for the reduction in milk yield of cows fed silage only. Cederberg (2017) pointed out increasing consumer interest in environmentally friendly milk and beef production in grass-based certified systems that can achieve long-term sustainability.

Silage nutritive value

Grass yield and nutritional and ensiling quality are important factors in optimising ruminant livestock production systems. To optimise forage utilisation on a dairy farm, it is important to know the quantitative effects of digestible organic matter concentration (D-value) and harvest time of grass silage on intake and milk production, and how these responses influence other factors, such as production level and concentrate supplementation (Pang, 2018). Fermentation-related factors are indicative of the efficiency of forage conservation and of modifications in the carbohydrate and nitrogen fractions during conservation, and they affect silage intake (Huhtanen *et al.*, 2007; Krizsan and Randby, 2007). The Swedish growing season for grass, i.e. average temperature above +5 °C, is now more than 10 days longer than it was 50 years ago (Gustavsson, 2017). This extended growing season is generating interest in using leys for forage production, including new varieties, and is creating demand for better knowledge of forage nutritive value and harvesting regimes.

Intake

Feed intake is the most important single nutritive factor influencing production of dairy cows (Huhtanen *et al.*, 2011; Mertens, 1994). Variations in the nutrient supply for ruminants are mostly related to forage characteristics and intake potential (Huhtanen *et al.*, 2007; Mertens, 1994). Feed is also often the largest single cost for dairy farmers. It is not always clear whether a given feeding regimen/diet, or the inherent potential of the cow, is limiting milk production. Friggens *et al.* (1998) conducted a cross-over trial examining the effect of feed quality on the relationship between intake and stage of lactation in dairy cows, and found that milk yield was significantly lower for cows fed the lower-quality diet than for cows offered the high-quality diet. After cross-over of diets, the cows adapted their milk yield to the quality of the new diet, and not to their production potential.

Krizsan *et al.* (2014) used individual milk yield data from dairy cow cross-over trials to evaluate feed intake prediction models. In their novel meta-analysis approach, individual milk yield data from previous period (prospective), observed period (retrospective) and average overall periods were combined with current feed data in intake prediction models for dairy cows and were compared with observed intake in the current period. They concluded that using observed milk yield as a model input in predictions of feed intake can be substantially affected by dietary factors. However, using a standardised milk yield according to Huhtanen *et al.* (2011) improved the predictions of feed intake and provided a more robust model reflecting the potential of the cow (Krizsan *et al.*, 2014). Milk yield as a model input in intake predictions can be substantially affected by current diet, generating errors in estimates of future feed intake and milk production if the economically optimal diet deviates from the current diet (Krizsan *et al.*, 2014).

Digestibility

Faecal energy is a greater and more variable loss of feed gross energy than CH_4 and urinary energy, and therefore accurate determination of digestibility is essential in determination of silage metabolizable energy concentration and forage intake potential. Variations in forage organic matter digestibility (OMD) cannot be satisfactorily predicted from feed chemical composition (Huhtanen *et al.*, 2006). In *in vivo* approaches, the feeding value of diets is determined from tabulated digestibility coefficients, determined in trials with sheep fed a maintenance level of intake, and proximate analysis. However, due to the large amounts of forage and labour required when conducting digestibility trials, different *in situ* and *in vitro* methods have been developed and successfully related to *in vivo* data on OMD. Krizsan *et al.* (2012) compared the use of different *in vitro* and *in situ* methods in empirical and mechanistic predictions of *in vivo* OMD for a wide variety of forage types. They achieved the smallest prediction error of forage *in vivo* OMD by using *in situ*-determined indigestible neutral detergent fibre (iNDF) content when forage-specific equations for lucerne and straw were used. Krizsan *et al.* (2015) showed that the iNDF concentration is not altered during ensiling of grass (Figure 1). A close relationship between near-infrared reflectance spectroscopy (NIRS) values and iNDF in grass silage has been reported by Nousiainen *et al.* (2004). These results support the development of a high-throughput NIRS application by farm service laboratories to predict forage OMD based on *in situ*-determined iNDF. Huhtanen *et al.* (2010) were able to predict forage OMD from iNDF and NDF with a prediction error of only 0.008 g kg^{-1}.

Pang (2018) found that every 10 g kg^{-1} DM increase in silage D-value resulted in a 0.39 and 0.24 kg increase in milk yield in cows fed primary growth and regrowth silage, respectively. These values

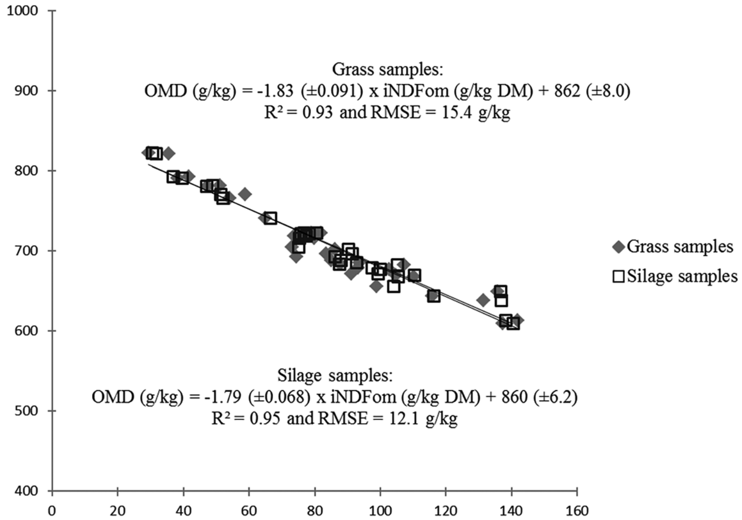

Figure 1. Linear relationship between *in vivo* organic matter digestibility (OMD) and ash-free indigestible neutral detergent fibre (iNDFom) (both g kg^{-1} dry matter (DM)) in grass and corresponding silage samples. Coefficient of determination (R^2) and root mean square error (RMSE) of the regression equations are given (from Krizsan *et al.*, 2015).

correspond to the increase of 0.26 kg by Huhtanen (1994), 0.27 kg by Rinne (2000) and 0.30 kg by Pang *et al.* (2019). Milk yield is positively related to dietary intake of metabolizable energy and the response to changes in silage D-value is positively correlated to intake of metabolizable energy (Pang *et al.*, 2019). The response of 0.109 kg energy-corrected milk yield per MJ additional metabolisable energy intake reported by Pang *et al.* (2019) compares well with the 0.114 kg energy-corrected milk yield per MJ found by Huhtanen *et al.* (2003). This suggests that the additional metabolisable energy from increased feed intake resulting from improved silage quality is used with at least the same relative efficiency for milk production as additional metabolizable energy from concentrate.

Flaten (2002) and Gunnarsson *et al.* (2014) identified economic initiatives related to choice of harvesting strategy following early harvest of the spring-growth grass. The profits obtained by farms applying a three-cut system were greater than those of farms applying a two-cut system, despite the greater costs of crop management and labour input for the three-cut system. Flaten (2002) also found that more expensive concentrate feeds have to be supplied in Norwegian dairy production when the two-cut system is applied. This is in line with suggested greater yield of grass in three-cut systems (Martinsson and Eriksson, 2010) and good milk yield response of cows fed third-cut silage (Pang, 2018; Sairanen *et al.*, 2016).

Dietary CH_4 mitigation strategies

Today, livestock supply 13% of the energy in human diets (Smith *et al.*, 2013) and are responsible for around 14% of total anthropogenic GHG emissions (7.1 Gt CO_2-equivalents per year; Gerber *et al.*, 2013). It is clear that cattle (beef and dairy) produce more total GHG emissions than other animal species (Figure 2). Enteric fermentation in cattle contributes most to these GHG emissions, followed by N_2O emissions from soil and manure management (Gerber *et al.*, 2013). Since global production of meat is projected to increase from 229 million ton in 2000 to 465 million ton in 2050, and milk production is projected to increase from 580 to 1,043 million ton, it is likely that ruminants will continue to contribute to GHG emissions in the future, even if monogastrics meet much of the increase in demand for meat. Regional average CH_4 emission intensity ranges from 1.2 to 7.5 kg CO_2-eq. kg^{-1} of milk (FAO, 2010). This range reflects the variable intensity and productivity of global livestock production and

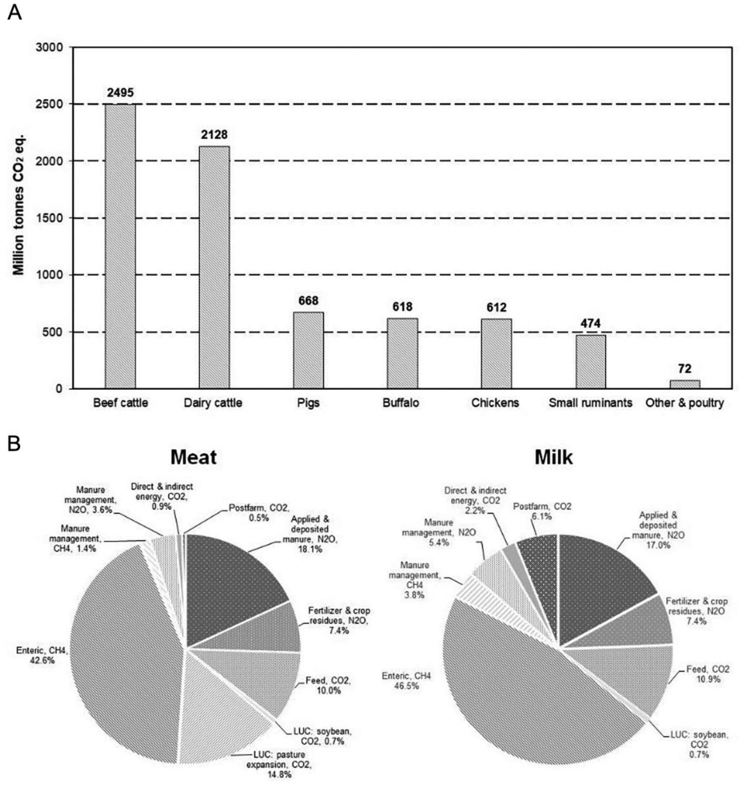

Figure 2. (A) Global estimated emissions by species. (B) Emissions from cattle milk and beef supply chains. Source: GLEAM (modified from Gerber *et al.*, 2013).

indicates that GHG emissions could still be reduced through increases in agricultural productivity and efficiency. Bryngelsson *et al.* (2016) concluded that major reductions (50% or more) in ruminant meat consumption are unavoidable if EU climate targets are to be met, and that high dairy consumption is only compatible with the targets if there are substantial advances in technology. Reducing food waste would play a minor role in meeting the climate targets, lowering emissions by only an additional 1-3%.

Hedenus *et al.* (2014) suggested that future implementation of efficient mitigation technologies can avert the need for dietary change. In future predictions, they included dietary mitigation of enteric CH_4 from supplementation of fat, estimated to reduce emissions by up to 20% in non-pasture fed animals. Chagas *et al.* (2019) evaluated different dietary mitigation strategies in a large *in vitro* experiment that included chemical inhibitory compounds, plant-derived inhibitory treatments and different potentially CH_4-reducing diets. They found that inclusion of the natural anti-methanogenic red seaweed *Asparagopsis taxiformis* at a level of 0.5 mg g^{-1} (organic matter (OM) basis) resulted in almost complete inhibition of CH_4 production, with little impact on rumen fermentation parameters. These results are in line with earlier findings that production of CH_4 was decreased by 84.7% at an *A. taxiformis* inclusion level of 1 mg g^{-1} (OM basis), while doses greater than 2 mg g^{-1} (OM basis) decreased CH_4 production by more than 99% (Machado *et al.*, 2016). A recent *in vivo* experiment by Stefenoni *et al.* (2019) involving inclusion of *A. taxiformis* at 0.5 g kg^{-1} of DM intake decreased CH_4 emissions in lactating dairy cows by 80%, with no negative effects on DM intake or milk yield. These results suggest that natural bioactives produced by

A. taxiformis can act as a strong natural inhibitor of CH_4 production and could form part of an effective strategy to mitigate CH_4 emissions caused by domesticated ruminants.

Nitrogen efficiency

In ruminant production systems, nitrogen (N) inputs from fertiliser, feed and manure have been identified as the major source of N excretion and associated environmental concerns (Powell *et al.*, 2013). European data suggest that agriculture is the main contributor to N pollution, accounting for approximately 78% of total N entering the surrounding ecosystems (Sutton *et al.*, 2011a). The N in manure deposited on pasture and on flooring in freestall housing can run off into surface water, leach into soil or volatilise to gases such as ammonia, which affects air quality, and N_2O, a potent GHG with a much higher CO_2 equivalence factor (298) than CH_4 (IPCC, 2007). The main sources of N_2O from agriculture are connected with nitrification and denitrification processes in soil. Farms emit N_2O which originates mostly from N fertilisers (organic manure or inorganic fertilisers), direct N deposition by confined animals or manure storage (Adler *et al.*, 2015). There is also enteric N_2O release by ruminants, but it has a 9-fold lower impact on total N_2O emissions than manure (Prusty *et al.*, 2014).

The proportion of feed N consumed by dairy cows that goes to producing milk is on average 28%, representing poor efficiency of converting feed N into milk (Foskolos and Moorby, 2018). Thus, increased N use efficiency (NUE; feed N consumed that is secreted as milk N) in ruminant production systems can be a key factor to improving N management and reducing the environmental impact (Sutton *et al.*, 2011b). Reductions in dietary crude protein (CP) or feeding CP:energy balanced diets can increase the apparent NUE and can be considered as a feasible N mitigation option (Hynes *et al.*, 2016). Kidane *et al.* (2018) reported that gradually decreasing dietary CP from 175 to 130 g kg^{-1} DM increased NUE and reduced urinary N excretion without affecting production. Colmero and Broderick (2006) reported that feeding more than 165 g CP kg^{-1} diet DM did not increase yield of milk and protein, while urinary N excretion increased linearly with increased dietary CP concentration. Although feeding management seems to be the most important approach to decrease N losses, rational use of fertiliser and improved manure management practices on dairy farms should also be considered as ways to decrease the N impacts on the environment.

Conclusions

Milk production in Sweden holds a high standard, but is currently based on resource-intensive dairy feed production with consequences for the environment. Milk yield per cow has more than doubled within the past half century. Still it is likely that the future possibility to decrease the environmental footprint of dairy production in Sweden through increased productivity will be marginal. Dairy cows have the ability to utilise human-inedible resources and can sustain a large part of their milk production from high-quality grass-based diets. It is crucial to develop sustainable grassland productivity through better management, feed evaluation and improved varieties, which can exploit changes in climate and seasonal variations to increase productivity. Grass-based ruminant food production with implementation of efficient methane mitigation strategies could be a sustainable part of an ecological carbon cycle and preserve important agricultural ecosystem services.

References

Adler A.A., Doole G.J., Romera A.J. and Beukes P.C. (2015) Managing greenhouse gas emissions in two major dairy regions of New Zealand: A system-level evaluation. *Agricultural Systems* 135, 1-9.

Bryngelsson D., Wirsenius S., Hedenus F. and Sonesson U. (2016) How can the EU climate targets be met? A combined analysis of technological and demand-side changes in food and agriculture. *Food Policy* 59, 152e164.

Cederberg C., Henriksson M. and Rosenqvist H. (2018) *Ekonomi och ekosystemtjänster i gräsbaserad mjölk- och nötköttsproduktion. Institutionen för Rymd-, geo- och miljövetenskap Avd. Fysisk resursteor*. Chalmers Tekniska Högskola, Göteborg, Sweden.

Cederberg C., Persson U.M., Schmidt S., Hedenus F. and Wood R. (2019) Beyond the borders e burdens of Swedish food consumption due to agrochemicals, greenhouse gases and land use change. *Journal of Cleaner Production* 214, 644-652.

Chagas J., Ramin, M. and Krizsan S.J. (2019) *In vitro* evaluation of different dietary methane mitigation strategies. *Animal* 9, 1129.

Colmenero J.J. and Broderick G.A. (2006). Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. *Journal of Dairy Science* 89, 1704-1712.

Dann H.M., Tucker H.A., Cotanch K.W., Krawczel P.D., Mooney C.S., Grant R.J. and Eguchi T. (2014) Evaluation of lower-starch diets for lactating Holstein dairy cows. *Journal of Dairy Science* 97, 7151-7161.

Ertl P., Knaus W., Metzler-Zebeli B.U., Klevenhusen F., Khiaosa-Ard R. and Zebeli Q. (2015a) Substitution of common concentrates with by-products modulated ruminal fermentation, nutrient degradation, and microbial community composition *in vitro*. *Journal of Dairy Science* 98, 4762-4771.

Ertl P., Zebeli Q., Zollitsch W. and Knaus W. (2015b) Feeding of by-products completely replaced cereals and pulses in dairy cows and enhanced edible feed conversion ratio. *Journal of Dairy Science* 98, 1225-1233.

Ertl P., Zebeli Q., Zollitsch W. and Knaus W. (2016) Feeding of wheat bran and sugar beet pulp as sole supplements in high-forage diets emphasizes the potential of dairy cattle for human food supply. *Journal of Dairy Science* 99, 1228-1236.

Ferris C.P., Gordon F.J., Patterson D.C., Kilpatrick D.J., Mayne C.S. and McCoy M.A. (2001) The response of dairy cows of high genetic merit to increasing proportion of concentrate in the diet with a high and medium feed value silage. *Journal of Agricultural Science* 136, 319-329.

Flaten O. (2002) Adjustments to changing economic conditions in Norwegian dairy farming. In: *Proceedings of the 13th International Farm Management Congress*. Wageningen, The Netherland, pp. 1-12.

Food and Agriculture Organization of the United Nations (2010) Greenhouse gas emissions from the dairy sector – a life cycle assessment. FAO, Rome, Italy.

Food and Agriculture Organization of the United Nations (2018) The state of food security and nutrition in the world. FAO, Rome, Italy.

Foskolos A. and Moorby J.M. (2018) Evaluating lifetime nitrogen use efficiency of dairy cattle: a modelling approach. *PLoS ONE* 13(8): e0201638.

Friggens N.C. Emmans G.C. Kyriazakis J. Oldham D. and Lewis M. (1998) Feed intake relative to stage of lactation for dairy cows consuming total mixed diets with a high or low ratio of concentrate to forage. *Journal of Dairy Science* 81, 2228-2239.

Gerber P.J., Hristov A.N., Henderson B., Makkar H., Oh J., Lee C., Meinen R., Montes F., Ott T., Firkins J., Rotz A., Dell C., Adesogan A.T., Yang W.Z., Tricarico J.M., Kebreab E., Waghorn G., Dijkstra, J. and Oosting, S. (2013) Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: a review. *Animal* 7, 220-234.

Gill M., Smith P. and Wilkinson J.M. (2010) Mitigating climate change: the role of domestic livestock. *Animal* 4, 323-333.

Gunnarsson C., Nilsdotter-Linde N. and Spörndly R. (2014) Två, tre eller fyra skördar av vallfoder per år – kostnader och fodervärde till kor. *JTI-rapport, Lantbruk and Industri* 419.

Gustavsson A.-M. (2017) System för att skatta skördetiden med hjälp av väderdata i ett varmare klimat. In: *Proceedings of Forage Conference* 2017. Uppsala, Sweden, pp. 104-107.

Hedenus F., Wirsenius S. and Johansson D.J.A. (2014) The importance of reduced meat and dairy consumption for meeting stringent climate change targets. *Climatic Change* 124, 79-91.

Huhtanen P. (1994) Forage influences on milk composition. In: *Proceedings of Nova Scotia Forage Conference; Forage: Seeding to feeding.* The Nova Scotia Forage Council, Dartmouth, N.S., Canada, pp. 144-162.

Huhtanen P., Hetta, M. and Swensson C. (2011) Evaluation of canola meal as a protein supplement for dairy cows: a review and a meta-analysis. *Canadian Journal of Animal Science* 91, 529-543.

Huhtanen P., Nousiainen J. and Rinne M. (2006) Recent developments in forage evaluation with special references to practical applications. *Agricultural and Food Science* 15, 293-323.

Huhtanen P., Nousiainen J., Khalili H., Jaakkola S. and Heikkilä T. (2003) Relationships between silage fermentation characteristics and milk production parameters: Analyses of literature data. *Livestock Production Science* 81, 57-73.

Huhtanen P., Rinne M. and Nousiainen J. (2007) Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. *Animal* 1, 758-770.

Huhtanen P., Südekum, K.-H., Nousiainen J. and Shingfield K. (2010) Forage conservation, feeding value and milk quality. In: Schnyder, H. *et al.* (eds). *Grassland Science in Europe* 15, 379-400.

Huhtanen P., Rinne M., Mäntysaari P. and Nousiainen J. (2011) Integration of the effects of animal and dietary factors on total dry matter intake of dairy cows fed silage-based diets. *Animal* 5, 691-702.

Hynes D.M., Stergiadis S., Gordon A. and Yan T. (2016) Effects of crude protein level in concentrate supplements on animal performance and nitrogen utilization of lactating dairy cows fed fresh-cut perennial grass. *Journal of Dairy Science* 99, 8111-8120.

Intergovernmental Panel on Climate Change (2007) Working group III. Climate change 2007: mitigation of climate change.

Jordbruksverket (2014) Foderstatistik – 2014, dated 2015-12-14.

Jordbruksverket (2019) Jordbruksstatistik sammanställning 2019. Available online: http://www.jordbruksverket.se/statistik/statistikomr/jordbruksstatistisksammanstallning/jordbruksstatistisksammanstallning2019.4.26abb9db16b94164c6c43954.html.

Kidane A., Øverland M., Mydland L.T. and Prestløkken E. (2018) Interaction between feed use efficiency and level of dietary crude protein on enteric methane emission and apparent nitrogen use efficiency with Norwegian Red dairy cows. *Journal of Animal Science* 96, 3967-3982.

Krizsan S.J. and Randby Å.T. (2007) The effect of fermentation quality on the voluntary intake of grass silage by growing cattle fed silage as sole feed. *Journal of Animal Science* 85, 984-996.

Krizsan S.J., Nyholm, L., Nousiainen J., Südekum K.-H. and Huhtanen P. (2012) Comparison of *in vitro* and in situ methods in evaluation of forage *in vivo* organic matter digestibility in ruminants. *Journal of Animal Science* 90, 3162-3173.

Krizsan S.J., Rinne M., Nyholm L. and Huhtanen P. (2015) New recommendations for the ruminal in situ determination of indigestible neutral detergent fibre. *Animal Feed Science and Technology* 205, 31-41.

Krizsan S.J., Sairanen A., Höjer A. and Huhtanen P. (2014) Evaluation of different feed intake models for dairy cows. *Journal of Dairy Science* 97, 2387-2397.

Machado L., Magnusson M., Paul N.A., Kinley R., De Nys R. and Tomkins N. (2016) Dose-response effects of *Asparagopsis taxiformis* and *Oedogonium* sp. on *in vitro* fermentation and methane production. *Journal of Applied Phycology* 28, 1443-1452.

Martinsson K. and Ericson L. (2010) Skördesystem i vall. Slutrapport SLF projekt (H0541203). Available online: http://www.lantbruksforskning.se/projektbanken/.

Mertens D.R. (1994) Regulation of forage intake. In: *Forage quality, evaluation, and utilization.* American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc. Madison, Wisconsin, USA, pp. 450-493.

Nousiainen J., Ahvenjärvi S., Rinne M., Hellämäki M. and Huhtanen P. (2004) Prediction of indigestible cell wall fraction of grass silage by near infrared reflectance spectroscopy. *Animal Feed Science and Technology* 115, 295-311.

Pang D. (2018) Improved utilization of grass silage in milk production. SLU Service/Repro, Umeå 2018, Doctoral thesis, Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, Publication 53, 89 pp.

Pang D., Krizsan S.J., Nousiainen J., Sairanen A. and Huhtanen P. (2019) Modelling feed intake and milk yield responses with different grass ley harvesting strategies. *Grass and Forage Science* 74, 509-524.

Pang D., Yan T., Trevisi E., and Krizsan S.J. (2018) Effect of grain- or by-product-based concentrate fed with early or late harvested first-cut grass silage on dairy cow performance. *Journal of Dairy Science* 101, 7133-7145.

Powell J.M., MacLeod M., Vellinga T.V., Opio C., Falcucci A., Tempio G., Steinfeld H. and Gerber P. (2013) Feed-milk-manure nitrogen relationships in global dairy production systems. *Livestock Science* 152, 261-272.

Prusty S., Sontakke U. and Kundu S. (2014) Methane and nitrous oxide emission from livestock manure. *African Journal of Biotechnology* 13, 4200-4207.

Randby A.T., Weisbjerg M.R., Nørgaard P. and Heringstad B. (2012) Early lactation feed intake and milk yield responses of dairy cows offered grass silages harvested at early maturity stages. *Journal of Dairy Science* 95, 304-317.

Regeringskansliet (2017). En livsmedelsstrategi för Sverige – fler jobb och hållbar tillväxt i hela landet. Prop. 2016/17:104. Available online: https://www.regeringen.se/rattsliga-dokument/proposition/2017/01/prop.-201617104/.

Rinne M. (2000) Influence of the timing of the harvest of primary growth grass growth on herbage quality and subsequent digestion and performance in the ruminant animal. Helsinki University Press, Helsinki 2000, Academic dissertation, University of Helsinki, Department of Animal Science, Publications 54, 42 pp.

Rinne M., Jaakkola S., Kaustell K., Heikkila T. and Huhtanen P. (1999) Silages harvested at different stages of grass growth v. concentrate foods as energy and protein sources in milk production. *Animal Science* 69, 251-263.

Sairanen A., Palmio A. and Rinne M. (2016) Milk production potential of regrowth grass silages. *Grassland Science in Europe* 21, 379-381.

Smith P., Haberl H., Popp A., Erb K., Lauk C., Harper R., Tubiello F.N., de Siqueira Pinto A., Jafari M., Sohi S., Masera O., Bötcher H., Berndes G., Bustamante M., Ahammad H., Clark H., Dong H., Elsiddig E.A., Mbow C., Ravindranath N.H., Rice C.W., Robledo Abad C., Romanovskaya A., Sperling F., Herrero M., House J.I. and Rose S. (2013) How much land based greenhouse gas mitigation can be achieved without compromising food security and environmental goals? *Global Change Biology* 19, 2285-2302.

Stefenoni H., Räisänen S., Melgar A., Lage C., Young M. and Hristov A. (2019) Dose-response effect of the macroalga *Asparagopsis taxiformis* on enteric methane emission in lactating dairy cows. In: *Proceedings of the American Dairy Science Association Annual Meeting*, Cincinnati, OH, USA, pp. W163, 378.

Sutton M.A., Billen G., Bleeker A., Bouwman A.F., Bull K. and Erisman J.W. (2011a) Summary for policy makers. In: *The European nitrogen assessment.* New York: Cambridge University Press, USA, pp. 24-34.

Sutton M.A., Howard C.M., Erisman J.W., Billen G., Bleeker A. and Grennfelt P. (2011b) Assessing our nitrogen inheritance. In: *The European nitrogen assessment.* New York: Cambridge University Press, USA, pp. 1-6.

Swensson C, Lindmark-Månsson H., Smedman A., Henriksson M. and Modin Edman A.-K. (2017) Protein efficiency in intensive dairy production: a Swedish example. *Journal of the Science of Food and Agriculture* 97, 4890-4897.

Whelan S.J., Carey W., Boland T.M., Lynch M.B., Kelly A.K., Rajauria G. and Pierce K.M. (2017) The effect of by-product inclusion level on milk production, nutrient digestibility and excretion, and rumen fermentation parameters in lactating dairy cows offered a pasture-based diet. *Journal of Dairy Science* 100, 1055-1062.

Effect of digestibility of silage and concentrate intake on milk yield: a metanalysis

Álvarez C.[1,2], Weisbjerg M.R.[3], Nielsen N.I.[4], Prestløkken E.[1] and Volden H.[1,2]
[1]Department of Animal and Aquacultural Sciences, NMBU, Ås, Norway; [2]TINE SA, Ås, Norway; [3]Department of Animal Science, AU Foulum, Aarhus University, Tjele, Denmark; [4]Livestock Innovation, SEGES, Aarhus, Denmark

Abstract

Our study evaluated the effect of grass silage digestibility and concentrate intake on milk yield of dairy cows. Eighty group mean treatments comprising different grass silage digestibility and concentrate levels were collected from nine published studies. Linear mixed models were developed with study as random effect. Silage quality was included in the models as organic matter digestibility (OMDs) or energy concentration. Concentrate intake (DMIc), concentrate proportion of total dry matter intake or energy intake were further tested. Best fit model included curvilinear effect of DMIc and linear effect of OMDs. Moreover, there was an interaction between DMIc and OMDs, where higher digestible silages resulted in higher milk yield, but marginal response in milk to increase of DMIc was lower. For low digestible silages, highest milk production was achieved with higher DMIc as compared to high digestible silages.

Keywords: milk production, substitution rate, dairy cow, meta-analysis

Introduction

Due to long winters, northern European countries depend on conserved roughage, mainly grass silage, for winter feeding (Bernardes *et al.*, 2018). Prioritising the use of grass silage over concentrate in rations for dairy cattle is important for several reasons: grasslands have higher carbon sequestration (Soussana *et al.*, 2004), and more dry matter (DM) and protein yield per hectare than most annual crops. Further, grass is often cheap compared with other crops. Grass silage quality varies significantly due to management strategies. Maturity at harvest is one of the most important factors affecting digestibility due to changes in cell wall content and lignification (Rinne *et al.*, 1997). We hypothesise that lower concentrate levels can be used with highly digestible silages, without compromising milk production. For this, the objective of this study was to evaluate the relationship between grass silage digestibility and concentrate level on milk production based on previous experiments evaluating these two parameters.

Materials and methods

The dataset included 80 group means with mixed parity from nine published studies conducted after 1990. The criteria to be included in this metanalysis study were grass silage as the predominant forage source, at least two levels of silage digestibility and two levels of concentrate within digestibility. A summary of the studies included is shown in Table 1. The evaluated dependent variable was average daily milk production (kg per day) expressed as energy corrected milk (ECM, 3.14 MJ kg^{-1}). Feed ration values were calculated based on feedstuff and animal characteristics of each experiment using the NorFor model (Volden, 2011). Data were analysed using mixed models using 'lmer' package in R software with study as random effect. Evaluated independent variables included silage organic matter digestibility (OMDs) or energy concentration (NELs), concentrate intake in kg DM/day (DMIc) or energy/day (NELc), concentrate proportion of total DM intake (DMIt) and chemical components of the ration. The level of significance for inclusion of an effect in the model was 0.05. Model fit comparison was done using Bayesian information criterion (BIC) statistic, R squared statistics (R^2) for mixed models and root mean square error (RMSE).

Table 1. Summary of trails included in the dataset.

Reference	Cows	Breed[2]	OMD silage (%)[3]	Concentrate level[4]
Aston *et al.* (1994)	40	H	68.8, 82.5	3, 6, 9, 12 kg
Rinne *et al.* (1999)[1]	32	FA	68.2, 74.9, 77.9, 79.6	7, 10 kg
Ferris *et al.* (2001)	48	H	71.2, 81.4	Hi: 10, 30, 50, 70%
				Me: 32, 48, 64, 80%
Kuoppala *et al.* (2008)	24	FA	67.3, 68.9, 69.2, 72.7, 73.4, 76.7	8, 12 kg
Prestløkken *et al.* (2008)	32	NR	74.1, 74.7, 81.4, 81.6	4, 10 kg
Randby *et al.* (2012)	66	NR	69.3, 76.3, 80.6	0, 4, 8, 12, 16 kg
Sairanen and Juutinen (2013) exp 1	36	FA	72.7, 74.2, 77.3	9, 11, 13 kg
Sairanen and Juutinen (2013) exp 2	40	FA	69.2, 76.3	4, 6.5, 8, 11.5 kg
Alstrup *et al.* (2016)	24	H	73.9, 74.2, 76.2, 79.1	20, 50%

[1] The treatments without rapeseed effect were taken into consideration.

[2] H: Holstein, FA: Finnish Ayrshire, NR: Norwegian Red.

[3] Range of *in vivo* organic matter digestibility tested in each study.

[4] Concentrate levels presents as % correspond to total mixed ration feeding systems. Hi: High digestible silage. Me: medium digestibility silage.

Results and discussion

The model which fitted best the ECM response presented a curvilinear effect of DMIc, a linear effect of OMDs and an interaction between DMIc and OMDs. Predicted ECM from this model to increased concentrate level using 3 different OMDs is shown in Figure 1. Maximum milk yield for 82% OMDs (32.2 kg ECM) is achieved with 10.0 kg DMIc and 12.4 kg silage intake (22.4 kg DMIt), which corresponds to a 0.45 concentrate: forage ratio. For the lowest digestibility silage, maximum milk yield was lower (29.8 kg ECM) and achieved with DMIt of 21.2 kg DM, of which 12.0 kg DM is DMIc, resulting in a concentrate: forage ratio of 0.57. Results confirm our hypothesis, showing higher milk yield in higher digestibility silages achieved with lower concentrate levels. Higher digestibility silages reported higher DMIt, agreeing with Huhtanen *et al.* (2007), and increased linearly with DMIc. However, silages with higher digestibility showed lower marginal response to increased concentrate level. The curvilinear response of ECM to DMIc could be explained by the decreased digestibility of the fibre (Kristensen and Aaes, 1989) together with a shift of nutrient partitioning to body weight gain (Ferris *et al.,* 2001; Randby *et al.,* 2012). Silage intake decreased by 0.53 kg DM per kg DMIc (substitution rate), which is similar

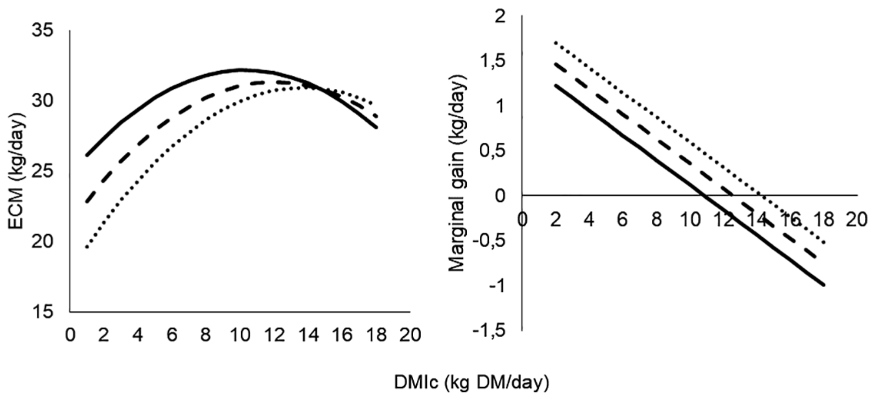

Figure 1. Predicted milk production (ECM) from the resulted model to increase concentrate intake (DMIc in kg) for 3 different silage digestibilities (OMDs in %): 82% OMDs (——), 75% OMDs (- - -), 68% OMDs (••••). Model: $y=-16.1+4.2\ DMIc - 0.07\ DMIc^2 + 0.50\ OMDs - 0.03\ DMIc \times OMDs$.

to the value of 0.47 kg DM reported by Huhtanen *et al.* (2008) although in our study substitution rate was not affected by concentrate level. Moreover, it was not affected by silage digestibility, in contrast to Faverdin *et al.* (1991).

Conclusions

Milk yield showed a curvilinear response to concentrate level, with decreasing increments. The response in milk was lower in silages with higher digestibility, achieving the same milk yield with lower concentrate level as compared with lower digestible silages. Thus, practices that increase silage digestibility also increase milk yield and reduce the need for concentrates in dairy cow diets.

References

Alstrup L., Søegaard K. and Weisbjerg M.R. (2016) Effects of maturity and harvest season of grass-clover silage and of forage-to-concentrate ratio on milk production of dairy cows. *Journal of Dairy Science* 99, 328-340.

Aston K., Thomas C., Daley S.R., Sutton, J.D. and Dhanoa, M.S. (1994) Milk production from grass silage diets: effects of silage characteristics and the amount of supplementary concentrate. *Animal Science* 59, 31-41.

Bernardes T.F., Daniel J.L.P., Adesogan A.T., McAllister T.A., Drouin P., Nussio L.G., Huhtanen P., Tremblay G.F., Bélanger G. and Cai Y. (2018) Silage review: unique challenges of silages made in hot and cold regions. *Journal of Dairy Science* 101, 4001-4019.

Faverdin P., Dulphy J.P., Coulon J.B., Vérité R., Garel J.P., Rouel J. and Marquis B. (1991) Substitution of roughage by concentrates for dairy cows. *Livestock Production Science* 27, 137-156.

Ferris C., Gordon F., Patterson D., Kilpatrick D., Mayne C. and McCoy M. (2001) The response of high genetic merit dairy cows to increasing proportion of concentrate in the diet with a high and medium feed value silage. *Journal of Agricultural Science* 136, 319-329.

Huhtanen P., Rinne M. and Nousiainen J. (2007) Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. *Animal* 1, 758-770.

Huhtanen P., Rinne M. and Nousiainen J. (2008) Evaluation of concentrate factors affecting silage intake of dairy cows: a development of the relative total diet intake index. *Animal* 2, 942-953.

Kristensen V.F. and Aaes O. (1989) Basis for choice of breeding goal and matching production system within dairy herds. In: Østergaard V. and Neimann-Sørensen A. (eds.) Report 660. Beretning fra Statens Husdyrbrugsforsøg (in Danish), pp 10-44.

Kuoppala K., Rinne M., Nousiainen J. and Huhtanen P. (2008) The effect of cutting time of grass silage in primary growth and regrowth and the interactions between silage quality and concentrate level on milk production of dairy cows. *Livestock Science* 116, 171-182.

Prestløkken E., Randby Å.T., Eknas M. and Garmo T.H. (2008) Effect of harvesting time and wilting on feed intake and milk production. In: Hopkins A., Gustafsson T., Bertilsson J., Dalin G., Nilsdotter-Linde N. and Spörndly E. (eds.) *Biodiversity and animal feed.* 22nd General Meeting of the European Grassland Federation, Uppsala, Sweden, pp. 849-851.

Randby Å.T., Weisbjerg M.R., Nørgaard P. and Heringstad B. (2012) Early lactation feed intake and milk yield responses of dairy cows offered grass silages harvested at early maturity stages. *Journal of Dairy Science* 95, 304-317.

Rinne M., Jaakkola S. and Huhtanen P. (1997) Grass maturity effects on cattle fed silage-based diets. 1. Organic matter digestion, rumen fermentation and nitrogen utilization. *Animal Feed Science and Technology* 67, 1-17.

Rinne M., Jaakkola S., Kaustell K., Heikkilä T. and Huhtanen P. (1999) Silages harvested at different stages of grass growth v. concentrate foods as energy and protein sources in milk production. *Animal Science* 69, 251-263.

Sairanen A. and Juutinen E. (2013) Feeding value of late autumn cut timothy-meadow fescue silage under Nordic conditions. In: Helgadóttir Á. and Hopkins A. (eds.) *The role of grasslands in a green future: threats and perspectives in less favoured areas.* 17th Symposium of the European Grassland Federation, Akureyri, Iceland, pp. 267-269.

Soussana J.-F., Loiseau P., Vuichard N., Ceschia E., Balesdent J., Chevallier T. and Arrouays D. (2004) Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use and Management* 20, 219-230.

Volden H. (2011) Feed calculations in NorFor. In: Volden H. (ed.) *NorFor – The Nordic Feed Evaluation System*, Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 55-58.

Effect of early or very early harvest date of tall fescue and timothy on performance of dairy cows

Sousa D.O.[1], Murphy M.[2], Hatfield R.[3] and Nadeau E.[1]

[1]Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Box 234, 532 23 Skara, Sweden; [2]Lantmännen Lantbruk, von Troils väg 1, 205 03 Malmö, Sweden; [3]U.S. Dairy Forage Research Center (retired), 1925 Linden Drive, Madison, WI 53706, USA

Abstract

The objective of this study was to evaluate the effect of early and very early harvest date of tall fescue and timothy on dairy cows' performance. Tall fescue and timothy were harvested on 25 May and 31 May 2015, resulting in a 2×2 factorial arrangement of treatments. Forty Holstein dairy cows (110 days in milk) were blocked by lactation number, lactation stage and energy corrected milk (ECM) into four groups. The continuous experiment lasted for six weeks with three registration periods. Diets contained 49% of grass silage, 49% of concentrate and 2% of mineral-vitamin premix on dry-matter (DM) basis. Overall, harvest date did not affect performance of dairy cows. There were no differences in DM intake, measured in kg d^{-1}. However, there was a tendency (P=0.06) for greater DM intake when related to body weight for cows fed very early harvested forage, compared with early, but only for timothy. The energy corrected milk yield (P=0.06), feed efficiency (P=0.01), and milk protein yield (P=0.02) were greater for cows fed timothy compared to tall fescue. In conclusion, timothy silage improved cow performance compared to cows fed tall fescue silage, regardless of harvest date.

Keywords: dairy cow, feed efficiency, grass, silage

Introduction

There is a balance between dry-matter (DM) yield and feed value when choosing types of grasses on a farm depending on the acreages of farmland and types of ruminants to be fed. Therefore, optimisation of the harvest strategy for grass silage is important for profitable dairy farming. Tall fescue has high DM yield (Niemeläinen *et al.,* 2001), and can replace traditional grasses, such as timothy, in northern Europe in a changing climate as it has been shown to be more drought tolerant. As differences in climate responses previously have been related to differences in cell wall structure between grass species and, consequently, in digestibility (Buxton, 1996), it is highly relevant to compare these species at similar maturity stages and to investigate if a very early harvest date will diminish potential differences between the species. The objective of this study was to evaluate the effect of early or very early harvest date of tall fescue and timothy for silage on performance of dairy cows.

Materials and methods

The experiment was conducted at Lantmännen Research Station Nötcenter Viken, Sweden, where forty dairy cows of Holstein and Swedish Red breed at 110 days in milk at start of the experiment, were fed silages of tall fescue cv. Swaj (SW Lantmännen) and timothy cv. Switch (SW Lantmännen) continuously for six weeks. Cows were blocked by lactation number, lactation stage and energy corrected milk (ECM) into four groups with 10 cows in each group. The grass species were harvested at a very early maturity stage (leaf-to-stem elongation stage) on May 25 (Harvest 1 (H1)) and at an early maturity stage (18% flag leaf stage) on May 31, 2015 (Harvest 2 (H2)), resulting in a 2×2 factorial arrangement of treatments in a randomized block design. Grasses were wilted to circa 35% DM before being pressed in square bales, treated with an acid (formic acid, propionic acid and formate; Perstorp AB, Perstorp) at 4 l $tonne^{-1}$ forage and wrapped with eight layers of plastic film. Silages were well fermented with no butyric acid present and ammonia-N concentrations below 70 g kg^{-1} of total N. Cows were fed a total mixed ration containing

49% of one of the four grass silages, 49% of a grain-based complementary feed and 2% of a salt/mineral/vitamin mixture of DM. Data on intake, milk yield and milk composition were registered on weeks 3, 5 and 6 and means for each week were used in the statistical analysis. The diets for tall fescue H1, timothy H1, tall fescue H2 and timothy H2 contained 300, 288, 314 and 322 g NDF and 173, 170, 171 and 166 g crude protein kg^{-1} DM intake (DMI), respectively. The dietary starch and crude fat contents were 164 g and 40 g kg^{-1} DMI, respectively. Data were analysed in Proc Mixed (SAS ver. 9.3) with silage and harvest date as fixed factors and block as a random factor. Pairwise comparisons between least-square means were analysed according to Tukey's test when a significant *F*-value occurred (P<0.05).

Results and discussion

The *in situ* digestion rate of potentially digestible neutral detergent fibre (NDF) was slower for tall fescue than for timothy, possibly corresponding to a higher indigestible NDF (iNDF) : acid detergent lignin (ADL) ratio, indicating that the lignin in tall fescue is more inhibitory to digestion per unit of lignin than the lignin of timothy (Table 1). However, this needs to be interpreted carefully as *in situ* fibre digestion kinetics only was performed on one sample per crop.

There were no differences in DMI, measured in kg day^{-1} between the treatments (Table 2). However, there was a tendency (P=0.06) for greater DMI when expressed in g kg^{-1} BW for cows fed very early harvested forage compared to early, but only for timothy, which might be related to the greater dietary NDF concentration (322 vs 288 g kg^{-1} DM) and the lower digestion rate of potentially digestible NDF of early harvested timothy silage (Table 1; Allen, 1996).

Cow performance was widely affected by forage species. Cows fed timothy silage yielded 1.8 kg more ECM (P=0.06), had 8% higher feed efficiency (P=0.01) and yielded 90 g more milk protein per day (P=0.02) compared with cows fed tall fescue silage (Table 2). Cows fed silage of tall fescue had higher content of milk urea (P<0.001) than cows fed timothy silage, which shows better protein utilisation for cows fed timothy silage. Furthermore, cows fed tall fescue silage lost body weight whereas cows fed silage of timothy gained body weight during the experiment (-3.1 vs +8.6 kg, P=0.02), which indicates differences in nutrient partitioning between the cows fed the different grass silages. The improved performance observed when cows were fed timothy silage seems to be related to a greater organic matter digestibility *in vitro* compared to cows fed tall fescue silage (933 vs 893 g kg^{-1}, P<0.001). The greater organic matter digestibility depends on a more efficient fibre degradation in terms of a higher digestion rate, which might be related to a less inhibitory structure of the lignin-polysaccharide matrix to cell-wall digestion in timothy than in tall fescue (Krämer *et al.,* 2012). Lignin structure, such as the syringyl/guaiacyl-unit ratio and cross linkings between lignin and the xylans in the cell wall can differ between

Table 1. *In situ* fibre digestion kinetics and the iNDF:ADL ratio of the ensiled crops (n=1).[1]

Item	Very early harvest May 25		Early harvest May 31	
	Tall fescue	Timothy	Tall fescue	Timothy
EFD, g kg^{-1} NDF	590	660	560	640
in situ iNDF, g kg^{-1} DM	39	33	47	36
in situ iNDF, g kg^{-1} NDF	99	89	112	82
pdNDF, g kg^{-1} NDF	901	911	888	918
$k_d$pdNDF, h^{-1}	0.064	0.082	0.054	0.075
iNDF:ADL ratio	2.8	2.2	2.9	2.0

[1] Effective fibre degradation (EDF) in rumen at 3% passage rate; indigestible neutral detergent fibre (iNDF) determined after 288 h rumen incubation *in situ*; potentially digestible NDF (pdNDF) = 1000 – iNDF (g kg^{-1} NDF) *in situ*; $k_d$pdNDF = fractional degradation rate of potentially digestible NDF from curve fitting of *in situ* values. DM = dry matter; ADL = acid detergent lignin.

Table 2. Performance of dairy cows fed a total mixed ration containing silage of tall fescue or timothy harvested at an early or a very early date in spring growth cycle (n=10).

Item[1]	Very early harvest May 25		Early harvest May 30		SEM	*P*-value[2]		
	Tall fescue	Timothy	Tall fescue	Timothy		H	S	H × S
DMI, kg day^{-1}	24.3	25.1	24.4	23.9	0.89	0.50	0.85	0.42
DMI, g kg^{-1} BW	4.06	4.25	4.09	3.73	0.16	0.10	0.55	0.06
Milk, kg	35.4	36.1	34.7	36.2	1.51	0.81	0.37	0.76
ECM, kg	33.9	36.5	34.2	35.2	1.21	0.57	0.06	0.37
Feed efficiency, milk, kg DMI, kg^{-1}	1.49	1.54	1.42	1.54	0.06	0.46	0.09	0.54
Feed efficiency, ECM, kg DMI, kg^{-1}	1.38	1.53	1.42	1.49	0.04	0.99	0.01	0.32
Milk fat, g kg^{-1}	38.3	40.5	40.6	38.6	1.60	0.88	0.92	0.17
Milk fat, kg	1.35	1.46	1.38	1.39	0.06	0.67	0.20	0.27
Milk protein, g kg^{-1}	34.4	36.4	35.9	36.0	0.70	0.49	0.15	0.21
Milk protein, kg	1.21	1.32	1.23	1.30	0.05	0.94	0.02	0.62
Milk urea, mmol l^{-1}	4.21	3.71	4.16	3.80	0.11	0.90	<0.001	0.50

[1] DMI = dry matter intake; ECM = energy-corrected milk; BW = body weight.

[2] H = harvest date (May 25 vs May 31); S = species (tall fescue vs timothy).

forage species (Hatfield *et al.,* 2007; Jung and Allen, 1995). These differences in the chemical structure of the lignified cell walls could possibly be the reason why lignin is more inhibitory to digestion of the cell wall in tall fescue compared to timothy. However, more research is needed to explain the differences in inhibitory effects of lignin structure on ruminal digestion.

Conclusions

Performance of the dairy cows was affected by forage species, where timothy silage resulted in improved feed efficiency compared to cows fed tall fescue, regardless of harvest date. The very early harvest date had no production advantage compared to the early harvest date.

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References

Allen M.S. (1996) Physical constraints on voluntary intake of forages by ruminants. *Journal of Animal Science* 74, 3063-3075.

Buxton D.R. (1996) Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science and Technology* 59, 37-49.

Hatfield R.D., Jung H.G., Broderick G. and Jenkins T.C. (2007) Nutritional chemistry of forages. In: Barnes, R.F, Nelson, C.J., Moore K.J. and Collins M. (eds.) *Forages, the science of grassland agriculture*, Vol. II, 6th edition Blackwell Publishing, Ames, IA, USA, pp. 467-485.

Jung H.G. and Allen M.S. (1995) Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. *Journal of Animal Science* 73, 2774-2790.

Krämer M., Weisbjerg M.R., Lund P., Jensen C.S and Pedersen M.G. (2012) Estimation of indigestible NDF in forages and concentrates from cell wall composition. *Animal Feed Science and Technology* 177, 40-51.

Niemeläinen O., Jauhiainen L. and Miettinen E. (2001) Yield profile of tall fescue (*Festuca arundinacea*) in comparison with meadow fescue (*F. pratensis*) in Finland. *Grass and Forage Science* 56, 249-258.

In vivo grass digestibility prediction from biochemical criteria and the sum of temperatures at cutting

Deroche B.[1,2], Salis L.[1], Le Morvan A.[1], Bernard M.[3], Wyss U.[4], Aoun M.[2] and Baumont R.[1]
[1] Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, 63122 Saint-Genès-Champanelle, France; [2] IDENA, 44880 Sautron, France; [3] INRAE, UE Herbipôle, 63122 Saint-Genès-Champanelle, France; [4] AGROSCOPE, 1725 Posieux, Switzerland

Abstract

In vivo digestibility (OMd) of forages is highly dependent on plant maturity stage at cutting, which is linked to the sum of temperatures (ST). In this study, we aimed to test if combining biochemical criteria, such as chemical composition and *in vitro* enzymatic digestibility, and ST improves the prediction of digestibility of hays harvested in three different locations (Massif Central and Jura in France and Fribourg foothills in Switzerland). *In vivo* OMd on sheep, chemical composition, *in vitro* enzymatic digestibility and ST were measured on 32 multispecies grassland hays harvested from 2015 to 2017. Spearman's correlations indicated that ST was negatively linked with OMd of hays harvested during the first vegetation cycle, whereas *in vitro* enzymatic digestibility was the biochemical criteria most strongly related to *in vivo* OMd. The best prediction models of *in vivo* OMd using biochemical criteria were obtained with *in vitro* enzymatic digestibility and crude protein (R^2=0.884; RMSE=0.0173), and the addition of ST slightly improved the model (R^2=0.910; RMSE=0.0153).

Keywords: hay, *in vivo* digestibility, *in vitro* enzymatic digestibility, sum of temperatures

Introduction

The determination of feed value of forage, especially organic matter digestibility (OMd), has an important role in the context of ruminant feeding, and it needs to be predicted precisely. The plant maturity stage at cutting highly influences OMd (Buxton, 1996). The sum of temperatures (ST; in growing degree-days) is positively linked to plant maturity stage at cutting, and negatively to chemical composition and *in vitro* enzymatic digestibility (Michaud *et al.*, 2012). Chemical composition and/or *in vitro* digestibility are commonly used to predict *in vivo* OMd in most ruminant feeding systems. In a 32-year survey of *in vivo* OMd of hays harvested at the same location in Massif Central (France), it was shown that ST explained part of the variability in OMd that chemical components did not (Deroche *et al.*, 2020). In this study, we aimed to test if combining biochemical criteria and ST can improve the prediction of digestibility of hays harvested in different locations.

Materials and methods

A collection of 32 hays harvested on multispecies grasslands was used in the study. Twenty-six hays were harvested during the first vegetation cycle: two in 2015 and four in 2017 in Fribourg foothills (Switzerland), 8 in 2017 in Jura (France) and 12 in Massif Central (France) (4 each year between 2015 and 2017). In addition, six regrowth hays were used, including two regrowth hays cut during the third vegetation cycle in 2015 in Fribourg foothills (Switzerland) and four regrowth hays cut during the second vegetation cycle in 2017 in Massif Central (France). The *in vivo* OMd of the 32 hays was measured on sheep at INRA according the method described by Demarquilly *et al.* (1995). The chemical composition of the hays offered to the sheep was analysed for determination of crude protein (CP), total soluble carbohydrates (TSC), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents, and the *in vitro* enzymatic dry matter (DM) digestibility (eDMd) was analysed according to Aufrère *et al.* (2007). The ST at cutting was calculated according to Theau and Zerourou (2008) by accumulation of mean daily air temperatures comprising between 0 to 18 °C from 1 February to the cutting date for hays harvested during the 1st vegetation cycle, and from the previous cutting date to the regrowth cutting date for hays harvested during 2nd and 3rd vegetation cycles. Correlations between OMd and biochemical criteria and ST were assessed using Spearman's correlation (SAS 5.1). Stepwise linear regression was used to predict OMd by (1) chemical components, (2) *in vitro* enzymatic digestibility, (3) chemical components and *in vitro* enzymatic digestibility, and (4) biochemical criteria and ST. The effect of ST was nested within the vegetation cycle (1st cycle and 2nd or 3rd cycles). Stability and validity of each model was checked by a leave one out cross validation (R software – package lm).

Results and discussion

The *in vivo* OMd of the herbage from 32 grasslands conserved as hays ranged from 0.539 to 0.725 (Table 1). *In vivo* OMd was highly correlated with *in vitro* enzymatic digestibility and NDF content and to a lesser extent with the other chemical components. The *in vivo* OMd was negatively correlated with ST for the 26 hays harvested during the first vegetation cycle, in accordance with Michaud *et al.* (2012). The positive correlation between ST and *in vivo* OMd on regrowth hays might be explained by the presence of leafy species (e.g. *Taraxacum* sp.) whose digestibility remains high in advanced maturity stages (Schubiger *et al.*, 2001). The *in vitro* enzymatic digestibility confirmed to be a very good predictor of *in vivo* OMd (R^2=0.871), better than the combination of CP and ADF contents (R^2=0.786) (Table 2) in accordance with Aufrère *et al.* (2007). The prediction of *in vivo* OMd by *in vitro* eDMd was slightly improved by the addition of the CP content in the model (R^2=0.884) or by the addition of CP content and ST (R^2=0.910; *RMSE*=1.531), which was the best model established on our dataset (Table 2). The negative coefficient of ST in the prediction model for hays harvested during the first vegetation cycle is consistent with literature (Michaud *et al.*, 2012), and with previous study (Deroche *et al.*, 2020).

Table 1. Descriptive statistics of the variables measured on the 32 multispecies grassland hays and Spearman's correlation between *in vivo* OMd and other criteria.

Variables[1]	n	Mean	SD	Minimum	Maximum	Correlation with OMd[2]
In vivo OMd (g g^{-1})	32	0.642	0.051	0.539	0.725	-
Crude protein (g kg^{-1} DM)	32	107	21.1	77.5	161	0.81***
Neutral detergent fibre (g kg^{-1} DM)	32	572	72.0	430	678	-0.93***
Acid detergent fibre (g kg^{-1} DM)	32	303	43.1	228	376	-0.90***
Total soluble carbohydrate (g kg^{-1} DM)	32	159	53.8	104	308	0.80***
eDMd (g g^{-1})	32	0.607	0.100	0.444	0.770	0.95***
ST_1st cycle hay (degree.day)	26	914	177	634	1325	-0.73***
ST_regrowth hay (degree.day)	6	1012	168	765	1295	0.85*

[1] DM = dry matter; eDMd = enzymatic dry matter digestibility; OMd = organic matter digestibility; ST = sum of temperatures; ST_1st cycle hay or ST_regrowth hay = ST is analysed within vegetation cycle.

[2] Significance: * = P<0.05; ** = P<0.01; *** = P<0.001; ns = non-significant.

Table 2. *In vivo* hay OMd prediction models (n=32), and cross validation with the leave one out method.[1]

Model components	Calibration		Cross validation
	RMSE	R^2	RMSPE
$0.767 - 7.087 \times 10^{-4} \times$ ADF (g kg^{-1} DM) + $8.431 \times 10^{-4} \times$ CP (g kg^{-1} DM)	0.0235	0.786	0.0246
$0.354 + 0.475 \times$ eDMd (g g^{-1})	0.0186	0.871	0.0194
$0.349 + 0.388 \times$ eDMd (g g^{-1}) + $0.054 \times$ CP (g kg^{-1} DM)	0.0173	0.884	0.0181
$0.371 + 0.316 \times$ eDMd (g g^{-1}) + $1.041 \times 10^{-3} \times$ CP (g kg^{-1} DM) – $2.94 \times 10^{-5} \times$ ST_1st cycle hay (degree.days) – $5.025 \times 10^{-5} \times$ ST_regrowth hay (degree.days)	0.0153	0.910	0.0166

[1] ADF = acid detergent fibre; CP = crude protein; DM = dry matter; OMd = organic matter digestibility; RMSE = root mean square error; RMSPE = root mean square of prediction error.

Conclusions

This study confirmed on hays from various origins that the *in vitro* enzymatic dry matter digestibility is a powerful criterion to predict *in vivo* digestibility. Agro-climatic criteria such as ST, which can be easily obtained from weather stations, are also correlated with *in vivo* digestibility of grass. Nevertheless, when combined with *in vitro* digestibility they improve only slightly the predictions of *in vivo* digestibility of grass.

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References

Aufrère J., Baumont R., Delaby L., Peccatte J.R., Andrieu J., Andrieu J.P. and Dulphy J.P. (2007) Prévision de la digestibilité des fourrages par la méthode pepsine-cellulase. Le point sur les équations proposées. *INRA Productions Animales* 20, 129-136.

Buxton D.R. (1996) Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science and Technology* 59, 37-49.

Demarquilly C., Chenost M. and Giger S. (1995) Pertes fécales et digestibilité des aliments des rations. In: Jarrige R., Ruckebusch Y., Demarquilly C., Farce M.H. and Journet M. (eds.) *Nutrition des ruminants domestiques, ingestion et digestion.* INRA, Paris, France, pp. 601-647.

Deroche B., Pradel P. and Baumont R. (2020) Long-term evolution and prediction of feed value for permanent mountain grassland hay: Analysis of a 32-year dataset in relation to climate change. *Grass and Forage Science* 75, 18-27.

Michaud A., Andueza D., Picard F., Plantureux S. and Baumont R. (2012) Seasonal dynamics of biomass production and herbage quality of three grasslands with contrasting functional compositions: Production and quality of permanent grasslands. *Grass and Forage Science* 67, 64-76.

Schubiger F.X., Lehmann J., Daccord R., Arrigo Y., Jeangros B. and Scehovic J. (2001) Valeur nutritive des plantes des prairies. 5. Digestibilité de la matière organique. *Revue Suisse d'Agriculture* 33, 275-279.

Theau J.P. and Zerourou A. (2008) Herb'âge, une méthode de calcul des sommes de températures pour la gestion des prairies. In: Cruz P., Jouany C. and Theau J.P. (eds.) *Les cahiers d'Orphée. Outils pour la Gestion des Prairies Permanentes*, INRA, Paris, France, pp. 91-102.

Future forages: differential effect of climate change scenarios on forage grasses for ruminant production.

Hart E.H.[1], Christofides S.[2], Rogers H.[2], Creevey C.[3], Müller C.[2] and Kingston-Smith A.H.[1]
[1]*IBERS, Aberystwyth University, United Kingdom;* [2]*School of Biosciences, Cardiff University, United Kingdom;* [3]*School of Biological Sciences, Queen's University Belfast, United Kingdom*

Abstract

Forage crops for livestock are essential for ruminant production. Human population growth will lead to an increase in demand for animal products together with pressure to decrease pollutant output. Increases in ruminant production have been achieved to date through continual improvement of forage feed germplasm. Climate models predict increases in temperature, atmospheric CO_2, precipitation and altered weather systems for 2050. Plants respond to abiotic stresses by physiological, biochemical, metabolic and molecular mechanisms. Thus, pre-exposure to stress can potentially prime the plants such that the stress response is altered in the rumen. We screened 10 forage grass varieties in 5 climate scenarios, including current climate (2020), future climate (2050), future (2050) plus flooding, future (2050) plus drought and future (2050) plus heat shock. When forage grasses were subjected to *in vitro* simulation of rumen conditions after being exposed to different climate scenarios there were significant differences in fermentation parameters and overall gas production. These results indicate that forage adaptation due to climate change will affect ruminant production systems, which is an important consideration regarding the appropriateness of current and future forage varieties for animal production in the future.

Keywords: climate change, future forages, ruminant production

Introduction

Human population growth predicts an increase to 9.7 billion by 2050, leading to an increased demand for animal products together with pressure to decrease pollutant output. Increases in ruminant production have been achieved to date through continual improvement of the forage feed germplasm. Therefore, the development of potential new forage varieties for livestock production is essential to meet future demands. However, climate change models in the near future (2050) predict increases in temperature (an increase of 4-6 °C in the UK), atmospheric CO_2 (from current 400 ppm to 500 ppm (Smith and Myers, 2018), precipitation and altered weather systems (e.g. extreme drought and flooding). Ingested forages are broken down in the rumen and react by inducing plant hormone-mediated stress responses (Kingston-Smith *et al.*, 2012), which in turn affect the overall efficiency of the rumen system. Previous studies have focused on the characterization of physiological changes of forage plant varieties under climate change conditions, but there has been relatively little research into how forage adaptation due to climate change will affect ruminant production systems, and the appropriateness of current and future forage varieties for animal production in the future. We aim to determine how 'stress memory' to a prior adverse environment may affect the contribution of the plant processes to feed degradation and so understand how ruminant feeding strategies might be affected by climate change.

Materials and methods

Ten different varieties of current forage grasses were grown for 3 months in five different climate scenarios consisting of control 2020 (400 ppm CO_2, 16/18 °C night/day temperature, 8 h photoperiod and watered regularly), control 2050 (500 ppm CO_2, 21/23 °C night/day temperature, 8 h photoperiod and watered regularly), Flood (as for control 2050 plus flood for 1 week before harvest), Drought (as for control 2050 but no water 1 week before harvest), Heat shock (as for control 2050 but temperature increased to 35 °C two days before harvest). Leaves were analysed for composition through measurement of plant dry matter, protein, water soluble carbohydrates (WSC), metabolites and chlorophyll levels before being incubated anaerobically at 39 °C with rumen fluid inoculum (mix from 4 cows) for 48 h (Theodorou *et al.,* 1994). Volatile organic compounds (VOCs) were collected from the head space at 24 h and analysed by mass spectrometry (BenchTOFdx, Markes International, Llantrisant, UK). Data analysis was conducted in R version 3.6.0 using RStudio (R Development Core Team 2011; RStudio Team 2016) using ANOVA.

Results and discussion

The physiological characteristics of the grass at point of harvest differed from each other among climate scenarios (P<0.001). This demonstrated the effect of abiotic stresses on the plants when grown in climate change scenarios. Previous research has shown that elevated levels of CO_2 improve the rate of photosynthesis in plants (Taub, 2010). However, high temperatures have been shown to inhibit photosynthesis by altering the structure of chloroplasts and by inactivating chloroplast enzymes by oxidative stress (Dekov *et al.*, 2000). Chlorophyll levels were highest in control 2020 and lowest in the flood scenario (P<0.05). When plants are flooded, the oxidative pathways move to fermentative and CO_2 levels, aerobic respiration and photosynthesis is decreased due to low light levels and limited gaseous exchange leading to an energy crisis within the plant (Yeung *et al.,* 2018).

Plants can 'remember' past occurrences (Kinoshita and Seki, 2014), and in agreement with this we found that subjecting plants to different environmental scenarios resulted in different responses during *in vitro* fermentation (P<0.001). Grasses previously subjected to a heat shock climate scenario resulted in more total gas produced compared to the other climate scenarios. Total CO_2 production was decreased in flood, drought and heat shock climate scenarios compared to the 2020 and 2050 controls. Interestingly, methane production was significantly lower during fermentation of grasses that had been previously subjected to flood and drought as compared to the 2020 control (Figure 1). It has been suggested that partitioning of hexose into fermentation end products (including methane) and microbial biomass is influenced by dietary carbohydrate and that increasing the proportion of WSC in the diet can significantly reduce the amount of methane produced (Moss *et al.,* 2001). No links were determined here between

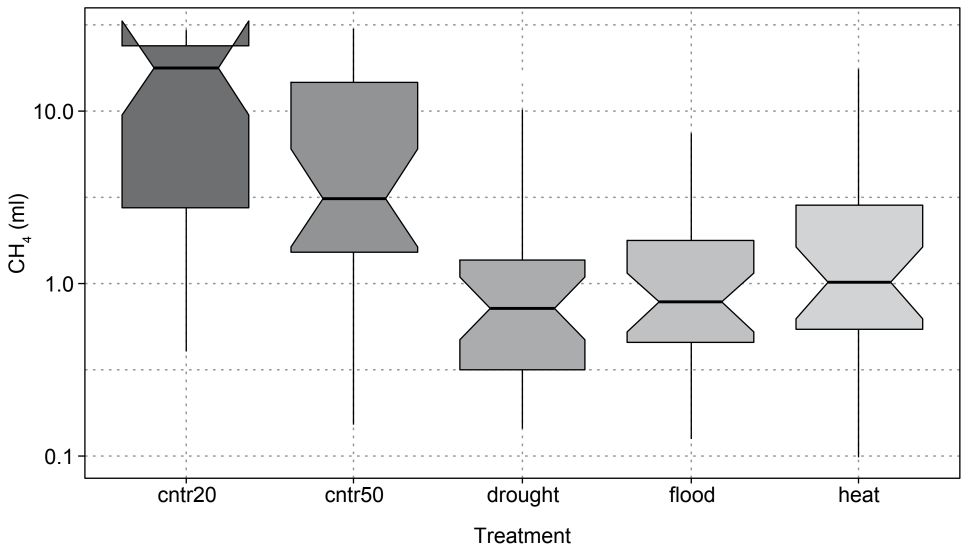

Figure 1. Cumulative methane production (ml g^{-1} dry matter) during *in vitro* fermentation (rumen microbial inoculum) of forage grown under different climate scenarios after 48 h.

WSC and methane outputs, however, overall plant physiological changes due to stress response to the climate scenario were observed to have significant effects. VOC profiles at 24 h *in vitro* fermentation revealed the most important compound in classification to be 3-pentanol, which activates pathogen defence responses via salicylic acid, jasmonic acid and ethylene signalling pathways (Song *et al.,* 2015).

Conclusions

Overall plant physiological changes due to stress response to climate scenario were observed to have significant effects. Further clarification will be required as to which parameters may be causing these changes within the rumen environment in the future.

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References

Dekov I., Tsonev T. and Yordanov I. (2000) Effects of water stress and high-temperature stress on the structure and activity of photosynthetic apparatus of *Zea mays* and *Helianthus annuus*. *Photosynthetica* 38, 361-366.

Kingston-Smith A.H., Davies T.E., Edwards J.E., Gay A. and Mur L.A.J. (2012) Evidence of a role for foliar salicylic acid in regulating the rate of post-ingestive protein breakdown in ruminants and contributing to landscape pollution, *Journal of Experimental Botany* 63, 3243-3255.

Kinoshita T. and Seki M. (2014) Epigenetic memory for stress response and adaptation in plants. *Plant and Cell Physiology* 55, 1859-186.

Moss A.R., Newbold C.J. and Givens D.I. (2001) The impact of hexose partitioning in sheep *in vivo*. *Proceedings of the British Society of Animal Science 2001,* pp. 157-157.

Smith M.R and Myers S.S. (2018) Impact of anthropogenic CO_2 emissions on global human nutrition. *Nature Climate Change* 8, 834-839

Song G.C., Choi H.K. and Ryu C.M. (2015) Gaseous 3-pentanol primes plant immunity against a bacterial speck pathogen, Pseudomonas syringae pv. tomato via salicylic acid and jasmonic acid-dependent signalling pathways in Arabidopsis. *Frontiers in Plant Science* 6, 821.

Taub D. (2010) Effects of rising atmospheric concentrations of carbon dioxide on plants. *Nature Education Knowledge* 3, 21.

Theodorou M.K., Williams B.A., Dhanoa M.S., McAllan A.B and France J. (1994) A simple gas production method using a pressure transducer to determine the fermentation kinetics of rumen feeds. *Animal Feed Science and Technology* 48, 185-197.

Yeung E., Van Veen H., Vashisht D., Paiva A.L.S., Hummel M., Rankenberg T., *et al.* (2018) A stress recovery signalling network for enhanced flooding tolerance in Arabidopsis thaliana. *Proceedings of the Natural Academy of Sciences* 115, E6085-E6094.

Concentration of phytoestrogens in red clover is affected by variety and season

Johansen M.[1], Jalůvka L.[2], Klitgaard G.[3] and Weisbjerg M.R.[1]
[1]Department of Animal Science, AU Foulum, Aarhus University, Tjele, Denmark; [2]DLF Seeds s.r.o., Hladké Životice, Czech Republic; [3]DLF Seeds & Science, Odense, Denmark

Abstract

The aim of this study was to investigate variation in phytoestrogen concentration in different varieties of red clover and the variation over the season. Eighteen varieties of red clover (12 diploid and 6 tetraploid) were sown in duplicates in a plot experiment in 2014. All plots were harvested four times a year in the growing season of 2015 and 2016. Samples were analysed for eight different phytoestrogens. Total amount of phytoestrogens decreased during the season. It was 86% higher in spring growth than in the third regrowth. Phytoestrogen composition also changed during the season. Differences between varieties were consistent over season and year, and the variety with the highest concentration had a concentration 57% higher than that with the lowest concentration. The considerable variation in phytoestrogen concentrations among varieties presents opportunities for selection of varieties with low concentrations for use when a high inclusion of red clover is required in ruminant diets.

Keywords: legume, forage, phytoestrogen, ruminant nutrition

Introduction

Phytoestrogens are a group of secondary metabolites in plants that in their active form can have oestrogenic effects in animals. The group includes isoflavones, coumestans and lignans, but isoflavones are the most important and are found in significant amounts in red clover (*Trifolium pratense* L.). Phytoestrogens can cause infertility, abnormal udder development and lactation, as well as uterine prolapse in sheep (Adams, 1995), whereby varieties with low phytoestrogen concentration are required if red clover constitutes a major proportion of the diet. The objective of this study was therefore to investigate the variation in phytoestrogen concentration in different varieties of red clover as well as the variation over the season when grown under Danish conditions.

Materials and methods

Eighteen varieties of red clover were used in a plot-experiment sown at the location of DLF (55°18'N, 12°25'E), Store Heddinge, Denmark. Varieties were selected from commercial varieties (Suez, Vendelín, Pavo, SW Ares and Lars) and the portfolio of DLF included commercial varieties and new varieties/candidates from DLF's breeding programme; twelve of the selected varieties were diploid and six were tetraploid. All varieties were sown in duplicated plots (plot size: 12 m^2) in a randomised complete block design in August 2014. Four cuts were harvested during each of the following two growing seasons: on 21 May, 6 July, 7 Sept, and 2 Nov of 2015, and 26 May, 30 June, 17 Aug, and 26 Oct of 2016. Dried samples were extracted with acidified methanol for 30 min and afterwards analysed for eight isoflavons: daidzin, daidzein, genistin, genistein, ononin, formononetin, sissotrin and biochanin A, with high-performance liquid chromatography (HPLC, Agilent Technology 1200 series) and tandem mass detection (MS/MS, Triple quadrupole QQQ Agilent 6460). Sum of the eight detected isoflavones was determined as total phytoestrogen (TP) concentration. For easier comparison with previous literature when analysing the results, the four aglycones where summed with their respective glycoside (i.e. daidzein and daidzin, genistein and genistin, formononetin and ononin, biochanin A and sissotrin, respectively). Statistical analyses were performed in R (version 3.6.0) using a linear model including variety (n=18), growth number (n=4), year (n=2) and block (n=2) as main effects as well as the two- and three-way interactions between variety, growth number and year.

Results and discussion

Concentration of TP was higher in 2016 than in 2015 in all four growth periods (Table 1), but in both years the concentration decreased during the season, with average 34.2 g kg^{-1} of dry weight (DW) in spring growth and 18.4 g kg^{-1} of DW in third regrowth. An interaction between year and growth appeared, as TP concentration was much higher (+ 11.3 g kg^{-1} of DW) in second regrowth in 2016 than in 2015, whereas for the other three growths the 2016 concentration was on average 1.87 g kg^{-1} of DW higher than in 2015. This may be because the regrowth period for the second regrowth was only 48 days in 2016, compared with 63 days in 2015. McMurray *et al.* (1986) showed that longer regrowth period reduces isoflavone concentration. Therefore, decreased concentration of TP over the season may be partly a result of an increased regrowth period later in season. Temperature and other environmental conditions can also affect phytoestrogen concentration, but under controlled conditions, lower temperature results in higher formononetin concentration (McMurray *et al.*, 1986). If temperature affected TP concentration over the season, lowest concentration should appear during summer (first and second regrowth) when temperatures are higher than in spring and autumn. In agreement with earlier studies (Mustonen *et al.*, 2018), formononetin was the most abundant isoflavone observed (565 mg g^{-1} of TP) followed by biochanin A (345 mg g^{-1} of TP), whereas the proportions of daidzein and genistein were minor. In both years, the proportion of formononetin was higher in spring growth than in the other growths, and generally, formononetin proportion was higher in 2016 than 2015. Proportion of biochanin A was higher in first and second regrowth than in spring growth and third regrowth. Variety influenced TP concentrations, varying from 21.6 g kg^{-1} in Vendelín to 33.9 g kg^{-1} in Hammon (Table 2) and tetraploid varieties had a higher TP concentration than diploid varieties (30.6 vs 25.9 g kg^{-1}). Variety × year interaction was not significant (P=0.91), whereas there were minor changes in the ranging of varieties over the season ($P_{variety \times growth}$=0.04). Overall, differences among varieties were constant, in agreement with previous studies (Mustonen *et al.*, 2018). Varieties also differed in proportion of single isoflavones, where Ganymed had lowest formononetin proportion (504 mg g^{-1} of TP) and highest biochanin A proportion (400 mg g^{-1} of TP). This can, besides TP concentration, be important when selecting species for ruminant feed, as formononetin in the rumen is converted to equol, which has an oestrogenic effect, whereas biochanin A is broken down to an inactive compound (Lundh, 1995).

Table 1. Total phytoestrogen (TP) concentration (g kg^{-1} of dry weight) and composition of TP (mg g^{-1} of TP) in two different years and in four growths, harvested during each year across eighteen varieties of red clover.

	Growth[1]	TP	Composition[2]			
			Formononetin	Biochanin A	Genistein	Daidzein
2015	Spring growth, 31d	33.0	580	366	35.6	18.1
	First regrowth, 46 d	31.3	514	446	24.1	16.3
	Second regrowth, 63 d	19.4	475	437	59.0	29.2
	Third regrowth, 63 d	17.7	502	329	121	48.1
2016	Spring growth, 36 d	35.4	634	258	79.0	28.9
	First regrowth, 35 d	32.9	598	337	50.2	14.6
	Second regrowth, 48 d	30.7	607	324	53.3	15.2
	Third regrowth, 70 d	19.2	611	264	87.1	38.7
SEM		1.02	3.65	3.86	2.07	1.37
P-value	Year (Y)	<0.001	<0.001	<0.001	<0.001	<0.001
	Growth (G)	<0.001	<0.001	<0.001	<0.001	<0.001
	Y × G	<0.001	<0.001	<0.001	<0.001	<0.001

[1] Days of growth period; for the spring growth calculated from April 20 to harvest. SEM = standard error of the mean.

[2] Sum of aglycone and respective glycoside for each isoflavone.

Table 2. Total phytoestrogen (TP) concentration (g kg^{-1} of dry weight) and composition of TP (mg g^{-1} of TP) in eighteen varieties of red clover across two years and four growths harvested during each year.

	Clover variety	TP	Composition[1]			
			Formononetin	Biochanin A	Genistein	Daidzein
Diploid	Vendelín	21.6	580	319	73.1	27.8
	Suez	23.3	535	364	72.9	27.4
	Kalyke	23.4	546	361	65.4	27.9
	DLF TPD-09-1001	24.2	585	314	72.7	28.0
	Ganymed	24.7	504	400	74.5	22.3
	Callisto	25.6	543	356	72.8	28.1
	SW Ares	25.6	590	313	67.2	29.4
	Pavo	25.8	553	358	62.7	26.6
	Elara	27.0	577	334	62.4	26.5
	Himalia	29.4	544	369	62.3	24.0
	Rajah	29.4	600	317	59.5	22.8
	Hegemon	30.8	565	351	57.7	26.2
Tetraploid	Nodula	27.6	582	333	59.7	25.7
	Bivoj	28.8	576	336	57.4	30.3
	Rezista	29.9	585	334	54.9	26.1
	Lars	31.6	552	362	59.3	26.3
	Vesna	31.6	578	343	55.1	24.5
	Hammon	33.9	574	348	56.9	20.7
SEM[2]		1.53	5.48	5.79	3.11	2.05
P-value	Variety	<0.001	<0.001	<0.001	<0.001	0.11
	Di- vs tetraploid	<0.001	<0.001	0.22	<0.001	0.43

[1] Sum of aglycone and respective glycoside for each isoflavone.

[2] SEM = standard error of the mean.

Conclusions

Concentration of phytoestrogens in red clover depended on variety, but it also varied during the season, and this was probably related to the regrowth period. Selection of varieties with a lower phytoestrogen concentration is possible, as differences between varieties are consistent over season and year.

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References

Adams N.R. (1995) Detection of the effects of phytoestrogens on sheep and cattle. *Journal of Animal Science* 73, 1509-1515.

Lundh T. (1995) Metabolism of estrogenic isoflavones in domestic animals. *Proceedings of the Society for Experimental Biology and Medicine* 208, 33-39.

McMurray C.H., Laidlaw A.S. and McElroy M. (1986) The effect of plant development and environment on formononetin concentration in red clover (*Trifolium pratense* L.). *Journal of the Science of Food and Agriculture* 37, 333-340.

Mustonen E., Tuori M., Kurki P., Isolahti M., Taponen J. and Vanhatalo A. (2018) Variety, time of harvest and conditions during growing season have impact on red clover isoflavone content. *Agricultural and Food Science* 27, 102-109.

Performance of mixtures of perennial ryegrass varieties evaluated under animal grazing

Tubritt T.[1,2], Delaby L.[3], Gilliland T.J.[2] and O'Donovan M.[1]

[1] *Teagasc, Animal and Grassland Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland;* [2] *The Institute for Global Food Security, Queen's University Belfast, Belfast, N. Ireland;* [3] *INRA, AgroCampus Ouest, UMR 1348, Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Elevage, Saint-Gilles, France*

Abstract

In Ireland, perennial ryegrass (*Lolium perenne* L., PRG) varieties are typically sown as mixtures but evaluation of variety performance takes place in monoculture plots. Two PRG variety studies were conducted to examine the performance of known varieties sown as mixtures under animal grazing. Within each study four varieties were examined. Ploidy was split equally within both studies. The first study contained varieties with a heading date range of nine days (2 June to 10 June). The second study contained varieties with a wider heading date range of eighteen days (23 May to 9 June). The studies were established in 2017 and examined over two grazing seasons. Within both studies, herbage accumulation, grazing efficiency and nutritive value ranking of mixtures differed significantly based on ploidy content. Variety mixtures tended to perform as the mean of the component varieties monoculture performance.

Keywords: perennial ryegrass, variety, mixture, grazing

Introduction

Reseeding levels within the Republic of Ireland are low with approximately 2% of grassland area reseeded annually (Creighton *et al.*, 2011). The Pasture Profit Index (PPI) has been developed as a variety selection support tool for the industry (O'Donovan *et al.*, 2017). The PPI ranks the highest economic performing varieties in Ireland based on their agronomic performance recorded as monocultures, by the Department of Agriculture, Food and the Marine (Recommended list trials). Traditionally grass seed is sown as mixtures of varieties. Previous research has examined how variety ratios sown as mixtures change over time (Griffith *et al.*, 2016). That study examined varieties under a mechanical harvesting (cutting) protocol which differs from how varieties are actually employed on-farm, as 75% of grass is utilised under grazing within the Republic of Ireland (O'Brien *et al.*, 2019). The aim of our study was to investigate the performance of perennial ryegrass (*Lolium perenne* L., PRG) mixtures compiled from the leading varieties of the PPI managed under a rotational grazing system.

Materials and methods

Two plot studies (Study 1 and 2) were sown in 2017 with plot size measuring 3×5 m. Table 1 displays the varieties examined and their respective ploidy and heading date classification. The maximum heading date range of the mixtures in Study 1 was 9 days and in Study 2 it was 18 days. Each replicate (n=3) contained 15 plots with the following treatments at sowing: monoculture (n=4), two varieties of equal proportions (n=6), three varieties of equal proportions (n=4) and four varieties of equal proportions (n=1). Each treatment was assigned a ploidy characterisation: Diploid (>50% diploid at sowing), Tetraploid (>50% tetraploid at sowing) and Equal (50% of both diploid and tetraploid at sowing). The plots were rotationally grazed by dairy cows when average herbage cover was estimated to be 1,400 kg dry matter (DM) ha^{-1} (above 3.5 cm). Cows were removed when the target average post-grazing height (4 cm) was reached (visually assessed). The plots were grazed on 8 occasions in both 2018 and 2019. On average 50 cows grazed each study separately with residency time never exceeding 12 hours within each study. DM yield, herbage accumulation, pre- and post-grazing height and herbage quality were

Table 1. Varieties examined within both studies and their respective ploidy and heading date.

Study 1			Study 2		
Variety	**Ploidy**	**Heading date**	**Variety**	**Ploidy**	**Heading date**
AberChoice	Diploid	June 9th	AberChoice	Diploid	June 9th
Astonenergy	Tetraploid	June 2nd	AberClyde	Tetraploid	May 25th
Oakpark	Diploid	June 2nd	Astonenergy	Tetraploid	June 2nd
Solas	Tetraploid	June 10th	Rosetta	Diploid	May 23rd

measured as in Tubritt *et al.* (2019). Proportion of herbage consumed was calculated as height consumed (pre-grazing height – post-grazing height) multiplied by herbage density (DM yield / pre-grazing height – cutting height), expressed as a proportion of pre-grazing DM yield. Data were analysed using the statistical analysis software SAS (SAS Inst. Inc., Cary, NC, USA). A mixed model was applied including sward treatment, ploidy, heading, grazing event and year as fixed factors. Block was used as a random factor.

Results and discussion

Significant differences (P<0.001) were found in mixture performance for all the measured variables in both studies (Table 2 and Table 3), and while there were significant seasonal differences between grazings, the predominant factor was always the ploidy composition of the mixtures. Swards of equal ploidy performed within the range of both ploidy dominant mixtures for all traits. Herbage accumulation was lower for tetraploid dominant swards than for diploid swards. Lower post-grazing height of tetraploid swards may explain the lower herbage yield as these swards have lower initial regrowth levels due to less leaf being available for photosynthesis (Tubritt *et al.*, 2019). Lower post-grazing height and higher

Table 2. Agronomic performance of Diploid (>50% diploid), Tetraploid (>50% tetraploid) and Equal (50% diploid: 50% tetraploid) swards in Study 1.[1,2]

	Diploid	Equal	Tetraploid	SE	Treatment	Ploidy	Year
Herbage accumulation (kg DM ha^{-1})	12,098	11,805	11,393	58	***	***	***
Pre-grazing height (cm)	11.0	10.7	10.0	0.1	NS	***	NS
Post-grazing height (cm)	4.4	4.1	3.8	0.1	***	***	***
Proportion consumed	0.89	0.93	0.97	0.01	***	***	NS
OMD (g kg^{-1} DM)	825	841	842	3	NS	***	NS

[1] Levels of significance: NS = P>0.05; * = P<0.05; ** = P<0.01; *** = P<0.001.

[2] OMD = organic matter digestibility; DM = dry matter; SE = standard error.

Table 3. Agronomic performance of Diploid (>50% diploid), Tetraploid (>50% tetraploid) and Equal (50% diploid: 50% tetraploid) swards in Study 2.[1,2]

	Diploid	Equal	Tetraploid	SE	Treatment	Ploidy	Year
Herbage accumulation (kg DM ha^{-1})	12,001	11,810	11,520	35	***	***	***
Pre-grazing height (cm)	10.5	10.4	9.9	0.2	NS	*	NS
Post-grazing height (cm)	4.1	4.1	3.8	0.1	NS	***	***
Proportion consumed	0.91	0.92	0.96	0.01	NS	***	***
OMD (g kg^{-1} DM)	861	867	876	2	NS	***	NS

[1] Levels of significance: NS = P>0.05; * = P<0.05; ** = P<0.01; *** = P<0.001.

[2] OMD = organic matter digestibility; DM = dry matter; SE = standard error.

proportion consumed indicated that tetraploid swards had increased grazing efficiency. Figure 1 displays the mean post-grazing height of each sward treatment within Study 1. Tetraploid-dominant swards had lower post-grazing height than the diploids, with equal ploidy treatments lying within the mid-range of both. A similar result was found in Study 2 for mean post-grazing height. Heading date in Study 1 and Study 2 had no significant effect on the measured variables. In both studies, mixture performance was consistent with the proportional performance values of their component varieties in monoculture (data not shown). The results from these studies are in agreement with previous monoculture studies examining the grazing efficiency of PRG varieties. These studies found that tetraploid varieties had higher grazing efficiency than diploids. Tetraploids have higher levels of organic matter digestibility (OMD), larger free leaf lamina and lower tiller density, which are allied to higher grazing efficiency (Tubritt *et al.*, 2019).

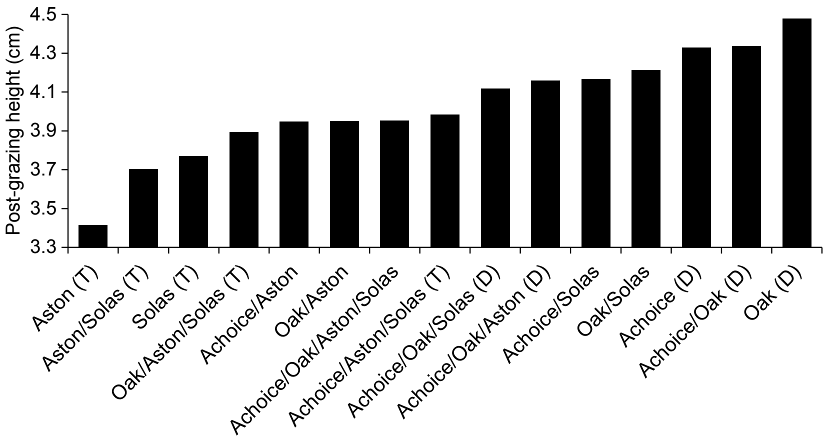

Figure 1. The mean post-grazing height of all treatments in Study 1.

Conclusions

Mixtures of PRG varieties performed to the mean of the component varieties. Tetraploid varieties displayed greater grazing efficiency (better graze out) than diploids and this was expressed in the ploidy dominant mixtures. Farmers selecting mixtures for use in grazing should select those with a high proportion of tetraploid varieties, and seeds merchants wishing to compile high grazing efficient mixtures should optimise the tetraploid content.

References

Creighton P., Kennedy E., Shalloo L., Boland T.M. and O'Donovan M. (2011) A survey analysis of grassland dairy farming in Ireland, investigating grassland management, technology adoption and sward renewal. *Grass and Forage Science* 66, 251-264.

Griffith V., Gilliland T.J., McEvoy M. and O'Donovan M. (2016) Competitive dynamics in mixtures of perennial ryegrass cultivars during two growing seasons under two cutting managements. *Crop and Pasture Science* 67, 225-235.

O'Brien D., Moran B. and Shalloo L. (2019) *Grass-fed Irish milk. Irish Dairying. Growing Sustainability.* Teagasc Animal and Grassland Research and Innovation Centre, Moorepark.

O'Donovan M., McHugh N., McEvoy M., Grogan D. and Shalloo L. (2017) Combining seasonal yield, silage dry matter yield, quality and persistency in an economic index to assist perennial ryegrass variety selection. *Journal of Agricultural Science,* 155(4), 556-568.

SAS (2014) *SAS version 9.4.* SAS Institute, Cary, NC, USA.

Tubritt T., Gilliland T.J., Delaby L. and O'Donovan M. (2019) Comparison of perennial ryegrass varieties for differences in grazing efficiency. *Grassland Science in Europe 24,* 527-529.

Effect of grazing management in autumn on the quality and quantity of perennial ryegrass in spring

Ankersmit E.[1], Ensing E.[2], Ter Horst A.C.[1], Bastiaansen-Aantjes L.M.[1] and Van den Pol-van Dasselaar A.[1]
[1]Aeres University of Applied Sciences, De Drieslag 4, 8251 JZ, Dronten, the Netherlands; [2]Barenbrug Holland BV, Stationsstraat 40, 6515 AB Nijmegen, the Netherlands

Abstract

Grazed grass is the cheapest feed available for pasture-based systems of milk production. An early start to grazing in spring can lead to reduced costs. Early spring grazing can be achieved by closing paddocks in autumn, and keeping the grass cover available for an early start in spring. However, this is not common in the Netherlands. The objective of this research was to examine the effects of autumn closing date on grass quality and quantity in early spring. A mixture of perennial ryegrass species was sown at Aeres Farms Dronten, the Netherlands, in autumn 2015. The study used a randomised block design with a factorial arrangement of eight treatments (four autumn closing dates × two spring opening dates) over a period of three years (autumn 2016 – autumn 2019). Results showed that under Dutch conditions early closing dates (September) are not favourable; they lead to lower dry matter yields than intermediate to late closing dates (October-November). It is possible, however, to close paddocks in September/early October and keep the grass cover available for an early start in spring. Early opening dates in spring also lead to higher crude protein contents of the fresh grass. Dutch farmers who would like to start early with grazing are recommended to use a balanced combination of early and late closing dates.

Keywords: autumn closing, grazing season, perennial ryegrass

Introduction

Grazed grass is the cheapest feed available for pasture-based systems of milk production (Van den Pol-Van Dasselaar *et al.*, 2013) in the Netherlands. In the Netherlands, grass starts to grow at 6-8 °C soil temperature, which means that there is little to no grass growth from Mid-November till March (Pellikaan, 2019). An early start to grazing in spring, when grass growth is still limited, could lead to reduced feeding costs. This could be achieved by closing paddocks in September/early October and keeping the grass cover available for an early start to grazing in spring, which is often done in Ireland (e.g. Hennessy *et al.*, 2006). However, this is not common in the Netherlands. The objective of this research was to examine the effects of autumn closing date on grass quality and quantity in early spring in the Netherlands.

Materials and methods

A mixture of perennial ryegrass species was sown at Aeres Farms Dronten, the Netherlands, in autumn 2015. The study used a randomized block design with a factorial arrangement of eight treatments (four autumn closing dates (CD) × two spring opening dates (OD)) over a period of three years (autumn 2016 – autumn 2019) in two paddocks differing in botanical composition (late heading varieties and intermediate heading varieties). The effects of autumn closing date on grass quality and quantity in early spring and throughout the year showed a similar pattern in each year. This paper mainly presents data of 2017/2018. In 2017/2018, CD1 was closed on 14 September, CD2 was 4 October, CD3 was 20 October and CD4 was 1 December. OD1 was opened on 27 March and OD2 on 11 April. The second cut for all plots was on 26 April. There were 4 replicates per treatment. In each paddock, 32 plots (300×70 cm) were established and randomly assigned to one of eight treatments (n=4). Grass height was measured twice per week with a rising plate meter (Jenquip EC09, Fielding, New Zealand) which was validated for Dutch conditions (Holshof and Stienezen, 2016). Grass was mown every 3-4 weeks. Dry matter

(DM), VEM (Dutch energy unit) and crude protein (CP) content of the grass were determined after each mowing. The fresh grass was dried for 48 h at 60 °C and then analysed. A 2-way MANOVA was used to test for significant differences between treatments with as dependent factors: DM (kg ha^{-1}), CP (g kg^{-1} DM) and VEM (kg^{-1} DM). Independent factors were closing date in autumn (CD) and opening date in spring (OD). For all tests an α=0.05 was used and Bonferroni correction was applied for post-hoc tests when applicable.

Results and discussion

The effects of autumn closing date on grass quantity throughout 2017/2018 are presented in Figure 1. OD2 had, as could be expected, a higher DM yield at opening than OD1 (P=0.017). Figure 1 shows the sum of the DM harvested in autumn and in the first two cuts in spring. The treatment CD1 led to the lowest DM yield for both paddocks (P<0.05). For paddock 1, there were no significant differences in DM yield between CD2, CD3 and CD4. At paddock 2, DM yield of CD3 was higher than of CD1 (P=0.000) and CD2 (P=0.003).

Before this study, the effect of different closing dates on available grass in early spring in the Netherlands was not known. Table 1 shows that it is possible to keep grass cover available for an early start in spring. Table 2 shows the effect of different closing dates in 2017 on CP and VEM in spring 2018. There was no significant effect of closing and opening dates on VEM and of closing dates on CP (P>0.05). However, an early opening date in spring increased CP (P=0.002). Unfavourable weather conditions in the Netherlands (and Europe) before and during the experiment, e.g. high temperatures and drought in summer 2018, might have affected the results. More experiments are therefore recommended. Table 1 shows that the largest grass covers in early spring can be reached by CD1 (September). However, as shown in Figure 1, CD1 also leads to less DM yield in the period September-April. This can be explained by

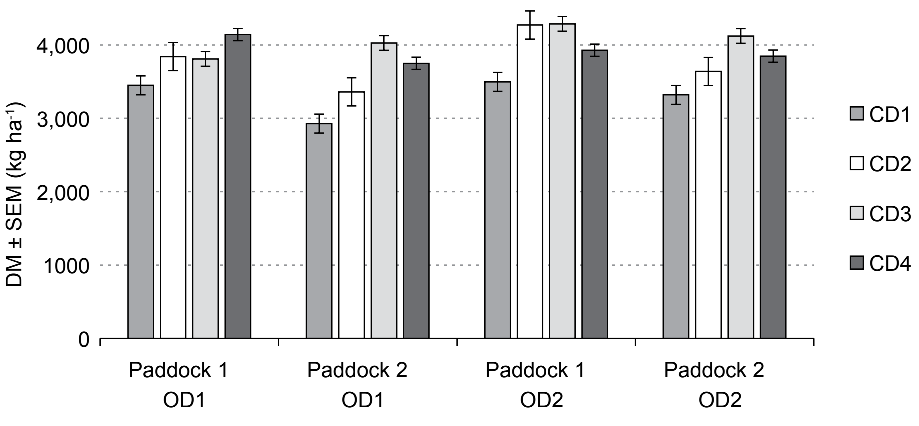

Figure 1. Dry matter harvested from 14 September 2017 – 26 April 2018 for two paddocks, four closing dates (CD) and two opening dates (OD) (kg dry matter (DM) ha^{-1} ± standard error of the mean).

Table 1. Effect of closing date (CD) in autumn on dry matter (DM) yield in early spring in the three experimental years (kg DM ha^{-1} at opening date 1 (OD1); average of two paddocks).

	CD1	CD2	CD3	CD4
OD1 2017: 13 March 2017	309	150	132	112
OD1 2018: 27 March 2018	462	331	336	315
OD1 2019: 1 April 2019	2,138	2,077	1,652	1,607

Table 2. Crude protein (CP) (g kg^{-1} dry matter) and VEM (kg^{-1} DM) in spring 2018 for four closing dates (CD) and two opening dates (OD) (average of two paddocks).

	CP		VEM	
	OD1	OD2	OD1	OD2
CD1	214	195	965	1,047
CD2	218	191	995	1,045
CD3	218	188	964	1,044
CD4	224	191	990	1,051

the fact that CD1, in contrast to the other CDs, has only one cut in autumn. Dutch farmers that would like to start early with grazing are therefore recommended to use a balanced combination of early and late closing dates.

Conclusions

The results show that under Dutch conditions early closing dates (September) are not favourable; they lead to lower DM yields than intermediate to late closing dates (October-December. It is possible, however, to close paddocks in the beginning of autumn and keep the grass cover available for an early start in spring. Swards opened early in spring (late March) had higher crude protein contents compared to those opened in April.

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References

Hennessy D., O'Donovan M., French P. and Laidlaw A.S. (2006) Effects of date of autumn closing and timing of winter grazing on herbage production in winter and spring. *Grass and Forage Science* 61, 363-374. https://doi.org/10.1111/j.1365-2494.2006.00543.x

Holshof G. and Stienezen M.W.J. (2016) Grasgroei meten met de grashoogtemeter. *Wageningen, Livestock Research report* 925, 23.

Pellikaan F. (2019) Geen piek in grasgroei in grasseizoen 2019. *Veeteelt* November 2, 42-43.

Van den Pol-van Dasselaar A., Philipsen A.P. and De Haan, M.H.A. (2013) Economics of grazing. *Grassland Science in Europe* 19, 662-664.

Predicting dairy cow dry matter intake and milk production on grass-only and grass-white clover swards

Hennessy D.[1], Hurley M.A.[1] and Delaby L.[2]
[1]*Teagasc, Animal and grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland;* [2]*INRA, Agrocampus Ouest, UMR Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Elevage, 35590 Saint Gilles, France*

Abstract

Incorporating white clover (*Trifolium repens* L.) into grass swards can increase milk production. Data from a four-year (2013 to 2016) grazing systems experiment with three treatments were compiled. The three treatments were grass-only receiving 250 kg N ha^{-1} yr^{-1} (Grass250), grass-white clover receiving 250 and 150 kg N ha^{-1} yr^{-1} (Clover250 and Clover150, respectively). Individual dairy cow dry matter intake (DMI) was measured in May and September each year, and in July in 2014, 2015 and 2016, using the *n*-alkane technique. Milk yield was measured daily during the same periods, and the milk yield potential (MYpot) and Unité Fourragère Lait (UFL) balance were calculated. The effect of parity, MYpot and body weight on herbage DMI, milk yield and UFL balance was examined. There was no effect of treatment on herbage DMI (16.9 kg), milk yield was greater (P<0.001) on Clover250 and Clover 150 compared to Grass250, and the Grass250 treatment had greater (P<0.05) UFL balance compared to the two grass-white clover treatments. In this 4-year database, introducing white clover had a positive effect on milk yield without clear effect on DMI.

Keywords: white clover, milk production, dry matter intake, prediction

Introduction

Recently there is renewed interest in the use of white clover (clover; *Trifolium repens* L.) due to factors including higher costs of fertiliser, limits on N application, highly volatile milk markets, as well as environmental regulations (Chapman *et al.*, 2017). Benefits of including clover in grass swards include increased herbage yield (Phelan *et al.*, 2013), substitution of inorganic N fertiliser with symbiotic N fixation (Enriquez-Hidalgo *et al.*, 2018), increased milk production (Egan *et al.*, 2018) and increased pasture quality (Guy *et al.*, 2018) which can increase the efficiency of conversion of herbage to animal protein (Lüscher *et al.*, 2014), and N-use efficiency (Hennessy *et al.*, 2019). The effects of clover on dry matter intake (DMI) vary; some authors have reported increased dry matter intake (DMI) (Egan *et al.*, 2018; Ribeiro-Filho *et al.*, 2003) while others have found no effect (Enriquez Hidalgo *et al.*, 2010). The objective of this study was to develop an equation to predict DMI and milk production from grass-clover swards.

Materials and methods

Data from a four-year (2013 to 2016) grazing systems experiment with three treatments (grass-only receiving 250 kg N ha^{-1} yr^{-1} (Grass250), grass-clover receiving 250 kg N ha^{-1} yr^{-1} (Clover250) and grass-clover receiving 150 kg N ha^{-1} yr^{-1} (Clover150)) were compiled. The experiment was undertaken at Teagasc, AGRIC, Moorepark, Fermoy, Co. Cork, Ireland (52°16'N; 8°25'W; 49 m asl). All treatments were stocked at 2.74 cows ha^{-1}. The design of the study and the production data used are described in detail in Egan *et al.* (2018) and Hennessy *et al.* (2018). Briefly, each February spring-calving dairy cows were selected and blocked according to calving date, parity (1, 2, 3+), two week pre-experimental daily milk yield, milk fat, milk protein and milk solids yield, and then randomly assigned to one of the three treatments. Cows were turned out to pasture from mid-February and remained at pasture until late November each year. No supplementation was fed during the measurement periods. Individual daily milk yield (kg) was recorded at each morning and evening milking (Dairymaster, Causeway, Co. Kerry, Ireland). Milk fat, protein and lactose concentrations were determined weekly from one successive evening and morning milking using Milkoscan 203 (Foss Electric, Hillerød, Denmark). Body weight (BW) and body condition score (BCS) were recorded fortnightly. Individual cow DMI was estimated using the *n*-alkane technique ((Mayes *et al.*, 1986) as modified by Dillon and Stakelum (1989)) in May and September each year, and in July in 2014, 2015 and 2016. The energy value required for maintenance, growth and milk production, expressed as Unité Fourragère Lait (UFL) in each measurement period and the milk yield potential (MYpot) was calculated for each cow using the equations described by Faverdin *et al.* (2007). The effect of parity, MYpot and body weight on herbage DMI, milk yield and UFL balance was examined using ANOVA in SAS.

Results and discussion

The database comprised 506 individual cow measurements. The database contained the following information for each DMI measurement: cow parity, daily milk yield, daily milk solids yield, milk fat, protein and lactose concentration, BW, BCS, DMI, peak milk yield, pre-grazing herbage mass, post grazing sward height, sward white clover content and herbage crude protein content. There was a significant effect of year on milk yield, DMI and UFL which were all lower (P<0.001) in 2014 compared to the other three years. Milk yield and DMI were greater for parity 2 and 3 cows compared with the parity 1 cows (Table 1 and Table 2). There was no treatment effect on daily DMI (16.9 kg DM). Daily milk yield was greater on the clover treatments compared to the Grass250 treatment. UFL balance was greatest on Grass250 and least on Clover150, with Clover250 intermediate (Table 1 and Table 2). The Clover250 and Clover150 treatments had greater milk yield response at the same level of DMI and a

Table 1. Effect of treatment (grass-only receiving 250 kg N ha^{-1} yr^{-1} (Grass250), grass-clover receiving 250 kg N ha^{-1} yr^{-1} (Clover250) and 150 kg N ha^{-1} yr^{-1} (Clover150)), year and parity on daily herbage dry matter intake (DMI), daily milk yield and Unité Fourragère Lait (UFL) balance.

		Herbage DMI	Daily milk yield	UFL balance
Treatment	Grass250	16.8	19.4^{a}	2.08^{a}
	Clover250	17.2	21.0^{b}	1.51^{b}
	Clover150	16.8	20.6^{b}	1.20^{c}
	Standard error	0.17	0.17	0.171
	P-value	N.S.	0.05	0.05
Parity	1	15.4^{a}	16.7^{a}	1.57
	2	17.0^{b}	20.6^{b}	1.81
	3	18.4^{c}	23.7^{c}	1.42
	Standard error	0.17	0.26	0.17
	P-value	0.001	0.001	NS

Table 2. Linear regression model used to predict herbage dry matter intake (DMI), daily milk yield and Unité Fourragère Lait (UFL) balance.

	Herbage DMI	Daily milk yield	UFL balance
n	506	506	506
R^2	0.480	0.857	0.264
RMSE	2.05	2.08	2.11
Intercept	6.61	1.13	1.92
Lact 1/2/3+	-1.5 / +0.5 / +1.0	-3.64 / +0.28 / +3.36	-0.03 / +0.21 / -0.18
Milk yield potential	+0.14	+0.74	-0.13
Bodyweight	+0.013	+0.004	+0.005

lower UFL balance compared to Grass250 suggests a more efficient use of energy for milk production by the clover treatments; this may be a consequence of protein nutrition. The MYpot (0.14) and the BW (0.013) coefficients on the DMI equation are similar to those proposed for the intake capacity by Faverdin *et al.* (2007). Including animal characteristics such as DMI, MYpot, parity and BW in the predication model will allow the model to adapt to changes in individual animal characteristics (McEvoy *et al.*, 2009).

Conclusions

Parity had a significant effect on milk yield. Incorporating clover into grass swards resulted in an increase in milk yield at the same DMI level as grass only. The lower UFL balance of the clover treatments compared with the grass-only treatment suggests that incorporating clover results in a more efficient use of energy for milk production.

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References

Chapman D.F., Lee J.M., Rossi L., Edwards G.R., Pinxterhuis J.B. and Minnee E.M.K. (2017) White clover: the forgotten component of high-producing pastures? *Animal Production Science* 57, 1269-1276.

Egan M., Galvin N. and Hennessy D. (2018) Incorporating white clover (*Trifolium repens* L.) into perennial ryegrass (*Lolium perenne* L.) swards receiving varying levels of nitrogen fertiliser: effects on milk and herbage production. *Journal of Dairy Science* 101, 3412-3427.

Enriquez-Hidalgo D., Gilliland T., O'Donovan M., Deighton M. and Hennessy D. (2014) The effect of grass white clover swards on herbage production, herbage dry matter intake, enteric methane emissions and milk production of dairy cows. *Journal of Dairy Science* 97, 1400-1412.

Faverdin P., Delagarde R., Delaby L. and Meschy F. (2007) *Alimentation des vaches laitières. Alimentation des bovins, ovins et caprins*. QUAE Editions, INRA, Versailles, France, pp. 23-55.

Guy C., Hennessy D., Gilliland T. J., Coughlan F., McClearn B., Dineen M. and McCarthy B. (2018) Comparison of perennial ryegrass, *Lolium perenne* L., ploidy and white clover, *Trifolium repens* L., inclusion for herbage production, utilization and nutritive value. *Grass and Forage Science* 73, 865-877.

Hennessy D., McAuliffe S. and Egan M. (2018) Herbage and milk production from grass-only and grass-clover swards. *Grassland Science in Europe*, 23, 222-224.

Hennessy D., McAuliffe S., Egan M. and Ruelle E. (2019) What is the effect of incorporating white clover (*Trifolium repens* L.) into grazed grassland on the farm-gate nitrogen balance? In: *Proceedings of the EGF EUCARPIA Joint Symposium: Improving sown grasslands through breeding and management*. Zurich, Switzerland. 24-27 June 2018, pp. 143-145.

Lüscher A., Mueller-Harvey I., Soussana J.F., Rees R.M. and Peyraud J.L. (2014) Potential of legume-based grassland-livestock systems in Europe: a review. *Grass and Forage Science* 69, 206-228.

McEvo, M., Delaby L., Kennedy E., Boland T.M. and O'Donovan M. (2009) Early lactation dairy cows: development of equations to predict intake and milk performance at grazing. *Livestock Science* 122, 214-221.

Phelan P., Casey I.A. and Humphreys J. (2013) The effect of target postgrazing height on sward clover content, herbage yield, and dairy production from grass-white clover pasture. *Journal of Dairy Science* 96, 1598-1611.

Ribeiro Filho H.M.N., Delagarde R. and Peyraud J.L. (2003) Inclusion of white clover in strip-grazed perennial ryegrass swards: herbage intake and milk yield of dairy cows at different ages of sward regrowth. *Animal Science* 77, 499-510.

Grass silage quality on Northern Ireland farms between 1998 and 2017

Patterson J.D.[1], Sahle B.[2], Archer J.E.[1], Yan T.[1], Grant N.[1] and Ferris C.P.[1]
[1]Agri-Food and Biosciences Institute (AFBI), Belfast, BT9 5PX Northern Ireland, United Kingdom;
[2]Queens University Belfast, BT7 1NN, Northern Ireland, United Kingdom

Abstract

In Northern Ireland (NI) 37% of the grassland area is used for silage production. This study examined the trends (1998-2017) in composition and nutritive value of NI silages (n=76,452 samples), and their relationship with harvest year, harvest number and farm location (east *vs* west). Across the 20-year period, within each of harvests 1, 2 and 3, silage dry matter (DM) and water-soluble carbohydrate (WSC) content increased, while fibre content decreased (P<0.001). Crude protein (CP) levels did not change over time, and largely followed the trend in fertiliser N usage within the period. Ammonia-N levels were higher in 3rd cut silages (P<0.001). There was no significant improvement in silage digestibility, while silage intake potential (for dairy cows) increased by approximately 8% (88.8 in 1998; 96.1 g DM kg^{-1}W$^{0.75}$ (live weight$^{0.75}$) in 2017). Silages made in the east of NI generally had higher CP, WSC and digestibility, and a lower fibre content, than those made in the west where weather conditions are generally less favourable. Despite overall improvements in DM, WSC and intake potential, digestibility parameters have not shown any significant improvement over the 20-year period.

Keywords: silage composition, silage fermentation, silage intake

Introduction

Grass silage was produced on 37% (298,480 ha) of the total NI grassland area (808,000 ha) in 2018 (DAERA, 2018), and is likely to remain the predominant winter forage for ruminants on NI farms. Despite improvements to silage making practices, including rapid wilting techniques, advances in silage additives, improved nutrient management, and timing of cutting, anecdotal evidence suggests silage quality has not improved on local farms. There is no single definition of silage quality. It is affected by fermentation and storage and is assessed by its chemical composition and the predicted animal performance. In this study silage quality is considered as a combination of nutritional quality, fermentation quality and predicted intake value. Previous work by Jackson *et al.* (1974) and Unsworth (1981) reported on silages made on NI farms between 1967-1972 and 1973-1979, respectively. These authors found that silage quality parameters such as dry matter (DM), crude protein (CP) and crude fibre did not differ greatly during these periods, and concluded that differences in chemical composition could be ascribed to weather patterns. In a recent survey of NI dairy farmers, Ferris *et al.* (2019) found that weather factors that delayed silage harvesting had the greatest negative impact on silage quality. This study examined the trends in silage quality parameters over a 20-year period.

Materials and methods

Between 1998 and 2017, 76,452 grass silage samples from NI farms were analysed in the Hillsborough Feeding Information System (HFIS) laboratory using Near Infrared Reflectance Spectroscopy (NIRS). Information on year of harvest, harvest number and location in NI (east vs west, based on the farm address) was recorded. The NIRS prediction provided estimates for fermentation variables (e.g. pH and ammonia nitrogen), nutrient concentrations (dry matter (DM), CP, neutral detergent fibre (NDF), acid detergent fibre (ADF) and water soluble carbohydrates (WSC)), DM digestibility (DMD), digestible organic matter in DM (D-value), and dairy cow intake potential (derived from mathematical models of

McNamee *et al.*, 2005). Data were analysed using GenStat 16th edition using an unbalanced analysis of variance.

Results and discussion

Silage DM content increased (P<0.001) over the period (Figure 1), as did WSC content. Mean pH did not change over time, which was perhaps unexpected given the increase in DM content. There was no significant long-term trend in silage CP over the period, with changes in CP levels within years largely reflecting trends in fertiliser N usage. This contrasts with Unsworth (1981) who reported an increase in CP levels in silages. There was no long-term change in silage digestibility over the 20-year period (Figure 1), which is disappointing since it is well established (e.g. Keady *et al.,* 2013) that silage digestibility is one of the most important determinants of its feeding value. Since 1998 the dairy cow intake potential of silages has increased by almost 10 g kg $W^{0.75}$ in first cut silages, which is likely associated with the increase in silage DM over this period.

Although silage DM content differed between harvests (P<0.001), the magnitude of the difference was small (Table 1). Crude protein content increased from harvest 1 to 3, while NDF content followed the opposite trend (P<0.001), both reflecting the leafier nature of third-cut silages. Surprisingly, the decreasing NDF content was not reflected in an increase in DMD or D-value, both of which were lower (P<0.001) with third cut compared to first cut silages.

Silages made in the east and west of NI were different in all of the traits presented in Table 1, with DM and CP content and intake potential being lower in the west, while NDF content was higher (P<0.001). Differences in DM generally reflect the wetter conditions which exist in the west of NI, while the lower protein content may reflect the less intensive agricultural systems that exist in the west. Surprisingly,

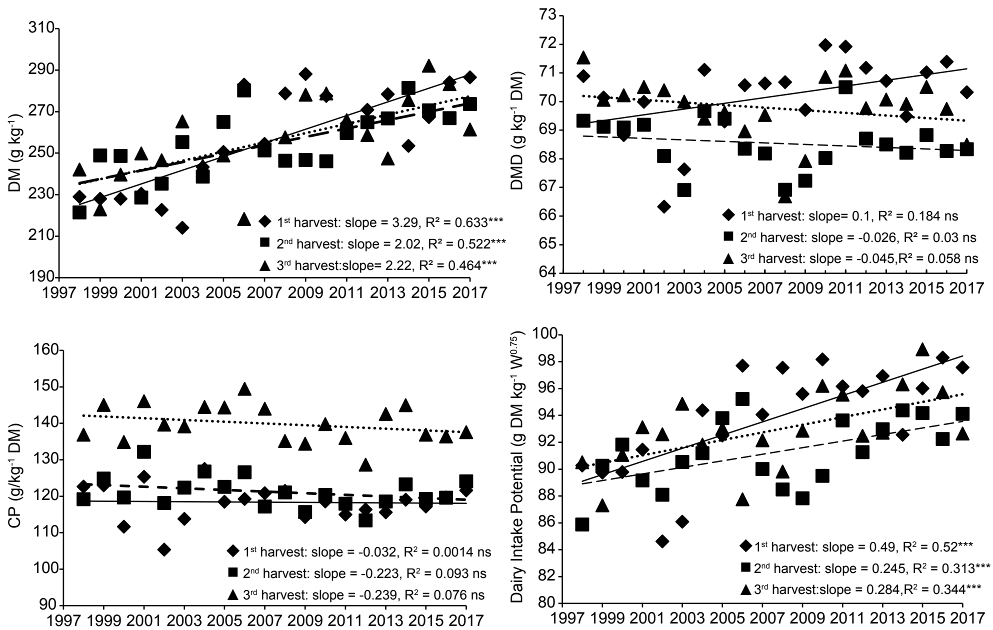

Figure 1. Changes in the dry matter (DM), dry matter digestibility (DMD), crude protein (CP) and dairy intake potential of grass silage in NI from 1998 to 2017. Solid line = 1st cut; dashed line = 2nd cut; dotted line = 3rd cut: * = P<0.05, ** = P<0.01, *** = P<0.001 and ns = non-significant.

Table 1. Effect of silage harvest number and farm location on silage quality between 1998-2017.

	1st cut	2nd cut	3rd cut	SEM	*P*-value	East	West	SEM	*P*-value
DM (g kg^{-1})	260^b	256^a	257^a	0.424	<0.001	268	244	0.166	<0.001
pH	4.03^b	3.97^a	4.05^c	0.004	<0.001	4.01	3.97	7×10^{-4}	0.008
Crude protein (g kg^{-1} DM)	118^a	121^b	140^c	0.113	<0.001	122	118	0.046	<0.001
NDF[1] (g kg^{-1} DM)	509^c	503^b	477^a	0.304	<0.001	496	516	0.120	<0.001
WSC (g kg^{-1} DM)	24.1^b	23.7^a	25.2^c	0.113	<0.001	26.8	20.6	0.046	<0.001
Ammonia-N (g kg^{-1} total N)	103^b	102^a	107^c	0.223	<0.001	101	106	0.088	<0.001
DMD (%)	70.3^c	68.5^b	69.7^a	0.032	<0.001	69.9	69.3	0.014	<0.001
D-value (%)	67.2^c	65.6^a	66.4^b	0.028	<0.001	66.7	66.5	0.010	<0.001
Dairy intake potential (g kg^{-1} W$^{0.75}$)	94.2^c	91.3^a	93.0^b	0.056	<0.001	94.7	91.2	0.025	<0.001

[1] DM = dry matter; DMD = dry matter digestibility; NDF = neutral density fibre; WSC = water soluble carbohydrate; D-value = digestible organic matter in DM; SEM = standard error of the mean.

differences in DMD and D-value of silage made in the east and west, although significant (P<0.001), were small.

Conclusions

Despite improvements in silage DM and dairy intake potential over the 20-year period, silage digestibility did not show any significant improvement. There is potential to improve the overall quality of grass silage, especially for third cut. Differences between silages from eastern and western counties may be partly explained by differing weather conditions during the silage-harvesting season.

References

DAERA (2018) The agricultural census in Northern Ireland results for June 2018. Available at: https://www.daerani.gov.uk/sites/default/files/publications/daera/AgriculturalCensusinNorthernIreland2017.pdf.

Ferris C.P., Laidlaw S. and Gordon A.W. (2019) Silage making practices on Northern Ireland dairy farms and factors perceived by farmers to influence silage quality. In: *Proceedings of the British society of animal science annual conference 2019*. Edinburgh, UK, p. 78.

Jackson N., O'Neill S.J.B. and Dawson R.R. (1974) The composition and quality of grass silages made in Northern Ireland – an analysis of six years results (1967-1972). *Record of Agricultural Research (Ministry of Agriculture for Northern Ireland)* 18, 45-50.

Keady T.W.J., Hanrahan J.P., Marley C.L. and Scollan N.D. (2013) Production and utilization of ensiled forages by beef cattle, dairy cows, pregnant ewes and finishing lambs – a review. *Agriculture and Food Science.* 22, 70-92.

McNamee B.F., Woods, V.B., Kilpatrick D.J., Mayne C.S., Agnew R.E. and Gordon F.J. (2005) *Livestock Production Science* 92, 233-240.

Unsworth E. (1981) The composition of grass silages made in Northern Ireland – an analysis of seven years' results (1973-1979). *Record of Agricultural Research (Ministry of Agriculture for Northern Ireland)* 21, 91-97.

Effect of different additives and temperature on fermentation of autumn-cut grass silage

Milimonka A. and Glenz G.
ADDCON Europe, Parsevalstr. 6, 06749 Bitterfeld, Germany

Abstract

Various factors, including grassland management and the impact of climate change push farmers towards late autumn cuts. Wilting is often impossible at that time, so that ensiling becomes risky. Autumn-cut grassland (310 g kg^{-1} dry matter (DM)) was ensiled in glass jars for 90 days. The plant material was treated with sodium nitrite-HMTA (KL) and KL + *Lactobacillus plantarum* (KL+LAC) at 3 different dosages. The ensiled material was stored at 25 or 12 °C. Common analytical methods for fermentation parameters were used. The reduced temperature resulted in lower DM losses and affected the fermentation in both main effects. This was reflected as higher residual sugar and slightly more lactic and lower acetic acid contents in the silage. The KL treatments had a tendency for higher lactic acid and lower acetic acid concentrations compared with the control. Ammonia-N was lower at low temperature and with higher application rate of KL. The combination of KL+LAC resulted in a slightly higher sugar consumption and lactic acid content. At lower temperatures, fermentation seemed to be less intense. The use of additives increased the residual sugar content and improved the protein quality of autumn-cut grass.

Keywords: chemical additive, inoculant, temperature, grass silage

Introduction

Grassland should be cut at the beginning of the winter to improve overwintering (field mouse population, fungal attacks, carbohydrate balance) and the quality of the spring growth (Jones and Lazenby, 1988), thus necessitating ensiling of autumn cuts. However, in autumn, the wilting conditions are often not suitable. In contrast, the higher sugar contents in autumn (Deinum, 1966) may support ensiling. The expected low temperatures in late autumn may decrease the fermentation intensity but allow the microbes to be active over a longer time (McDonald *et al.*, 1991). In the case of round bales, Lie *et al.* (2019) showed that lower temperatures reduced the intensity of fermentation, demonstrated by lower lactic acid (LA) production and a delayed pH drop. The number of bacteria may also vary widely, depending on environmental conditions (Lindgren *et al.*, 1985; Zhou, 2016). Low temperatures might cause lower numbers of lactic acid bacteria (LAB) in autumn. The success of fermentation of a grass cut can be described via its dry matter (DM) and ratio of water soluble carbohydrates (WSC) to buffering capacity (BC) (Weißbach *et al.*, 1974) and in the case of late autumn cuts, low DM and higher crude ash may be expected. As a result, fermentation might be more difficult in autumn and the use of additives can be recommended. Here, different concepts of additives were tested in autumn-cut grass.

Materials and methods

A late cut was taken on 18 October 2017 from a *Lolium*-dominated multi-species natural grassland in the district of Wittenberg, Germany. The crop traits for the grass were (g kg^{-1} DM): DM 310, crude protein 165, crude ash 141, WSC 124, nitrate 0.3, BC of 7.6 g LA/100 g DM, WSC/BC 1.6 and the fermentation coefficient was 44.

The crop material was ensiled in 1.5 litre jars. Each treatment was prepared from 5 kg fresh crop and treated with: (1) KL1, nitrite + HMTA with 1 litre Mg^{-1} fresh matter (FM), KOFASIL liquid, ADDCON Europe GmbH, (2) KL2 (2 litres Mg^{-1} FM), (3) KL3 (3 litres Mg^{-1} FM), (4) KL1 LAC (1 litre+1 g LAC Mg^{-1} FM, *Lactobacillus plantarum*, Kofasil LAC), (5) KL2 LAC (2 litres+1 g LAC Mg^{-1}

FM), (6) KL3 LAC (3 litres+1 g LAC Mg^{-1} FM) and (7) Control (no additive). Silages were fermented at 25 or 12 °C. The additives were applied at the dosages mentioned above but were filled up to 50 ml with water prior to dosing for better distribution. The jars were filled with an average of 0.97 kg chopped grass within a three-hour period.

Fermentation loss (FL) was measured by weighing each week and calculated according to Weißbach (2005). The silages were opened after 90 days. Crop parameters from the fresh crop as well as for the silage were evaluated based on VDLUFA (2011). Silage DM was measured and corrected for loss of volatiles during drying (Weißbach and Kuhla, 1995). The following parameters of the silage were analysed: pH, WSC (VDLUFA, 2011), LA, acetic acid (AA), butyric acid (HPLC; VDLUFA, 2011), and NH_3 (auto-analyser; VDLUFA, 2011). The setup of the experiment was a two-factorial random block design, 3 times replicated. Statistical analyses were done using the ANOVA procedure using SPSS. When the overall *P*-value was significant at the 5% level, main effects and pair wise comparisons between LSMEANS of treatments were done using Tukey´s test.

Results and discussion

The additive treatments controlled the FL after 7 and 90 days, compared with the control. There was a lower FL after 7 days with higher KL-dosage in the main effect. A main effect of temperature on FL was given after 7 days and after 90 days (Table 1). These results might come from a better control of enterobacteria via KL. The amount of AA dropped with higher KL-dosage whereas the additional use of an inoculant (KL×LAC) had no effect. LA decreased in tendency with higher KL-dosage, but in KL×LAC there was a tendency towards slightly higher LA. The WSC consumption was reduced at higher KL-dosage and at lower temperature.

Lie *et al.* (2019) described at higher temperature more LA combined with a faster pH drop. However, in the current experiment a higher LA was observed at low temperature which might affect the lower NH_3-N and lower protein solubility (PS). The higher WSC consumption at 25 °C storage temperature and an increase of AA as well as a weak temperature effect on pH may indicate a slow pH drop, longer activity of enterobacteria (more AA) and thus higher CO_2 losses. A slower fermentation agrees with the higher NH_3-N contents and higher PS measured at high temperature. These parameters, NH_3 and PS, were reduced with increased KL-dosing. Butyric acid was quite low and not affected by the treatments in

Table 1. Effects of different additive treatments and storage temperatures on fermentation parameters of a late, wet grass cut.[1,2]

	Main effect additive treatment							Main effect temperature	
	Control	KL1	KL2	KL3	KL1LAC	KL2LAC	KL3LAC	warm	cold
FL7 (%DM)	4.1^{f}	$3.6d^{e}$	$3.4b^{c}$	3.2^{a}	3.6^{e}	$3.4c^{d}$	3.2^{ab}	3.7^{a}	3.3^{b}
FL90 (%DM)	5.7^{b}	4.5^{a}	4.8^{a}	4.3^{a}	4.5^{a}	4.7^{a}	4.5^{a}	5.2^{a}	4.3^{b}
WSC (%DM)	1.2^{a}	1.9^{ab}	2.8^{b}	5.9^{c}	1.8^{ab}	2.5^{ab}	4.8^{c}	2.3^{a}	3.7^{b}
LA (%DM)	6.8^{a}	8.2^{bc}	7.8^{b}	6.7^{a}	8.6^{c}	7.8^{b}	7.0^{a}	7.2^{a}	7.9^{b}
AA (%DM)	3.1^{c}	2.4^{b}	2.1^{b}	1.2^{a}	2.4^{b}	2.2^{b}	1.4^{a}	2.4^{a}	1.8^{b}
BA (%DM)	0.16^{a}	0.16^{a}	0.16^{a}	0.16^{a}	0.16^{a}	0.16^{a}	0.16^{a}	0.16^{a}	0.16^{a}
pH	4.2^{b}	4.0a	4.2^{b}	4.5^{c}	4.0^{a}	4.2^{b}	4.4^{c}	4.2^{a}	4.3^{b}
PS (%)	53.3^{d}	50.3^{c}	47.7^{b}	45.2^{a}	49.3^{c}	47.1^{b}	44.7^{a}	50.7^{a}	45.8^{b}
NH_3-N (%N_t)	9.2^{c}	8.2^{b}	7.6^{b}	6.4^{a}	7.9^{b}	7.7^{b}	6.4^{a}	8.2^{a}	7.1^{b}
clost. (MPN)	$1,056^{b}$	915^{ab}	952^{ab}	308^{a}	561^{ab}	706^{ab}	506^{ab}	607^{a}	823^{b}

[1] Different letters indicate significant differences within the main effects ($P<0.05$). For additive treatment details see Methods section.

[2] Warm = 25 °C; cold = 12 °C; FL7, 90 = fermentation loss 7, 90 days; WSC = water soluble carbohydrates; LA = lactic acid; AA = acetic acid; BA = butyric acid; PS = protein solubility; clost. = clostridia.

the experiment. The additional inoculation barely impacted the fermentation. Clostridia counts showed a high degree of variation, but a tendency was seen towards clostridia reduction with higher KL-dosing.

Conclusions

Reduced temperature resulted in lower DM losses and affected the fermentation. This was reflected in higher residual WSC, slightly higher LA and lower AA contents in the silage. The additive treatments reduced the FL and the amount of AA. Parameters like NH_3-N and PS could be reduced in silage by using additives. Thus, protein quality of this autumn-cut grass could be improved. The number of clostridia can be reduced by using the recommended KL dosage of 3 l Mg^{-1}.

References

Deinum B. (1966) Effect of climate on composition and feeding value of herbage. In: *Proceedings of the 10th Int. grassland congress*, Helsinki, pp. 415-418.

Jones M.B. and Lazenby A. (1988) *The Grass Crop*. Chapman and Hall, London, UK, 315 pp.

Li P., Gou W., Zhang Y. and Shen Y. (2019) Fluctuant storage temperature increased the heterogeneous distributions of pH and fermentation products in large round bale silage. *Grassland Science* 65. https://doi.org/10.1111/grs.12232.

Lindgren S., Pettersson K. and Jonsson A. (1985) *Swedish Journal of Agricultural Research* 15, 9-18.

McDonald P., Henderson N. and Heron S. (1991) *The biochemistry of silage*. Chalcombe Publications, Marlow, UK, 340 pp.

VDLUFA (2011) VDLUFA *Methodenbuch Band III*, VDLUFA-Verlag, Darmstadt, 2190 pp.

Weißbach F., Schmidt L. and Hein E. (1974) Method of anticipation of the run of fermentation in silage making, based on the chemical composition of green fodder. In: *Proceedings of the 12th Int. grassland congress*, Moscow, pp. 663-673.

Weißbach F. and Kuhla S. (1995) Substance losses in determining the dry matter content of silages in green fodder: arising errors and possibilities of correction. *Übersichten Tierernährung* 23, 189-214.

Weißbach F. (2005) A simple method for correction of fermentation losses measured in laboratory silos. In: *Proceedings of the 14th International silage conference*, Belfast, p. 278.

Zhou Y., Drouin, P. and Lafreniere C. (2016) Effect of temperature (5-25°C) on epiphytic lactic acid bacteria populations and fermentation of whole-plant corn silage. *Journal of Applied Microbiology* 121, 657-671.

Modern cultivars of timothy produce more herbage with enhanced feeding value

Aavola R. and Pechter P.
Estonian Crop Research Institute, J. Aamisepa 1, 48309 Jõgeva, Estonia

Abstract

The newest timothy (*Phleum pratense* L.) cultivars (cv.) bred by Boreal Plant Breeding Ltd. have been selected for improved herbage production and quality. Dry matter yield (DMY), digestibility (DDM) and content of metabolisable energy (ME) of an Estonian reference cultivar were compared with four Finnish cultivars in a field trial. Treatment with limited plant nutrition was compared with more abundant and versatile mineral fertiliser application. Timothy was first harvested at early heading. The second harvest was seven weeks after the first. Both harvests were followed by delayed cuts. Additional three samples of senescent herbage were taken from cv. 'Tia' and 'Rhonia' at three to six-day intervals. Cultivar 'Dorothy' produced the highest total DMY (10.66 Mg ha^{-1}). Increase in the total DMY of timothy, caused by versatile fertilisation or by delayed harvests, was 0.4 and 1.3 Mg ha^{-1}, respectively. Modern timothy cultivars evidenced enhanced DMY and equal or superior herbage DDM and ME when compared with the Estonian cultivar. However, their digestibility also decreased after the optimal harvest time at both fertilisation regimes.

Keywords: dry matter, forage, digestibility, metabolisable energy

Introduction

Timothy is a widely cultivated forage grass species in northern latitudes. Despite its superior tolerance to adverse winter conditions and pathogens, its feeding value deteriorates rapidly after the optimal harvest stage. Timothy tends to form stems in regrowth, which decreases leaf to stem ratio and thus forage digestibility (Ball *et al.*, 2017). Synchronised enhancement of herbage production and digestibility are essential breeding goals. Since the releases of 'Tika' in 1992 and 'Tia' in 1993, timothy has not been bred further in Estonia. Meanwhile an active improvement programme has continued in Finland at Boreal Plant Breeding Ltd. Four Finnish cultivars included in the study were registered in 2002-2019. The aim of the study was to assess the progress of timothy breeding for productivity and feeding value.

Materials and methods

The experiment was established in a field on which 20 m^3 ha^{-1} cattle slurry had been incorporated into the soil. Two Estonian and four Finnish timothy cultivars were seeded on 7 June 2018. Two fertilisation regimes were tested: (1) 'Farmer' ('F') – N 69 as NH_4NO_3 and K 50 kg ha^{-1} as KCl on 10 April and N 52 kg ha^{-1} as NH_4NO_3 on 8 June for the second crop, and (2) 'Yara' crop nutrition system ('Y') composed of three applications of Yara Mila (YM) fertilisers with trace elements: N 90, P 10, K 56 and S 14 kg ha^{-1} as YM 20-5-15 on 27 March and two applications of YM 22-0-14 equivalent to N 66, K 35, S 9 and Mg 13 kg ha^{-1} on 8 June and N 33, K 17, S 5 and Mg 6 kg ha^{-1} on 1 August. Half of the plots were harvested at early heading stage on 4 June and the remaining half at full heading on 7 June. 'Tia' and 'Rhonia' were seeded on additional plots in three replicates. These were fertilised according to 'Y' regime and sampled on 10, 14 and 18 of June. Similarly, the second crop was harvested early on 25 July and late on 31 July. Thereafter 'Tia' and 'Rhonia' were sampled on 5, 9 and 13 of August. Third crop was harvested on 3 October whereby the treatment 'F' was raised without fertilisation. The following total nutrient rates were applied in 2019: 'F' – N 120 and K 50 kg ha^{-1}, 'Y' – N 189, P 10, K 108, Mg 19, S 27 kg ha^{-1} + B 315, Zn 45 and Se 14 g ha^{-1} yr^{-1}. Foss NIRS 6500 analyser was used for the estimation of chemical composition. Digestible dry matter (DDM) and metabolisable energy (ME) were computed (Moore and Undersander, 2002). Statistical differences were calculated using the package Agrobase 20™.

Results and discussion

The first cut was preceded by moderate air temperature and was conducted in the middle of a ten-day drought period. The harvesting was followed by a hot period with the 24-hour mean temperatures raised up to 23.8 °C and the five-day maximums up to 29.7 °C. This resulted in significant dry matter yield (DMY) gain in just three days for most cultivars, except for 'Tika' and 'Uula' at 'F' and for 'Tuure' at 'Y' treatment. A three-day delay after the optimal harvest stage in the first cut caused a mean increase in DMY of 789 and 648 kg ha^{-1} d^{-1} with the 'F' and 'Y' treatments, respectively. As a mean of all cultivars, postponed harvest enabled the stands to accumulate by 1.87 Mg ha^{-1} more DM at 'F' and by 1.30 Mg ha^{-1} at 'Y' treatment. Maximum gains in DMYs during the longer cutting interval were registered for 'Dorothy' (3.09 Mg ha^{-1} at 'F') and 'Tika' (2.49 Mg ha^{-1} at 'Y').

A six-day delay after the optimal harvest stage in second cut caused a mean decrease in DMY by 84 and 108 kg ha^{-1} d^{-1} with the 'F' and 'Y' treatments, respectively. As an average of the fertilisation regimes, the DMY gain in the first cut caused by harvest delay was 719 and a decrease in the second 102 kg ha^{-1} d^{-1}. Across the two fertilisation and harvest regimes, 'Dorothy' produced the highest DMY (10.66 Mg ha^{-1}) and exceeded significantly Estonian cultivars and 'Uula'. 'Tuure' yielded most at early and 'Dorothy' at late harvesting (Table 1).

Delayed harvest caused a decline in herbage DDM concentration in timothy from 647 to 628 g kg^{-1} and ME from 10.17 to 9.82 MJ kg^{-1} DM (not shown). 'Y' treatment reduced the herbage DDM from 640 to 635 g kg^{-1} and ME from 10.04 to 9.95 MJ kg^{-1} DM. 'Tuure' and 'Uula' distinguished from the check cv. 'Tia' by higher DDM and ME if cut early at 'F', 'Uula' also in the case of late harvesting but only at 'Y' treatment. All the changes were significant ($P<0.05$). All tested cultivars attained the required contents of ME (>9.5 MJ kg^{-1}). Only 'Tuure' and 'Uula' exceeded the DDM level set for high-quality forage (>650 g kg^{-1} DM) (Tamm, 2017). Early harvesting assured high ME; this varied between 10.03 and 10.39 MJ kg^{-1} DM.

Even postponed harvesting produced highly digestible forage in all cultivars, in which the ME content varied between 9.70 ('Dorothy') and 9.99 MJ kg^{-1} DM ('Uula'). In the first cut, the differences between 'Tia' and 'Rhonia' fertilised according to 'Y' regime became evident two weeks after the optimal harvest stage (Figure 1). 'Rhonia' demonstrated a significant decline in DDM (not shown) and ME contents towards 'Tia'. On 9 June the lasting drought ended with a week-long rain with 48 mm of precipitation. The

Table 1. Dry matter production and digestibility of timothy cultivars harvested at two stages.[1]

Cultivar	Dry matter yield, Mg ha^{-1}				Dry matter digestibility, g kg^{-1}			
	'Farmer'		'Yara'		'Farmer'		'Yara'	
	Early	Late	Early	Late	Early	Late	Early	Late
Tia	7.39	9.12	9.10	10.08	647	625	646	623
Tika	8.50	9.96	8.44	10.93	649	631	639	625
Dorothy	8.24	11.33	10.84	12.23	647	622	639	621
Rhonia	8.85	10.84	9.73	11.76	642	625	640	624
Tuure	8.86	10.51	10.92	10.35	658	635	648	632
Uula	8.33	9.61	9.04	10.52	656	637	649	632
LSD 5%	1.48		0.90		7.8		8.4	

[1] LSD = least significant difference.

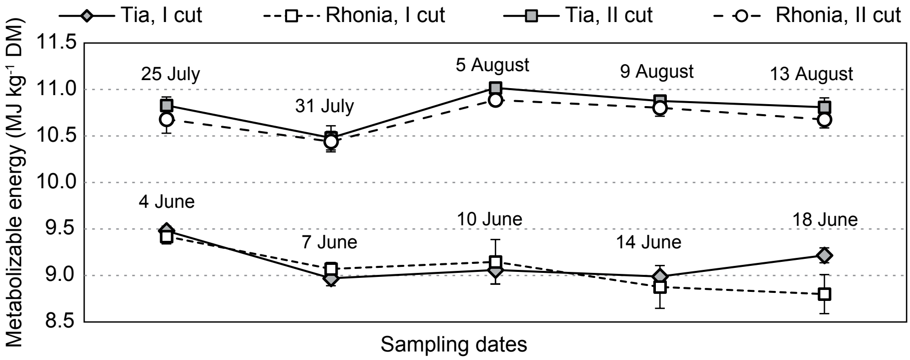

Figure 1. Dynamics of metabolizable energy in timothy cultivars after the onset of heading on 4 June and regrowth harvesting on 25 July. Means ± standard deviation.

rain induced new growth and therefore increased ME content for 'Tia' in samples collected on 18 June. YM-fertiliser that still remained on the ground was dissolved and could contribute to the improvement. In the second cut, 'Rhonia' had continuously lower ME and also DDM concentrations during the sampling period, whereas on 25 July, 5 and 13 August the differences were significant. However, based on these parameters the nutritional value of aftermaths meets the requirements.

Diurnal air temperatures rose to 32.7 °C during the three days before second sampling on 31 July. Rapid development reduced significantly the ME content in 'Tia' and 'Rhonia'. Moreover, a 10-day drought period preceding the sampling impeded the vegetative growth of stressed stands. Cooler weather from 30 July onwards along with scattered rain (7 mm in total) enabled the plants to recover from the prolonged stress and slowed their development.

Increase in ME content by the third sampling time of aftermath on 5 August was caused by regeneration of new digestible material. Therefore, ME content of aftermath sampled eleven to nineteen days past the optimum harvest stage exceeded that of timothy sampled just six days after optimum. Improvement in herbage nutritive value in course of senescence is uncommon. In case of ordinary weather conditions, the feeding value of forage crops steadily declines along with advancing maturity (Tamm, 2017).

Conclusions

The agronomic traits of Finnish cultivars, as featured in their descriptions, remained valid also in Estonia. 'Dorothy' and 'Rhonia' were differentiated by high productivity, 'Tuure' and 'Uula' by high DDM and ME, distinctly in the first cut. Heat stress and water deficiency caused a steep decline of DDM and ME in both harvest periods. All cultivars produced forage with above-required ME but most of them with satisfactory DDM. Based on the daily gain in DMY, 'F' treatment had an advantage over 'Y' in extremely dry periods. However, yearly DMY grown in the circumstances of 'Y' treatment exceeded that of 'F' by 1 Mg ha^{-1}.

References

Ball D.M., Collins M., Lacefield G.D, Martin N.P., Mertens D.A., Olson K.E., Putnam D.H., Undersander D.J. and Wolf M.W. (2017) *Understanding Forage Quality.* American Farm Bureau Federation Publication 1-01, Park Ridge, IL, USA, 21 pp.

Moore J.E. and Undersander D.J. (2002) A proposal for replacing relative feed value with an alternative: relative forage quality. In: *American Forage and Grassland Council Proc.*, vol. 11, Bloomington, MN, USA, pp. 171-175.

Tamm U. (2017) *Parema toiteväärtusega rohusööt*, Eesti Taimekasvatuse Instituut, Saku, Estonia, 57 pp.

Can we use miRNA to certify raw milk from fresh grass-fed cows?

Abou el Qassim L., Royo L.J., Martínez-Fernández A., Soldado A., De La Torre S., Forcada S., Baizán S., Gómez-Navarro N. and Vicente F.
Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), P.O. Box 13, 33300 Villaviciosa (Asturias), Spain

Abstract

MicroRNAs (miRNAs) are small, noncoding RNAs that hybridize mRNA by complementary sequences. miRNAs act as key regulators of diverse biological and developmental processes in eukaryotes, including cell proliferation and differentiation, apoptosis, immune system development, and immune responses. Their presence in all tissues and organic fluids make them good, non-invasive biomarkers for milk quality. We and others have demonstrated that miRNA profiles found in raw milk change according to the cow's diet and/or the milk production system. Our aim here is to study miRNA differential expression in milk in order to authenticate the production system of the milk. Tank milk from dairy farms managed under different production systems in Asturias (north of Spain) was collected, milk fractions separated (fat and cells), and total RNA isolated. Differential expression of previously identified miRNAs cells was analysed and compared among different production systems. Bta-mir-2285e was identified as a possible miRNA marker of milk produced by cows fed on fresh-grass. This study sets a precedent for the use miRNAs as a certification tool for agro-food products, but also can help us to understand the functionality of milk produced under different systems.

Keywords: milk quality, biomarkers, miRNA, production system, diet

Introduction

Consumers are more and more interested in the quality and origin of the products they consume. In dairy production, grazing is being promoted over cattle housed indoors for two main reasons: the improvement in animal welfare and the improvement in milk quality. Thus, mechanisms of evaluation and authentication are required to ensure consumer the quality of the milk they purchase. Finding biomarkers that allow a rapid and inexpensive authentication of milk quality and origin is of necessity. Cow milk is enriched in miRNAs, molecules that regulate gene expression in eukaryotes (He and Hannon, 2004). Several works describe how the miRNAs profile of cow mammary gland or milk varies in disease, physiology, diet, stress, and breed (Billa *et al.*, 2019; Colitti *et al.*, 2019; Lai *et al.*, 2017; Li *et al.*, 2012, 2015; Mobuchon *et al.*, 2017; Sun *et al,* 2015). Additionally, in the case of bovine milk, different works have demonstrated that miRNA content is different depending on the milk fraction (fat, cells or serum) (Canovas *et al.*, 2014; Li *et al.*, 2016). These and other works suggest that milk fat could be a non-invasive alternative source for miRNA studies of bovine mammary gland (Li *et al.*, 2016). We have previously investigated miRNA profiling in the milk fat fraction. Our aim here is to study miRNA differential expression in another milk fraction, cells. This would expand our knowledge of miRNA as biomarkers in milk and would allow the use of miRNA as a certification tool for dairy farms based on fresh-grass feeding, and also to understand the functionality of milk produced under different systems, being able to modulate targeted functions in the body by enhancing a certain physiological response and/or by reducing the risk of disease (Nicoletti, 2012).

Materials and methods

Sample collection and clustering

Tank milk from 22 dairy farms was sampled twice, during autumn and spring, (making a total of 44 samples). The milk was collected from farms that varied between the extensive system, with a feeding based on grazing and a minimum amount of concentrates, and intensive systems where the cows were permanently housed with a feeding based on high amounts of concentrates. Milk samples were clustered according to the amount of fresh grass consumed by the cow (kg of dry matter (DM) d^{-1} $animal^{-1}$) in the total mixed ration (TMR). The consumption of fresh grass was estimated by subtracting the amount of dry matter ingested from the ration ingredients, to the total theoretical consumption of the cows, calculated by the equation: TTC (kg DM d^{-1} $animal^{-1}$) = 12 + Milk Production / 3 (NRC, 2001). The three groups resulting were: Group 1 without fresh-grass (n=27), Group 2 with less than 10 kg per animal of fresh-grass (n=11), Group 3 with more than 10 kg per animal (n=6).

miRNA quantification and analysis

Total RNA was isolated from milk cells fractions using the mirVana miRNA Isolation Kit following the manufacturer's instructions (LifeTechnologies). The isolated RNA was used for cDNA synthesis using the TaqMan Advanced miRNA cDNA Synthesis Kit (LifeTechnologies). The levels of five different miRNA were determined by quantitative PCR (TaqMan Advanced miRNA Assays; ThermoFisher Scientific) in a StepOne thermocycler (LifeTechnologies). Differential expression of miRNAs was analysed using the software Qbase+ (Biogazelle) as previously described in Abou el Qassim (2017). The expression in cells of bta-miR-574, bta-miR-3432a, bta-miR-2285e, bta-miR-197 and bta-miR-2284y relative to the levels of control miRNAs (bta-miR-103-3p, bta-miR-181a and bta-miR-107) was determined and significant differences between the groups was established using T-test statistical at $P<0.05$.

Results and discussion

In order to identify whether milk from fresh grass-fed cows could be identified by means of miRNA we analysed the difference in the expression of 5 miRNAs obtained from cow milk sampled from three different farming systems. The statistical differences in the expression of 5 different miRNAs among the sampled farming categories are shown in Table 1. Considering the overall effect of quantity of fresh grass in all farms and seasons, we found one miRNA that could serve as a biomarker for cow's diet. Using miRNA quantification of the milk cellular fraction we were able to identify that expression of bta-mir-2285e varies upon diet. In a previous work, we described that the levels of another miRNA, bta-miR-215, in milk fat vary according to the production systems (Abou el Qassim *et al.,* 2018). Other studies have identified that the bta-mir-2285 miRNA family is related to the response to *Staphylococcus aureus* infection (Sun *et al.*, 2015), and in the regulation of milk yield and component traits (Do *et al.,* 2017), denoting the complicated putative functional pathways of miRNAs, and how complex an agro-food product certification process based on miRNA can be.

Table 1. Statistical *P*-value of miRNA expression among the three groups of dairy farms.[1]

Statistical *P*-value	bta-miR-574	bta-miR-3432a	bta-miR-2285e	bta-miR-197	bta-miR-2284y
Group 1 vs Group 2	0.402	0.734	0.571	0.879	0.678
Group 2 vs Group 3	0.851	0.270	0.142	0.092	0.150
Group 1 vs Group 3	0.635	0.172	0.044*	0.059	0.113

[1] Significance $P<0.05$. Group 1 without fresh-grass, Group 2 with less than 10 kg per animal of fresh-grass, Group 3 with more than 10 kg per animal.

Conclusions

Although more research could be necessary in this regard, the bta-mir-2285e miRNA has been identified as a possible marker of cow's milk produced based on fresh-grass.

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References

Abou el Qassim L., Vicente F., De la Torre S., Jiménez-Calderón J.D., Baizán S., Soldado A., Martínez-Fernández A. and Royo L.J. (2018) *¿Es posible certificar la leche producida en ecológico utilizando microARN*? XIX Reunión de Mejora Genética Animal. León, Spain.

Billa P.A., Faulconnier Y., Ye T., Chervet M., Le Provost F., Pires J.A.A. and Leroux C. (2019) Deep RNA-Seq reveals miRNome differences in mammary tissue of lactating Holstein and Montbéliarde cows. *BMC genomics*, 20(1), 621.

Colitti M., Sgorlon S., Licastro D. and Stefanon B. (2019) Differential expression of miRNAs in milk exosomes of cows subjected to group relocation. *Research in Veterinary Science* 122, 148-155.

Do D., Dudemaine P.L., Li R. and Ibeagha-Awemu E. (2017) Co-expression network and pathway analyses reveal important modules of miRNAs regulating milk yield and component traits. *International Journal of Molecular Sciences*, 18, 1560.

He L. and Hannon G.J. (2004) MicroRNAs: small RNAs with a big role in gene regulation. *Nature Reviews Genetics* 5, 522-531.

Lai Y.C., Fujikawa T., Maemura T., Ando T., Kitahara G., Endo Y., Yamato O., Koiwa M., Kubota C. and Miura N. (2017) Inflammation-related microRNA expression level in the bovine milk is affected by mastitis. *PLoS One*, 12(5), e0177182.

Li Z., Liu H., Jin X., Lo L and Liu J. (2012) Expression profiles of microRNAs from lactating and non-lactating bovine mammary glands and identification of miRNA related to lactation. *BMC Genomics*, 13, 731.

Li R., Beaudoin F., Ammah A.A., Bissonnette N., Benchaar C., Zhao X., Lei C and Ibeagha-Awemu E.M. (2015) Deep sequencing shows microRNA involvement in bovine mammary gland adaptation to diets supplemented with linseed oil or safflower oil. *BMC Genomics*, 16, 884.

Li R., Dudemaine P.L., Zhao X., Lei C. and Ibeagha-Awemu E.M. (2016) Comparative analysis of the miRNome of bovine milk fat, whey and cells. *PloS One* 11, e0154129.

Mobuchon L., Le Guillou S., Marthey S., Laubier J., Laloë D., Bes S., Le Provost F. and Leroux C (2017) Sunflower oil supplementation affects the expression of miR-20a-5p and miR-142-5p in the lactating bovine mammary gland. *PLoS One*; 12(12), e0185511.

Muroya S., Shibata M., Hayashi M., Oe M. and Ojima K. (2016) Differences in circulating microRNAs between grazing and grain-fed Wagyu cattle are associated with altered expression of intramuscular microRNA, the potential target PTEN, and lipogenic genes. *PLoS One,* 11(9), e0162496.

Nicoletti M. (2012) Nutraceuticals and botanicals: overview and perspectives. *International Journal of Food Sciences and Nutrition*, 63 (sup1), 2-6.

NRC (2001) *Nutrient requirements of dairy cattle*, 7th revised edition, National Research Council, National Academy Press, Washington, DC, USA, 381 pp.

Romao J.M., Jin W., He M., McAllister T. and Guan L.L. (2012) Altered microRNA expression in bovine subcutaneous and visceral adipose tissues from cattle under different diet. *PLoS One*, 7(7), e40605.

Sun J., Aswath K., Schroeder S.G., Lippolis J.D., Reinhardt T.A. and Sonstegard T.S. (2015) MicroRNA expression profiles of bovine milk exosomes in response to *Staphylococcus aureus* infection. *BMC Genomics* 16, 806.

Forage organic matter digestibility: NIRS predictions based on *in vivo* values and standardisation of *in vitro* determinations

Ampuero Kragten S.[1], Pacheco Aguirre J.A.[1,2], Wyss U.[2], Meisser M.[3], Probo M.[3] and Huguenin-Elie O.[4]
[1] *Agroscope, Feed Chemistry, 1725 Posieux, Switzerland;* [2] *Agroscope, Ruminant Research, 1725 Posieux, Switzerland;* [3] *Agroscope, Grazing Systems, 1260 Nyon 1, Switzerland;* [4] *Agroscope, Forage Production and Grassland Systems, 8046 Zurich, Switzerland*

Abstract

The determination of forage organic matter digestibility (OMD) is of great importance to ruminant production but can be expensive and time consuming. We evaluated an *in vitro* OMD determination and near infrared spectroscopy (NIRS) OMD prediction using 199 forage samples from 24 regions around Switzerland, which were selected according to the diversity of their fibre and protein content, botanical composition and geographical origin. The *in vitro* OMD was determined with a Daisy II incubator (Ankom Technology), by 48 h digestion of 250 mg sample (in a filter bag) with rumen inoculum (from three cows) in a buffer solution (pH=6.8) at 39.5 °C and anaerobic conditions. Five samples with known *in vivo* OMD values were repeated in each batch to standardise rumen inoculum activity. The standardisation procedure was evaluated with three validation samples. Near infrared spectrometry (NIRS) was performed with a FT-NIR (NIRFlex N-500, Büchi, Flawil, Switzerland) with 3 replicates per sample, 32 scans/replicate from 4,000 to 10,000 cm^{-1} and 8 cm^{-1} of resolution. The NIRS OMD model was based on *in vivo* values. The *in vitro* and NIRS OMD determinations showed moderate to good correlations with ADF, NDF and lignin content, R^2 ranging from 0.24 to 0.85.

Keywords: OMD; *in vitro* digestibility; digestibility with NIRS; permanent and intensive meadows

Introduction

With increasing pressure on animal production and environmental concerns, tools are needed to perform frequent determinations of forage organic matter digestibility (OMD), thereby enabling the optimisation of grassland management. Although tables with OMD values exist, they are based on average values, often not reflecting grasslands specificities, e.g. year, botanical and chemical composition etc., which ultimately influence OMD. This trial aimed to evaluate fast and economical techniques (*in vitro* and near infrared spectrometry (NIRS)) for determining OMD of forages. We focused on the standardisation of rumen inoculum activity for *in vitro* OMD determination and the predictability of NIRS models.

Materials and methods

Standardisation of rumen inoculum

Different rumen inoculums were used per batch, i.e. a mix of the ruminal fluid of one fistulated cow and of two additional cows (from a pool of four cows) vacuum probed through the oesophagus. In each *in vitro* batch, 5 standard samples were included for the standardisation of rumen inoculum activity, as well as 3 validation samples for the evaluation of the standardisation procedure, all 8 (standard and validation samples) with known *in vivo* OMD values. A Daisy II incubator (Ankom technology, Macedon, NY, USA) was used for the *in vitro* OMD determination. The incubator is designed to perform a 48 h digestion by slowly rotating four 2-litre bottles at 39 °C with a capacity of 25 samples each. To prevent oxidation the buffers and the ruminal fluid were maintained at 39 °C under anaerobic conditions at all times. The *in vitro* OMD determination was performed with 0.2500±0.0001 g of milled and dried sample, sealed inside a filter bag. The incubation bottles were each filled with 1,330 ml of buffer [266 ml (10 g l^{-1} KH_2PO_4, 0.5 g l^{-1} $MgSO_47H_2O$, 0.5 g l^{-1} NaCl, 0.1 g l^{-1} $CaCl_22H_2O$, 0.5 g l^{-1} urea) + 1,330 ml (15 g l^{-1} Na_2CO_3, 1 g l^{-1} Na_2S9H_2O)] and were maintained at pH 6.8 and 39 °C. To each incubation bottle 25 filter bags were added that contained either forage (standard and validation samples) or were empty (at least 3 empty bags: blanks). After about 30 min of mixing at 39 °C, 400 ml of ruminal inoculum was added to each bottle, fluxed with CO_2 and incubated for 48 h. The result was calculated as the mass lost during incubation, corrected for ash content. Three determinations per sample were performed, each in a different *in vitro* batch (nine batches in total).

Predictability of NIRS model

An FT-NIR (NIRFlex N-500, Büchi, Flawil, Switzerland) was used with 3 replicates per sample, 32 scans/replicate from 4,000 to 10,000 cm^{-1} and a resolution of 8 cm^{-1}. The NIRS OMD model was built in 2018 based on approximately 100 samples whose *in vivo* OMD values span back to 1990. The study was based on circa 800 forage samples from 32 meadows (23 permanent and 9 intensive) distributed within 24 regions across Switzerland, ranging from 400 to 1000 m in altitude. First, second and third cuts (year 2018) were sampled, with nine samples per meadow × cut. The evaluation of the botanical composition showed values ranging between 60 to 98% grass, 0 to 10% legumes and 1 to 35% forbs for the first cut and between 35 to 98% grass, 1 to 20% legumes and 1 to 74% forbs for the subsequent cuts. The variation of the botanical composition, within the same meadow and cut, could reach 17% for legumes and 25% for forbs. The samples were oven dried at 60 °C for 24 h and milled to pass through a 1 mm sieve. The nutrient and chemical composition of all samples was determined by NIRS (Ampuero Kragten and Wyss, 2014). From this parent database, 199 samples were chosen for *in vitro* (Daisy II incubator) and NIRS determination of OMD while maximising the diversity in fibre, protein and botanical content as well as the geographical origin of the sample subset. Based on the mass availability of forage, 3 samples were chosen for *in vivo* determinations (Pacheco *et al.*, 2018) and further evaluation of *in vitro* and NIRS procedures with recent values of OMD *in vivo*.

Results and discussion

Figure 1A shows the dispersion of the 9 *in vitro* batches by plotting the *in vitro* OMD of the 5 standard samples (single determination, but rumen inoculum from 3 cows) against their corresponding *in vivo* OMD values. Linear regressions per batch showed R^2 and slopes ranging from 0.961 to 0.998 and from 0.84 to 1.09 respectively (data not shown). Figures 1B and 1C show the corresponding values for the three validation samples per batch: B before and C after the correction by a batch-regression equation built with the standard samples. The potential of this form of standardisation should be more evident when using more diverse rumen inoculums. Indeed, a limited number of cows was used in this trial with limited variation in breed, feeding management, health status, etc. The precision was evidently improved by averaging the *in vitro* OMD values over 3 batches (Figure 1D). In Figures 1A and 1D, *in vitro* total average OMD and NIRS OMD values are respectively denoted by X and — signs.

As expected, high correlation coefficients were obtained for first-cut forages between ADF, NDF and lignin respectively and both: *in vitro* OMD values (0.81, 0.67 and 0.63), and NIRS OMD values (0.85, 0.72 and 0.84). However, when using all samples, R^2 decreased, e.g. NIRS: 0.36, 0.24 and 0.71. This probably reflects fibre quality and amount changes between primary growth and regrowth of forage, showing the necessity of including more *in vivo* OMD values of regrowth samples (higher cuts) in the NIRS prediction model.

The diversity of the chemical composition of the 199 samples used for testing the predictability of NIRS model is described in Table 1.

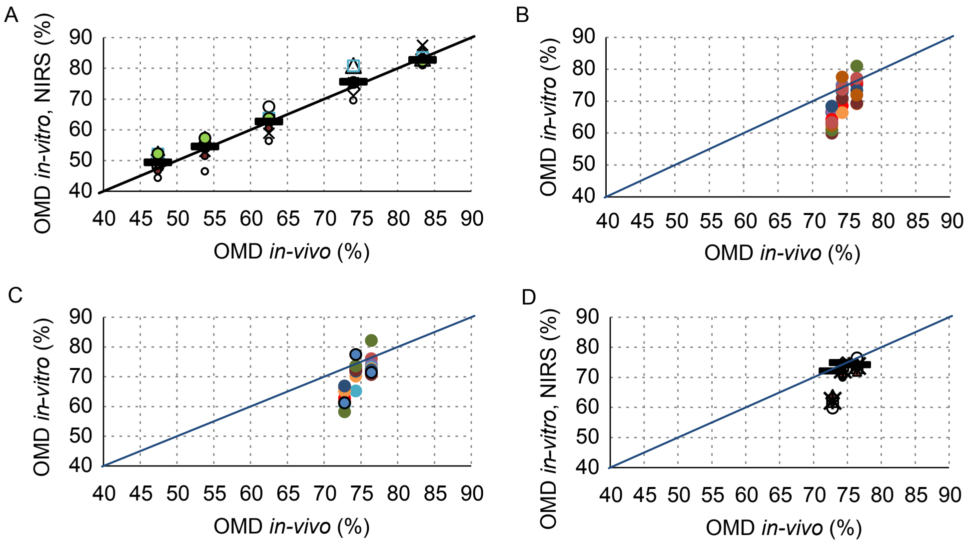

Figure 1. *In vitro* (and NIRS) organic matter digestibility (OMD) vs *in vivo* OMD. (A) Standard samples, per batch values (single determination, different rumen inoculum); (B) validation samples, per batch values; (C) validation samples, per batch values corrected by the corresponding linear regression equation of standard samples; (D) validation samples averaged over three batches. X: *in vitro* OMD total average; NIRS OMD values.

Table 1. Chemical composition (% in dry matter) of the 199 forage samples determined with NIRS.[1]

	DM	CP	CF	ADF	NDF	Lignin	CA	WSC
Average	93.8	15.9	23.2	27.1	45.7	2.9	10.5	9.9
SD	1.3	5.1	5.9	5.8	10.7	1.1	1.9	3.0
Min	91.3	6.8	8.7	11.7	18	0.5	6.5	0.3
Max	97.4	33.9	36.7	39.7	66.9	6.5	16.4	20.0
RMSEP	0.33	0.55	0.80	1.03	1.52	0.40	0.63	0.68

[1] DM = dry matter in % of fresh matter; CP = crude protein; CF = crude fibre; ADF = acid detergent fibre; NDF = neutral detergent fibre; CA = crude ash; WSC = water soluble carbohydrates, SD = standard deviation; RMSEP = NIRS root mean square of prediction error.

Conclusions

The averaging of three batches as a standardisation technique of rumen inoculum activity shows an interesting potential for improving the accuracy of *in vitro* OMD determination. However, the NIRS technique seems more efficient, more economical and less time consuming for OMD evaluation, provided that the set of samples used for the modelling contains all the necessary sample diversity.

References

Ampuero Kragten S. and Wyss U. (2014) Futtermittel im Nah-Infrarotlicht (NIRS). *Agrarforschung Schweiz* 5(05), 204-211.

Pacheco J.A., Pittet A., Ampuero Kragten S. and Arrigo Y. (2018) Verdaulichkeit der organischen Substanz: Vergleich von *in-vivo-* und *in-vitro*-Bestimmungen. *Agrarforschung Schweiz* 9(03), 92-97.

The effect of herbs in grass mixtures on the dry matter intake of dairy cows

Bastiaansen-Aantjes L.M.[1], Ankersmit E.[1], Nicolasen S.H.M.[2], Ter Horst A.C.[1] and Van den Pol-van Dasselaar A.[1]

[1]*Aeres University of Applied Sciences, De Drieslag 4, 8251 JZ Dronten, the Netherlands;* [2]*Barenbrug Holland BV, Stationsstraat 40, 6501 BH Nijmegen, the Netherlands*

Abstract

Grasslands in the Netherlands consist mainly of ryegrass (*Lolium perenne* L). Recent developments in the Dutch dairy sector have increased the interest in herb-rich grasslands. However, effects on dairy cow dry matter intake (DMI) of herb-rich grasslands are largely unknown and farmers fear that the intake might be lower. In September 2018, three different mixtures (*L. perenne* L. (LP), *L. perenne* L. + seven types of herbs (LP+) and *Festuca arundinacea* + seven types of herbs (FA+)) were sown on a clay soil at Aeres Farms, the Netherlands. Three groups of 14 dairy cows each grazed these mixtures during two consecutive grazings in June 2019 via strip grazing. During grazing, DMI was estimated by measuring pre- and post-grazing yield. In order to test for significant effects of the mixture used on DMI we used a three-way ANOVA with the factors: Mixture (LP, LP+, FA+), Time (Day, Night) and Period (P1, P2). Results showed no significant differences in DMI per cow between the different mixtures. This implies that the fear of farmers for lower DMI is unfounded.

Keywords: herb-rich grassland, dry matter intake, grazing trial, grass mixtures

Introduction

Grasslands in the Netherlands mainly consist of perennial ryegrass (*Lolium perenne* L.) Recent developments in the Dutch dairy sector have increased the interest in herb-rich grassland. Mixtures of grass, legumes and specific herb species may lead to higher dry matter (DM) productivity under dry weather conditions, less fertiliser use, more feed protein produced and more biodiversity (Wagenaar *et al.*, 2017). There are indications that herb-rich grass will increase the palatability of the roughage, and therefore the grass DM intake (DMI) of dairy cows. Especially in the late summer, the DMI of grass-clover is usually much higher than that of pure grass because leguminous plants are not susceptible to rust on leaves, and will also reduce rust on the grass. However, it is the composition of the total ration that determines the total intake (Wagenaar *et al.*, 2017). A German grazing experiment showed that cows do have a preference for *Lotus corniculatus, Plantago lanceolate,* and *Chichorium* in situations of high proportions of clover in the available grass, since the three mentioned herbs will reduce the risk of bloat caused by the high proportion of clover (Vereijken, 2010). *L. perenne* is strong in terms of sod formation, but dense swards give less space for herbs to develop and survive in the long term, so combining herbs with *Festuca arundinacea* may be positive. The deeper roots of *Festuca* will also contribute to synergy with deep rooting herbs (Deru *et al.*, 2011). However, effects of herb-rich grasslands on dairy cow DMI are largely unknown under Dutch conditions and farmers fear that the intake of herb-rich grasslands by dairy cows might be lower. Therefore, the objective of this research was to examine the effects of three different grass mixtures on DMI by dairy cows in the Netherlands.

Materials and methods

In the autumn of 2018, three different mixtures were sown on paddocks of 1 ha each on a clay soil at the organic research farm of Aeres University of Applied Sciences in Dronten, the Netherlands. The first mixture (LP) consisted of *L. perenne* L, the second mixture (LP+) consisted of *L. perenne* L + seven types of herbs and the third mixture (FA+) consisted of *F. arundinacea* + seven types of herbs. The herbs were similar for LP+ and FA+: (1) *Trifolium pratense* (red clover); (2) *Trifolium repens* (white clover); (3) *Onobrychis viciifolia* (common sainfoin); (4) *Carum carvi* (caraway); (5) *Cichorium* (common chicory); (6) *Lotus corniculatus* (bird's-foot trefoil); and (7) *Plantago lanceolate* (ribwort plantain).

Stellaria media emerged in all parcels in spring 2019 and was treated three times with a weeding harrow. In spring, the parcels were fertilised with 20 kg N ha^{-1} by an organic fertiliser based on sugar cane. The first cut was mown on 6 May 2019, followed by fertilisation of 76 kg N ha^{-1} from organic manure. Thereafter, grazing commenced. Three groups of 14 dairy cows each grazed one of the three mixtures during two consecutive grazing periods (P1: 31 May – 4 June, and P2: 17-20 June). Before grazing, the groups were randomised and blocked with respect to milk production (on average 30.7 kg cow^{-1} day^{-1}), combined fat and protein content (on average 2.2 kg cow^{-1} day^{-1}), somatic cell count (on average 112,000 cells ml^{-1}), days in milk (on average 121 days) and lactation number (on average 3.2 lactations). The same groups were used both in P1 and P2.

In P1, herbage allowance (above a stubble of 5 cm) was set at 20 kg DM cow^{-1} day^{-1}. In P2, herbage allowance was set at 22 kg DM cow^{-1} day^{-1}. Since the pre-grazing yield ha^{-1} was different for the different mixtures, the area provided to the cows was different for each group. Cows additionally received 4 kg concentrates day^{-1} in both P1 and P2. Pre-grazing yields were measured once a day in the afternoon by mowing 5 squares of 0.25 m^2 each on 5 cm stubble height. The fresh grass samples were weighed and then dried for 48 hours at 60 °C to calculate the DM yield. Post-grazing yields were measured twice a day after milking according to the same procedure.

In order to test for significant effects of the mixtures used on DM intake (kg DM cow^{-1}) we used a three-way ANOVA with the factors: Mixture (LP, LP+, FA+), Time (Day, Night) and Period (P1, P2). DM intake cow^{-1} was calculated by subtraction of pre-and post-grazing yields, and dividing this resultant by the number of cows (i.e. 14).

In addition, we tested on possible differences in grass quality indicators between the different mixtures. We used a one-way MANOVA with Mixture (LP, LP+, FA+) as independent variable and DM, VEM, sugar, crude protein and neutral detergent fibre (NDF) as dependent variables. Alpha was set at 0.05 and Bonferroni correction was applied when applicable.

Results and discussion

The average herbage DM intake was 12.6 kg cow^{-1} day^{-1}. The three mixtures showed different quality (Table 1), e.g. FA+ had the highest protein content, which was associated with more legumes visible in the field. Analyses showed no significant differences between the DMI in P1 and P2 and therefore the data from both periods was combined (Figure 1). Even though results showed a tendency to greater DMI for FA+, differences between the mixtures were not significant. The Partial Eta Squared for mixture was

Table 1. Grass quality indicators for three different mixtures (LP, LP+ and FA+) in June 2019. Values represent averages of P1 and P2 (standard error of the mean); means with different superscript letters within a row indicate significant differences of *(P*<0.05).[1]

Indicator	LP	LP+	FA+
DM (g kg^{-1} fresh grass)	212 (6.7)^{a}	166 (4.2)^{b}	135 (3.9)^{c}
VEM (kg^{-1} dm)	871 (8.6)^{a}	854 (7.2)^{ab}	826 (10.3)^{b}
Sugar (g kg^{-1} dm)	169 (9.0)^{a}	150 (7.8)^{a}	118 (8.6)^{b}
Crude protein (g kg^{-1} dm)	156 (4.0)^{b}	160 (2.4)^{b}	176 (5.5)^{a}
NDF (g kg^{-1} dm)	48 (0.9)^{a}	43 (0.7)^{b}	40 (0.9)^{b}

[1] VEM = Dutch energy unit; NDF= neutral detergent fibre; DM = dry matter.

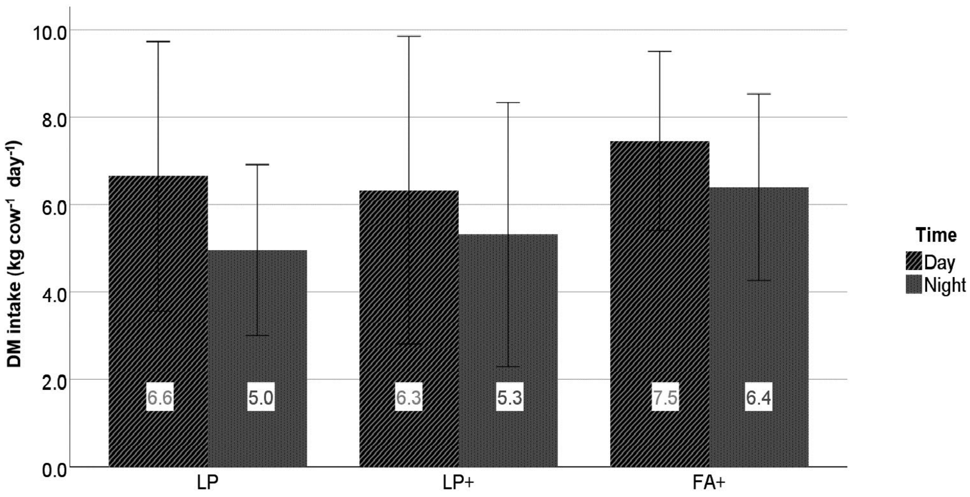

Figure 1. Average dry matter (DM) intake (kg cow^{-1} day^{-1} ± standard deviation) during the day and during the night for three different mixtures (LP, LP+ and FA+) in June 2019 (average of P1 and P2).

0.052, so more measurements could have resulted in significant differences between mixtures. The results imply that the fear of farmers for lower DMI when there are herbs in the sward is unfounded. Based on positive effects shown in other studies, herb-rich mixtures might play a role in Dutch animal husbandry in the future.

Conclusions

Three different mixtures (1) *L. perenne*, (2) *L. perenne* + herbs, and (3) *F. arundinacea* + herbs were tested during two consecutive grazing periods in June 2019. Results showed no significant differences between the three mixtures in dry matter intake by grazing dairy cows.

Acknowledgements

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References

Deru J., Schilder H., Van der Schoot J.R. and Van Eekeren N.J.M. (2014) Genetic differences in root mass of *Lolium perenne* varieties under field conditions. *Euphytica* 199, 223-232.

Vereijken H. (2010) Haben Milchkuhe Kräutervorlieben? *Lebendige Erde* 3, 18.

Wagenaar J.-P., De Wit J., Hospers-Brands M., Cuijpers W. and Van Eekeren N. (2017) *Van gepeperd naar gekruid grasland: Functionaliteit van kruiden in grasland*. Report 2017-022, Louis Bolk Instituut, Driebergen, 44 pp.

Ryegrass and red clover mixtures for dairy cows: impact of harvest stage for silage on intake, production and income

Brocard V.[1], Cloet E.[2], Tranvoiz E.[2] and Rouillé B.[1]
[1]Institut de l'Elevage, BP 85225, 35652 Le Rheu, France; [2]Chambre d'agriculture de Bretagne, 2 allée St Guénolé, 29000 Quimper, France

Abstract

During four years, an experiment was conducted in Western France on two systems, one conventional and one organic. The aim was to produce grass silage with a higher content in proteins by reducing cutting intervals and harvesting mixtures of hybrid ryegrass and red clover at an earlier stage (Early: 5 weeks of regrowth versus 7 for the Control). During winter, the silages were delivered to separate groups of cows. In the conventional system, grass silage represented 40% of the forage diet. In the Early group the total intake increased by 1.7 kg DM d^{-1} cow^{-1} and cows produced significantly more milk (+1.9 kg d^{-1}) with no impact on milk quality. In the organic system, grass silage represented 60 to 70% of the forage diet. Intake increased by 4.5 kg DM cow^{-1} d^{-1} and cows produced significantly more milk (+2.4 kg d^{-1} for primiparous and 4.8 kg d^{-1} for multiparous cows). The margin over feeding cost for a 75-cow herd was increased by 2,600 € per winter in the conventional system, and by 7,800 € in the organic one. The high increase in silage intakes remains a challenge for the latter. Whatever the production system, producing early cut grass silage of ryegrass and clover mixtures is an efficient way to improve feeding self-sufficiency and milk production.

Keywords: dairy cows, protein, self-sufficiency, grass silage, tall legumes

Introduction

In France increasing protein self-sufficiency is the major challenge for dairy farms. Producing more home-grown proteins can decrease the feeding cost, secure the production system, improve products traceability and reduce the potential negative impacts on environment (Rouillé *et al.,* 2014). Ryegrass and red clover mixtures can meet these expectations. Thus, the aim of our study was to produce grass silage with a higher content in proteins by harvesting grass silage at an earlier stage and more frequently than the usual practices of the dairy farmers. The impacts on forage quality and yields were previously presented by Brocard *et al.* (2019): the Early harvest system led to 5 cuts per year and resulted in reduced yield per ha in two of four experimental years (average -1.5 Mg DM ha^{-1}), compared to the Control system with 3 cuts per year. However, the early harvested forage always had higher nutritive values both in energy and proteins. But would the cows see the difference? These silages were introduced in their winter diet to measure the impacts on intake and milk production.

Materials and methods

Trevarez experimental farm (Brittany, France) has two different production systems, one is conventional, the other is organic, totally separated. This trial was thus performed in both systems with different modalities according to the production system and rules. In both systems, grass fields of mixtures of hybrid ryegrass and red clover were harvested in two different ways (Early and Control). On the 'Early' plots, grass was harvested to produce silage with a higher content in proteins by harvesting the first cut at an earlier stage (Brocard *et al.*, 2019) than in the Control plots (first cut at early heading stage of the grass). On the 'Early' plots, the next cuts occurred with a shorter interval than on the 'Control' plots: 5 weeks of regrowth versus 7 weeks. The two types of silages were stored in separate concrete clamps (the successive cuts displayed in layers to make a sandwich). In each system (conventional and organic), two separate groups of Holstein cows received during winter a diet including either the Early or the Control grass silage harvested in their system. The share of grass silage in both systems represented an 'average' diet used by farmers in western France, maize silage being the other forage delivered to the cows.

In the conventional herd, the experiment was carried out during 4 successive winters (2014/15-2017/18). Two groups of 20 cows were compared each winter. Altogether 159 lactations were analysed (with 36% first lactations, Table 1). The average stage of lactation at the start of the trials (early December) was around 70 days. The Early and Control groups received a diet made of the same maize silage representing 60% of the forage diet, together with 3.5 kg of concentrate d^{-1} (soybean and wheat) (Table 1). Each group then received 40% grass silage *ad libitum*, either early cut (Early group) or Control during 3 months. In the organic herd, the experiment was carried out during two successive winters (2016/17-2017/18). Two groups of 20 cows were compared each winter. Altogether 58 lactations were analysed (34% first lactations, Table 1). The average stage of lactation at the start of the trials was around 100 days. The Early and Control groups received a diet made of the same maize silage (around 5 kg DM d^{-1} c^{-1}) together with 2.5 kg of concentrate per day (barley and corn), Table 1. Then each group received grass silage *ad libitum*, either early cut (Early group) or Control. Grass silage represented around 60% of the forage delivered in the Control group. Group intakes, individual dairy production, milk fat and protein were recorded and analysed with Anova procedures (SAS® software). No difference in terms of weight, body condition score, reproduction or health were stated during these experiments.

Results and discussion

In the conventional herd, grass silage represented 39% of the forage intake in both compared groups. Thanks to the higher content in crude proteins in the Early grass silage (+48 g kg^{-1}), the total intake of the Early group was increased by 1.7 kg DM cow^{-1} d^{-1} (Table 2) resulting in a statistically significant increase in milk production by +1.9 kg cow^{-1} d^{-1} (P=0.02). No effect was found on milk composition. As a consequence, the milk sales per day were increased (Table 3) as well as the feeding cost in relation to the higher intake of the Early group and the higher cost of each kg of grass silage (Brocard *et al.,* 2019). But still the margin over feeding cost was higher by 0.4 € per cow per day in the Early group. For a herd of 75 cows during 3 months, it represents an increased benefit of around 2,635 €. In the organic herd, grass silage represented 57% of the forage intake in the Control group and 70% in the Early group. Thanks to the higher nutritional value in the Early grass silage, the total intake of the Early group was increased by 4.5 kg DM cow^{-1} d^{-1} (Table 2) resulting in a statistically significant (P<0.001) increase in milk production by +2.4 kg cow^{-1} d^{-1} for the primiparous cows and by 4.8 kg cow^{-1} d^{-1} for the multiparous cows. No effect was found on milk composition. As a consequence, the milk sales were increased (Table 3) as well as the feeding cost in relation to the higher intake of the Early group and the higher cost of each kg of grass silage (Brocard *et al.*, 2019). But the margin over feeding cost was still higher by 1.1 € per cow per day in the Early group. For a herd of 75 cows during 3 months, it represents a potential increased benefit of around 7,800 €. In both the conventional and the organic diets, the higher nutritional quality in the Early cuts led to higher intakes. The effect is particularly strong in the organic diet which is lacking N because it is based on home-grown feeds (maize silage, cereals). Reducing the cutting interval also improved the

Table 1. Experimental groups and average intakes in both conventional and organic herds in kg dry matter cow^{-1} d^{-1}.

	Conventional herd (4 winters)		Organic herd (2 winters)	
	Control	Early	Control	Early
Number of cows	79	80	29	29
Maize silage	10.3	11.0	5.0	4.8
Grass silage	6.6	7.5	7.1	11.9
Hay	0.2	0.3	0.3	0.3
Concentrates + minerals	3.7	3.7	2.8	2.7
Total intake	20.8	22.5	15.2	19.7
Difference Early-Control	+1.7		+4.5	
% grass silage in the forage diet	39	39	57	70
Crude protein in grass silage g kg^{-1}	113	161	114	166

Table 2. Effects of compared diets with either control or early grass silage on dairy production (tests at P=5%).[1]

Difference Early-Control	Conventional herd	Organic herd
Milk kg d^{-1}	+1.9 (P=0.02)	Primiparous +2.4 (P<0.001)
		Multiparous +4.8
Fat content %	ns	ns
Protein content %	ns	ns

[1] NS = not significant.

Table 3. Daily sales, feeding cost and margin over feeding cost in the conventional and in the organic herds; Calculations with 35% first lactations.

	Conventional herd		Organic herd	
	Control	Early	Control	Early
Milk kg d^{-1}	23.8	25.7	14.1	18.1
Milk sales (€ cow^{-1} d^{-1})	8.3	9.0	6.4	8.3
Feeding cost (€ cow^{-1} d^{-1})	2.0	2.3	1.5	2.2
Margin over feeding cost (€ cow^{-1} d^{-1})	6.3	6.7	4.9	6.1
Difference Early-Control if 75 cows × 3 months	+2,635 €		+7,803 €	

energy value as shown by Rinne *et al.* (1999) though it is not the most limiting factor in dairy cow diets in Western France thanks to the resort to maize silage and cereals. In the conventional herd, the economic impact was positive and easy to reach: most of the farms can afford a decrease of the grass yields by 1.5 Mg DM per ha when processing early cuts and turn cereals areas into forage areas to compensate and cover the total forage requirements of the herd. The situation in the organic system is different: because the diets used by farmers are usually too low in proteins and have a high fill value, the improvement brought by early cut silage leads to a great increase in intakes. For a 75-cow herd, it would require 33 Mg of DM extra grass silage to be harvested in order to cover winter intakes. Most of the organic Breton dairy farms cannot use extra areas as the whole farm is already used for forage production. Added to a potentially lower yield, turning the whole grass silage process to early cuts would lead to a serious deficit in forages or lead to a decrease in cow numbers. So, each organic farm will have to find a compromise between quality and quantity of grass harvested for silage according to its own situation, but there is a great potential increase in benefit for those organic farms with large areas available for forage production.

Conclusions

In both systems, producing early cut grass silage of ryegrass and clover mixtures is an efficient way to improve feeding self-sufficiency and milk production.

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References

Brocard V., Cloet E., Tranvoiz E. and Rouillé B. (2019) Ryegrass – red clover mixtures: impact of harvest stage for silage on yield and nutritional value. *Grassland Science in Europe* 24, 213.

Rinne M., Jaakkola S., Kaustell K. and Huhtanen P. (1999) Silages harvested at different stages of grass growth *v.* concentrate foods as energy and protein sources in milk production. *Animal Science* 69, 251-263.

Rouillé B., Devun J. and Brunschwig P. (2014) L'autonomie alimentaire des élevages bovins français. OCL 2014, 21(4) D404. Available at: www.ocl-journal.org.

Effect of grazing season length and stocking rate on milk production in the north-east region of Ireland

Cahill L.[1,2], Reilly B.[3], Patton D.[1], Pierce K.[2] and Horan B.[1]

[1]*Animal and Grassland Research and Innovation Centre, Teagasc Moorepark, Fermoy, Co. Cork, Ireland;* [2]*UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland;* [3]*Ballyhaise Agricultural College, Drumcrow, Ballyhaise, Co. Cavan, Ireland*

Abstract

Increasing the proportion of grass in the diet of the dairy cow is one of the fundamental objectives of Irish milk production systems. Significant potential exists to increase productivity from pasture by increasing stocking rate and extending grazing season length. The objective of the experiment was to quantify the effect of grazing season (GS) length and stocking rate (SR) on milk production and supplementary feed requirements within an intensive spring calving system. Over 2 years (2017 and 2018), 120 spring calving dairy cows were randomly assigned pre-calving based on breed, parity, calving date and previous lactation milk yield to one of four grazing systems. The systems evaluated comprised two GS lengths: average GS (AGS; 210 days; 15 March to 15 October) and extended GS (EGS; 279 days, 15 February to 20 November) and two SR treatments: medium SR (MSR; 2.5 cows ha^{-1}) and high SR (HSR; 2.9 cows ha^{-1}). GS had a significant effect on individual animal performance in terms of milk yield and milk composition. Higher SR resulted in significantly increased milk and milk fat plus protein production per hectare. As the AGS treatment was indoors for an additional 60 days between February and November, significantly more concentrate and silage were required during lactation compared with the EGS treatment. The results of this study show the potential of both extended grazing and higher SR to support increased milk productivity while reducing supplementary feed requirements in grazing systems.

Keywords: grazing season length, stocking rate, dairy systems

Introduction

Temperate grazing systems are characterized by seasonal calving, a prolonged grazing season (>275 days) and a primarily pasture-based diet (Dillon *et al.*, 2005). Such systems, based on a cheap feed source (Shalloo *et al.*, 2004), provide pasture-based milk producers with a competitive economic advantage over other production systems based on high milk output per hectare with reduced fixed and variable costs (Finneran *et al.*, 2010). It is also widely acknowledged that pasture utilisation is the most important factor influencing operating profit per hectare (Shalloo *et al.*, 2004) and that stocking rates (SR) is a critical factor (Macdonald *et al.*, 2008). In comparison with other regions of Ireland, dairy production systems in the north-east are characterised by lower SR, a shorter grazing season and reduced farm profitability (Lapple *et al.,* 2012; Ramsbottom *et al.*, 2015). The objective of this study was to quantify the impacts of alternative SR and grazing season length combinations on animal and pasture productivity on a wetland soil in the north-eastern region.

Materials and methods

This study was carried out at Ballyhaise Agricultural College (54° 015'N, 07° 031'W) during 2017 and 2018. In January 2017, 120 spring calving dairy cows were randomly assigned pre-calving, based on breed, parity, calving date and previous lactation milk yield, to one of four grazing systems. The systems evaluated comprised two grazing season (GS) lengths: average GS (AGS; 210 days; 15 March to 15 October) and extended GS (EGS; 279 days, 15 February to 20 November); and two SR treatments: medium SR (MSR; 2.5 cows ha^{-1}) and high SR (HSR; 2.9 cows ha^{-1}). Each experimental group had its own farmlet. While indoors, both AGS groups were fed a grass silage and concentrate diet. Weekly milk production was derived from individual milk yields recorded at each milking. Milk fat, protein and lactose concentrations were determined once weekly from successive morning and evening milk samples, while individual body weight (BW) and body condition score (BCS) was recorded on a bi-weekly basis. Pre-grazing sward height (PreGSH) and post-grazing sward height (PostGSH) were measured using a rising plate meter (Jenquip, Feilding, New Zealand). Herbage mass (>3.5 cm) was measured by cutting three quadrat samples per paddock before grazing. Least squares means for GS and SR were estimated using linear mixed models in SAS 9.4.

Results and discussion

There was no significant interaction between GS and SR on animal performance (Table 1). Grazing season length varied from 199 days for both AGS treatments to 257 and 255 days for the MSR EGS and HSR EGS treatments, respectively. Grazing season length had a significant effect on individual animal performance in terms of milk yield and milk solid production. Neither SR nor GS had a significant effect on individual animal performance in terms of milk composition, average BW or average BCS. Higher SR resulted in similar individual cow performance to MSR and significantly increased milk and milk fat plus protein production per hectare. There was no significant effect of SR or GS on growth of pasture herbage (Mg DM ha^{-1}) or utilisation. As both AGS treatments were indoors for an additional 60 days between February and November, significantly more concentrate and silage were required during lactation compared with the EGS treatments. The MSR and HSR average grazing season treatments consumed 2.1 to 2.7 Mg silage DM ha^{-1} yr^{-1}, respectively, compared to 1.2 and 1.1 Mg DM ha^{-1} $year^{-1}$ for the respective extended grazing treatments. In addition, the average grazing season length treatments required 20% more concentrate than the comparable SR groups.

In a meta-analysis review of the effects of SR on animal performance McCarthy *et al.* (2011) reported a significant linear decline in individual animal performance as SR increased, whereas no reduction was evident in the current study. Similar to previous studies (Kennedy *et al.*, 2005; O'Donovan *et al.*, 2004), there was also no significant difference in pasture utilisation between the different grazing treatments in this experiment. The results suggest that extending the grazing season by 60 days delivers increased milk production performance based on 1.4 Mg DM ha^{-1} of additional pasture herbage utilisation and requiring 100 kg less concentrate and 468 kg DM less silage per cow per year. A full economic appraisal of the production systems will be undertaken at the end of the project.

Table 1. Effect of stocking rate (SR) and grazing season length (GS) on animal performance and feed requirements (2017 and 2018).[1]

	Average grazing season length		Extended grazing season length		SE	*P*-value		
Stocking rate	Medium	High	Medium	High		GS	SR	GS×SR
Milk production and composition								
Milk yield (kg cow^{-1} $year^{-1}$)	5,040	5,211	5,178	5,243	121.5	0.001	0.09	0.42
Fat + protein yield (kg cow^{-1} d^{-1})	1.44	1.49	1.53	1.59	0.022	0.001	0.01	0.68
Fat + protein (kg ha^{-1})	1,058	1,298	1,134	1,327	28.4	0.06	<0.0001	0.40
Grass utilised (Mg DM ha^{-1})	9.4	10.0	10.3	11.6	0.76	0.27	0.15	0.88
Total forage utilised (Mg DM ha^{-1})	14.4	14	13.9	14.7	285.39	0.77	0.5	0.02
Supplementary feed requirements								
Concentrate (Mg DM ha^{-1})	1.7	2.0	1.6	1.9	0.04	0.001	0.001	0.78
Lactation silage (Mg DM ha^{-1})	4.2	5.2	3.2	3.5	0.12	0.01	0.001	0.22

[1] SR = medium (2.5) and high (2.9); GS = average (210 days) and extended (279 days).

Conclusions

The results of this study show the potential of both extended grazing and higher SR to support increased milk productivity while reducing supplementary feed requirements. Increasing SR resulted in similar milk production per cow and significantly increased milk output per hectare.

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References

Dillon P., Roche J.R., Shalloo L. and Horan B. (2005) Optimising financial return from grazing in temperate pastures. In: *Utilization of Grazed Grass in Temporal Animal Systems. The International Grassland Conference,* Wageningen Academic Publishers, Wageningen, the Netherlands, 131-147.

Finneran E., Crosson P., Wallace M., O'Kiely P., Forristal D. and Shalloo L. (2010) Simulation modelling of the cost of producing and utilising feeds for ruminants on Irish farms. *Journal of Farm Management* 14, 95-116.

Kennedy E., O'Donovan M., Murphy J.P., Delaby L. and O'Mara F.P. (2005) Effects of grass pasture and concentrate based feeding systems for spring calving dairy cows in early spring on lactation performance. *Grass and Forage Science* 60, 310-318.

Läpple D., Hennessy T. and O'Donovan M. (2012) Extended grazing: a detailed analysis of Irish dairy farms. *Journal of Dairy Science* 95, 188-195.

Macdonald K.A., Penno J.W., Lancaster J.A.S. and Roche J.R. (2008) Effect of stocking rate on pasture production, milk production and reproduction of dairy cows in pasture-based systems. *Journal of Dairy Science* 91, 2151-2163.

McCarthy B., Delaby L., Pierce K.M., Journot F. and Horan B. (2011) Meta-analysis of the impact of stocking rate on the productivity of pasture-based milk production systems. *Animal* 5, 784-794.

O'Donovan M., Delaby L. and Peyraud J.L. (2004) Effect of time of initial grazing date and subsequent stocking rate on pasture production and dairy cow performance. *Animal Research* 53, 489-502.

Ramsbottom G., Horan B., Berry D.P. and Roche J.R. (2015) Factors associated with the financial performance of spring-calving, pasture-based dairy farms. *Journal of Dairy Science* 98, 3526-3540.

Shalloo L., Dillon P., O'Loughlin J., Rath M. and Wallace M. (2004) Comparison of a pasture-based system of milk production on a high rainfall, heavy-clay soil with that on a lower rainfall, free-draining soil. *Grass and Forage Science* 59, 157-168.

Methane emissions from dairy cows fed maize- or grass silage-based diets with or without rapeseed oil supplementation

Chagas J., Ramin M. and Krizsan S.
Swedish University of Agricultural Sciences (SLU), Skogsmarksgränd 90183 Umeå, Sweden

Abstract

The study aimed to determine the effects of rapeseed oil supplementation on methane (CH_4) yield and intensity when partially replacing grass silage with maize silage in diets of lactating dairy cows. Twenty Nordic Red cows averaging 71 days in milk and 34.2 kg milk d^{-1} pretrial were assigned to a replicated 4×4 Latin square design. Dietary treatments were in a 2×2 factorial arrangement. The four experimental dietary treatments were: grass silage (GS), GS supplemented with rapeseed oil (GSO), grass silage and maize silage (GSMS), and GSMS supplemented with rapeseed oil (GSMSO). The partial replacement of grass silage with maize silage decreased dry matter intake, milk yield, energy corrected milk (ECM), and yields of fat, protein, and lactose ($P \leq 0.04$), but no effect ($P \geq 0.13$) of forage source was observed on daily CH_4 emission, yield (g kg^{-1} dry matter; DM) or intensity (g kg^{-1} ECM). The rapeseed oil supplementation decreased ($P \leq 0.01$) DM intake, milk fat, and CH_4 yield and intensity. There were no significant ($P \geq 0.16$) interactions between forage source and rapeseed oil supplementation on any traits.

Keywords: dairy cattle, grass silage, maize silage, methane, rapeseed oil

Introduction

Methane (CH_4) is a greenhouse gas (GHG) that plays an important role in global climate change, and its concentration has been rising rapidly in the atmosphere over the past decade. Data suggest that the increased global CH_4 emission is due to shale gas, and that natural gas and oil industry is the main contributor rather than agriculture (Howarth, 2019). However, the livestock sector is responsible for about 14.5% of the total anthropogenic GHG emissions (Gerber *et al.*, 2013). Population growth, urbanization, and income are driving the demand of livestock products. Methane production grows in direct proportion to increases in livestock numbers, and with more food produced from ruminants the global livestock related CH_4 emission is expected to increase by 60% by 2030 (FAO, 2003). This indicates a need to decrease CH_4 in the atmosphere caused by ruminant livestock production.

Enteric CH_4 production is a natural byproduct of feed degradation and fermentation in the rumen. Using more grain and whole crop silages have been identified as potentially efficient low-CH_4 diets when fed to dairy cows (Beauchemin *et al.*, 2008). Furthermore, rapeseed oil supplementation has been shown to reduce enteric CH_4 emission without affecting feed digestion and milk production (Brask *et al.*, 2013). The objective of this experiment was to determine the effects of rapeseed oil supplementation on CH_4 yield and intensity when partially replacing grass silage with maize silage in diets of lactating dairy cows. We hypothesised that cows fed a diet with maize silage would produce less CH_4 than if only fed grass silage, and that supplementation with rapeseed oil would additionally mitigate CH_4 emission.

Materials and methods

The trial was conducted at Röbäcksdalen experimental farm of the Swedish University of Agricultural Sciences in Umeå (63°45'N; 20°17'E) in 2019. Twenty Nordic Red cows averaging 71±37.3 days in milk and 34.2±5.26 kg milk d^{-1} pretrial were blocked by parity and milk yield (MY) and assigned to a replicated 4×4 Latin square design. The experimental periods lasted for 28 d and were divided into 14 d of adaptation and 14 d of data recording and sampling. Dietary treatments were in a 2×2 factorial arrangement: grass silage (GS), GS supplemented with rapeseed oil (GSO), GS plus maize silage (GSMS), and GSMS supplemented with rapeseed oil (GSMSO) (Table 1). Diets were fed *ad libitum* as total mixed ration (TMR). The GS was harvested in June 2018 in Umeå, preserved using an acid-based additive (PromyrTM XR 630, Perstorp, Sweden; 3.5 l Mg^{-1}) and stored in a bunker silo. The maize silage (MS) was purchased from Denmark where it was harvested in 2017, stored in bunker silos and baled in 2018 before transportation to Umeå. The chemical analysis indicated a good quality MS, holding 367 g dry matter (DM) kg^{-1}, 11.3 MJ metabolizable energy kg^{-1} DM, 320 g starch kg^{-1} DM, 65 g crude protein (CP) kg^{-1} DM and 393 g neutral detergent fibre (NDF) kg^{-1} DM. Feed intake was recorded individually on a daily basis throughout the trial, but only data from d 15 to 28 of each period were used for statistical analysis. Milk samples were collected from d 19 to 21 and d 26 to 28 to calculate yield and milk composition, and energy corrected milk (ECM) was calculated according to Sjaunja *et al.* (1990). Mass fluxes of CH_4 were measured daily by the GreenFeed emissions monitoring system (GreenFeed; C-Lock Inc., Rapid City, SD, USA). Experimental data were subjected to analysis of variance using the GLM of SAS (SAS Inc. 2002-2003, Release 9.2; SAS Inst., Inc., Cary, NC) and the following model:

$$Y_{ijkl} = \mu + B_i + P_j + C_k(B)_i + D_l + \varepsilon_{ijkl},$$

where Y_{ijkl} is the dependent variable and μ is the mean for all observations, B_i is the effect of block i, P_j is the effect of period j, $C_k(B_i)$ is the effect of a cow k within block i, D_l is the effect of diet l, and $\varepsilon_{ijkl} \sim N(0, \sigma_e^2)$ is the random residual error. Least square means are reported and mean separation was done by orthogonal contrasts.

Results and discussion

The chemical composition of the experimental diets are presented in Table 1. The partial replacement of grass silage with maize silage decreased dry matter intake (DMI), MY, ECM, and yields of fat, protein, and lactose ($P \leq 0.04$; Table 2). Including MS did not decrease ($P \geq 0.13$) the CH_4 emission, yield or intensity. This is in contrast to previous statements in the literature (Schils *et al.*, 2007; Beauchemin *et al.*, 2008). However, it is important to highlight that the maize inclusion in the experimental diets was ~300 g kg^{-1} DM basis, which may not be enough to modify the ruminal condition to mitigate CH_4 emission. More starch in the diet is reported to promote propionate rather than acetate fermentation in the rumen, and thereby decreases on CH_4 emission in dairy cows (Hatew *et al.*, 2015). The rapeseed oil supplementation decreased ($P \leq 0.01$) DMI, milk fat, and CH_4 daily emission, yield and intensity, while lactose content and yield increased ($P < 0.01$). CH_4 reduction due to dietary fat profile is well

Table 1. Ingredient composition of the formulated diets (g kg^{-1} of dry matter) and diets chemical composition (g kg^{-1} of dry matter unless otherwise stated).

Item		Diets[1]			
		GS	GSO	GSMS	GSMSO
Ingredients	Grass silage	749	738	420	414
	Maize silage	0	0	300	294
	Barley	207	201	230	224
	Rapeseed meal	38	16	44	17
	Rapeseed oil	0	40	0	45
	Mineral mixture	6	5	6	6
Chemical composition	Dry matter, g kg^{-1} as fed	357	364	414	425
	Crude protein	150	150	128	128
	NDF	426	421	421	386

[1] In addition to the TMR, the cows received concentrate pellets during the visits to the Greenfeed system. GS = grass silage; GSO = grass silage with rapeseed oil supplementation; GSMS = grass silage and maize silage; GSMSO = grass silage and maize silage with rapeseed oil supplementation. NDF = neutral detergent fibre.

Table 2. Effect of maize- or grass silage-based diets with or without rapeseed oil supplementation on dry matter intake (DMI), milk yield (MY), energy corrected milk (ECM), milk composition, and methane (CH_4) emission.

Item	Diets[1]				SEM	*P*-value[2]	
	GS	GSO	GSMS	GSMSO		Forage	Oil
DMI, kg d^{-1}	21.0	19.0	21.1	18.8	0.44	0.92	<0.01
MY, kg d^{-1}	31.5	32.0	28.7	29.9	0.50	<0.01	0.13
ECM	31.6	31.0	29.1	28.3	0.82	<0.01	0.37
Fat	1,336	1,316	1,295	1,110	57.3	0.03	0.08
Protein	1,051	1,077	929	913	34.4	<0.01	0.89
Lactose	1,275	1,455	1,153	1,330	43.5	<0.01	<0.01
Milk composition, g kg^{-1}							
Fat	42.7	39.0	44.9	37.9	1.36	0.71	<0.01
Protein	33.6	32.5	33.5	32.6	0.66	0.97	0.13
Lactose	41.3	44.4	41.9	44.1	0.96	0.76	<0.01
CH_4 emission, g d^{-1}	453	353	443	338	9.6	0.20	<0.01
CH_4 yield, g kg^{-1} DMI	21.4	18.6	21.7	17.9	0.55	0.69	<0.01
CH_4 intensity, g kg^{-1} ECM^{-1}	14.7	11.2	15.5	12.3	0.49	0.06	<0.01

[1] GS = grass silage; GSO = grass silage with rapeseed oil supplementation; GSMS = grass silage and maize silage; GSMSO = grass silage and maize silage with rapeseed oil supplementation.

[2] Probability of a significant effect of forage source and rapeseed oil supplementation. The interaction between forage source and rapeseed oil supplementation was not significant for any trait ($P \geq 0.16$). SEM = standard error of mean.

known, and unsaturated fat, such as rapeseed oil, depress CH_4 by the biohydrogenation and decreasing of methanogenic activity in the rumen (Martin *et al.*, 2010). In addition, daily CH_4 emission is closely related to DMI (Oss *et al.*, 2016), which can also explain the reduced CH_4 emissions from cows fed rapeseed oil and agrees well with the results of Bayat *et al.* (2018).

Conclusions

We did not observe reductions in CH_4 emission when partially replacing grass silage with maize silage in dairy cow diets. On the other hand, inclusion of rapeseed oil clearly reduced daily CH_4 emissions, and CH_4 yield and intensity. Including maize silage in the diets reduced yield of milk and milk components, while rapeseed oil only decreased daily milk fat content.

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References

Bayat A.R., Tapio I., Vilkki J., Shingfield K.J. and Leskinen H. (2018) Plant oil supplements reduce methane emissions and improve milk fatty acid composition in dairy cows fed grass silage-based diets without affecting milk yield. *Journal of Dairy Science* 101, 1136-1151.

Beauchemin K.A., Kreuzer M., O'Mara F. and McAllister T.A. (2008) Nutritional management for enteric methane abatement: a review. *Australian Journal of Experimental Agriculture* 48, 21-27.

Brask M., Lund P., Weisbjerg M.R., Hellwing A.L.F., Poulsen M., Larsen M.K. and Hvelplund T. (2013) Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows. *Journal of Dairy Science* 96, 2356-2365

FAO (2003) *World agriculture: towards 2015/2030. An FAO perspective*. FAO, Rome, 97 pp.

Gerber P.J., Steinfel H., Henderson B., Motte A., Opio C., Dijkman J., Falcucci A. and Tempio G. (2013) *Tackling climate change through livestock – a global assessment of emissions and mitigation opportunities*. Food and Agriculture Organization of the United Nations (FAO), Rome, 139 pp.

Hatew B., Podesta S.C., Van Laar H., Pellikaan W.F., Ellis J.L., Dijkstra J. and Bannink A. (2015) Effects of dietary starch content and rate of fermentation on methane production in lactating dairy cows. *Journal of Dairy Science* 98, 486-499.

Howarth R.W. (2019) Ideas and perspectives: is shale gas a major driver of recent increase in global atmospheric methane? *Biogeosciences* 16, 3033-3046.

Martin C., Morgavi D.P. and Doreau M. (2010) Methane mitigation in ruminants: from microbe to the farm scale. *Animal* 4, 351-365.

Oss D.B., Marcondes M.I. Machado F.S., Pereira L.G.R., Tomich T.R., Ribeiro Jr. G.O.R., Chizzotii M.L., Ferreira A.L., Campos M.M., Maurício R.M., Chaves A.V. and McAllister T.A. (2016) An evaluation of the face mask system based on short-term measurements compared with the sulfur hexafluoride (SF6) tracer, and respiration chamber techniques for measuring CH_4 emissions. *Animal Feed Science and Technology* 2016, 49-57.

Schils R.L.M., Olesen J.E., Del Prado A. and Soussana J.F. (2007) A review of farm level modelling approaches for mitigating greenhouse gas emissions from ruminant livestock systems. *Livestock Science* 112, 240-251

Sjaunja L.O., Baevre B., Junkkarinen L., Pedersen J. and Setala J. (1990) A Nordic proposal for an energy corrected milk (ECM) formula. In: *27th Session of the ICRPMA*, pp. 156-157.

Evaluation of NIR technique for the estimation of fibre digestibility in lactating cow diets

Colombini S.[1], Gislon G.[1], Dal Prà A.[2], Rota Graziosi A.[1], Pacchioli M.T.[2] and Rapetti L.[1]
[1]University of Milan, Department of Agricultural and Environmental Science, Via Celoria 2, 20133, Italy; [2]Centro Ricerche Produzioni Animali, Viale Timavo 43/2 Reggio Emilia, 42121, Italy

Abstract

The aim of the present study was to assess the near infrared (NIR) technique for the evaluation of fibre digestibility in lactating cows. The undigested neutral detergent fibre (uNDF) content of faeces and diets obtained from an *in vivo* total tract collection trial was determined by NIR spectroscopy and the values were used to estimate total tract fibre digestibility (TTNDFD). The diets were characterised by their different forage basis: maize silage (MS); wheat silage (WS); lucerne and grass silages (LGS) or hays (LGH). Using the same methodology, total mixed ration and faeces samples were later collected on commercial farms from cows fed diets with different forage: grass silage (GS); wheat silage (WS); sorghum and lucerne in an organic system (ORG). The average values of TTNDFD were 42.8 vs 43.2%, respectively for NIR and *in vivo* data (P=0.784). The TTNDFD was affected by diet with highest values for MS (48.2) and LGS (48.8), intermediate for WS (40.8) and lowest for LGH (34.2) (P<0.05). The regression equation between NIR-TTNDFD and *in vivo*-TTNDFD% was: y=0.898 *in vivo*-TTNDFD + 3.91 (R^2=0.809). In the commercial farms, although the difference was not significant, TTNDFD (%) of cows fed ORG (59.3) was higher than GS (55.0) and WS (53.1).

Keywords: NIR, fibre digestibility, forage, lactating cow

Introduction

Estimation of *in vivo* total-tract nutrient digestibility of neutral detergent fibre (TTNDFD) is important for improving the efficiency of milk production of lactating cows. A positive effect of increased neutral detergent fibre (NDF) digestibility on cow performances was observed (Oba and Allen, 1999). An approach to estimate TTNDFD is based on the use of markers and among these indigestible NDF (iNDF) is commonly used. iNDF is the part of the forage cell wall unavailable to microbial digestion, even if total tract residence time of fibre could be extended to infinite time (Allen and Mertens, 1988). As it is impossible to reach infinite time, an approximation of iNDF can be determined utilising long fermentation time points to determine undigested aNDFom (uNDF) (Raffrenato *et al.*, 2018). The main disadvantage is the time required for the analysis (usually from 240 to 288 h). With this regard, near infrared reflectance spectroscopy (NIR) has the advantage of velocity and inexpensiveness. NIR technology, therefore, enables farmers to obtain results in a short time providing a method that is easily applicable on a commercial scale. Nousiainen *et al.* (2004) showed that NIR technique had a great potential in predicting uNDF of grass silage. Similarly, Brogna *et al.* (2018) showed that faecal uNDF can be well predicted by NIRS.

The aims of the present study were: (1) to evaluate NIR technique for the estimation of TTNDFD in lactating cow diets, in comparison with data collected by *in vivo* total tract collection; (2) to evaluate TTNDFD of cows fed diets with different forage basis in commercial farms.

Materials and methods

In Experiment 1, eight multiparous lactating Holstein cows were used in a replicated 4×4 Latin Square design for TTNDFD determination based on total tract collection. The experimental diets consisted of forage systems identified as: (1) MS, forage system based on maize silage (*Zea mays* L.) (maize silage

49.3% on dietary dry matter (DM)); (2) LGS, forage system based on lucerne (*Medicago sativa* L.) and ryegrass (*Lolium* spp.) silages (lucerne 26.8%, ryegrass 19.1%; on DM); (3) WS, forage system based on wheat silage (*Triticum aestivum* L.) (wheat silage 20.0%; on DM); (4) LGH, forage system for Parmigiano Reggiano PDO cheese production (ryegrass hay 25.3%; lucerne hay 25.3%; on DM). Animals were housed in 4 individual chambers to enable the individual measurement of dry matter intake (DMI) and collection of total faeces. Total mixed ration (TMR), orts and faeces were analysed for fibre concentration.

In Experiment 2, TMR and faeces samples of lactating cows from three commercial dairy farms characterised by a different forage basis were collected once a month during a 4-month period. The diets were characterised as follows: (1) GS, ryegrass silage, as main forage of diet; (2) WS wheat silage, as main forage; (3) ORG, organic diet based on sorghum (*Sorghum bicolor* spp.) and lucerne (silage or green).

Faeces and TMR samples of both experiments were dried in a ventilation oven at 60 °C until constant weight. After drying, the samples were ground at 0.5 mm, using a Fritsch mill (Pulverisette 19, Fritsch). The samples were scanned on Foss NIR System 5000 monochromator in the 1,098-2,500 nm spectral region. The aNDFom and the uNDF contents of faeces and TMR were estimated by NIR equation calibrated as reported by Brogna *et al.* (2018).

The TTNDFD values were estimated as follows:

$$\text{TTNDFD (\%)} = 100 - \{100 \times [(\text{TMR uNDF/faecal uNDF}) \times (\text{faecal aNDFom / TMR aNDFom})]\}$$

Data were statistically analysed by PROC REG and PROC GLM procedures of SAS (ver 9.2). Main effects of diet and method were tested in Exp. 1; the main effect of diet was tested in Exp. 2.

Results and discussion

Experiment 1

The average TTNDFD was not affected by method (P=0.784); the average values were 42.8 vs 43.2%, respectively for NIR and *in vivo* data. The TTNDFD was affected by diet with highest values for MS (48.2) and LGS (48.8), intermediate for WS (40.8) and lowest for LGH (34.2) (P<0.05). The lowest TTNDFD for the diet LGH is probably due to the forage composition of the diet (i.e. only hay); the result is in line with Broderick (1995), who showed higher value of TTNDFD for silage than hay. The regression analysis between TTNDFD obtained by NIR and the total tract collection data is presented in Figure 1. The correlation coefficient (R^2=0.809) shows that NIR can be a suitable method for the *in vivo* determination of TTNDFD.

Experiment 2

Data about DMI, milk production and digestibility of cows fed the different diets are given in Table 1. The ORG system was characterized by lower milk production, DMI and feed efficiency than GS and WS. The NFC dietary content was also lower for ORG than GS and WS; whilst aNDFom had the opposite trend. Although dairy cows require forage NDF in diets for maximum productivity, excess dietary NDF often limits voluntary intake because of physical fill in the rumen (Oba and Allen, 1999) which in turn decreases DMI. Although the difference was not significant (P=0.164), TTNDFD of cows fed ORG was higher than GS and WS; this can be due to a different quality of the forages (for example green lucerne) but also to a higher retention time in the rumen for ORG diet.

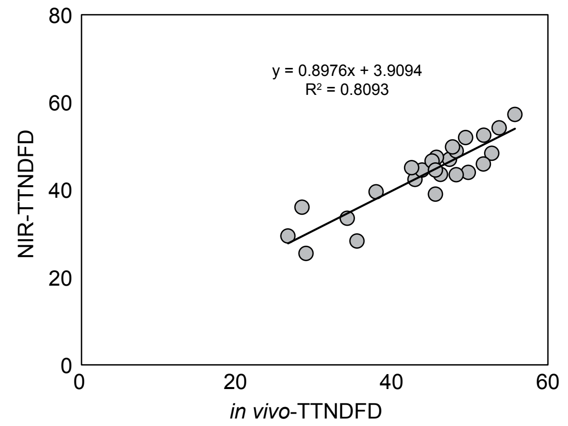

Figure 1. Linear regression between total tract NDF digestibility (%) calculated by *in vivo* total collection (*in vivo*-TTNDFD) and by NIR analysis (NIR-TTNDFD).

Table 1. Milk production, dry matter intake (DMI), diet chemical composition and digestibility of cows fed diets based on different forage basis.[1]

Diet	Milk	DMI	Feed efficiency[2]	Diet crude protein	Diet aNDFom	Diet NFC	TTNDFD[3]
	kg d^{-1}	kg d^{-1}		g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM	%
Grass silage	32.5[a]	23.7[a]	1.37[a]	156	252[b]	474[a]	55.0
Wheat silage	33.5[a]	25.2[a]	1.45[a]	147	282[b]	446[a]	53.1
Organic	20.7[b]	18.1[b]	1.16[b]	134	404[a]	329[b]	59.3
Standard error	0.635	1.52	0.06	7.69	24.1	39.9	2.98
P-value diet	<0.001	0.006	0.030	0.130	<0.001	0.008	0.164

[1] Means in the same column with different superscript differ at $P<0.05$.

[2] Feed efficiency was calculated as milk (kg d^{-1})/DMI (kg d^{-1}).

[3] Total tract neutral detergent fibre (NDF) digestibility was estimated based on the indigestible NDF content of faeces and diets.

Conclusions

The study shows a great potential for NIR to estimate TTNDFD in commercial farms based on the uNDF contents of faeces and TMR. A further validation on a wide data set of samples in commercial farms would be useful to better characterize the potential of NIR technique.

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References

Allen M.S. and Mertens D.R. (1988) Evaluating constraints on fiber digestion by rumen microbes. *Journal of Nutrition* 118, 261-270.

Broderick G.A. (1995) Performance of lactating dairy cows fed either alfalfa silage or alfalfa hay as the sole forage. *Journal of Dairy Science* 78, 320-329.

Brogna N., Palmonari A., Canestrari G., Mammi L., Dal Prà A. and Formigoni A. (2018) Near infrared reflectance spectroscopy to predict fecal indigestible neutral detergent fiber for dairy cows. *Journal of Dairy Science* 101, 1234-1239.

Nousiainen J., Ahvenjärvi S., Rinne M., Hellämäki M. and Huhtanen P. (2004) Prediction of indigestible cell wall fraction of grass silage by near infrared reflectance spectroscopy. *Animal Feed Science and Technology* 115, 295-311.

Oba M. and Allen M.S. (1999) Evaluation of the importance of the digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. *Journal of Dairy Science* 82, 589-596.

Raffrenato E., Ross D.A. and Van Amburgh M.E. (2018) Development of an *in vitro* method to determine rumen undigested aNDFom for use in feed evaluation. *Journal of Dairy Science* 101, 9888-9900.

Effect of silage type and combination with grazing on antioxidant profile of cow milk

De La Torre-Santos S., Royo L.J., Martínez-Fernández A. and Vicente F.
Regional Service for Agri-food Research and Development (SERIDA), P.O. Box 13, 33300 Villaviciosa (Asturias), Spain

Abstract

Milk production must be efficient in the use of natural resources, respecting the environment and offering a better quality of products for an increasingly demanding society. Pastures provide a natural environment for ruminants and improve the animal welfare and milk quality. Legumes are a source of high-quality protein and guarantee less dependence on nitrogen fertilisers, less water usage, and reduced pollution. The cultivation of forage legumes allows producers to reduce costs and protect the environment. The aim of this study was to evaluate the effect of legume inclusion in the diet of dairy cows, with and without grazing, in the North of Spain on milk production and composition. Three total mixed rations including ryegrass, faba bean or pea silages were offered *ad libitum* for nine continuously housed cows or during two hours after each milking for another nine grazing cows. Differences were seen between legumes and ryegrass silages with a better proportion of γ-tocopherol in the pea diet. Grazing cows tend to have higher dry matter intake, greater milk yield and greater proportion of xanthophylls and carotenes than housed cows, but lower milk protein and urea concentration. In conclusion, the effect of grazing was stronger than type of legume.

Keywords: ryegrass, faba bean, pea, grazing, milk yield, antioxidants

Introduction

Faced with the challenge of increasing production, a change in the agricultural sector is needed to meet the growing demand for food while at the same time being more efficient. This requires enhancing the use of natural resources, making a positive contribution to the environment, and offering better quality food, health and well-being to a demanding society (Elgersma *et al.*, 2013). Milk is of great importance for humans, not only for its nutritional characteristics but also with the relevance of other components, such as antioxidants on human health. Fresh forages are an important natural source of antioxidants, vitamins and fatty acids in ruminant diets. Their concentrations in forage have an important function in the composition and quality of milk and dairy products because some of them, such as tocopherols and carotenoids can be directly transferred into milk (Elgersma *et al.*, 2015). Higher levels of antioxidants (α-tocopherol, β-carotene and retinol) have been reported in milk from cows that consume fresh grass compared with diets rich in concentrate or silage (Agabriel *et al.*, 2007; Havemose *et al.*, 2004). Milk fat antioxidants have an important role in the maintaining the pro-oxidant/antioxidant balance in the human body (Grażyna *et al.*, 2017). The objective of the present study was to evaluate the effect of the inclusion in the diet for dairy cows, with and without grazing, of legumes that are increasing in their presence in the North of Spain, on milk production and composition.

Materials and methods

Two trials were carried out in parallel in a cross-over design, with three periods of 19 days each, which were distributed in 13 days of adaptation and six days of sampling and measurements. Eighteen Holstein dairy cows in the second half of lactation were selected and randomly distributed into two groups, and subdivided into three subgroups of three cows each. Experimental treatments consisted total mixed rations (TMR) with Italian ryegrass (*Lolium multiflorum* Lam.), faba bean (*Vicia faba* L.) or pea (*Pisum* sativum L.) silages, all offered *ad libitum* indoors or with limited access for two hours after each milking, and grazing the rest of the day. Grazing was carried out in paddocks with rotational management. During the assay period, paddocks were assigned to the corresponding group of animals taking into account the availability of forage for them. All TMRs were sampled daily. Paddocks were sampled on the first day in each sampling period. Dry matter intake of TMR was recorded daily. Herbage intakes on pasture were estimated following the animal performance method (Macoon *et al.,* 2003). Milk production was also recorded and sampled daily on both milking sessions (morning and afternoon), and pooled proportionally according to the milk produced. Milk samples were analysed by a MilkoScan FT6000. Carotenoids and vitamins E and A in the milk were determined according to the methodology proposed by Gentili *et al.* (2013). All results were analysed by ANOVA using R statistical package (R Core Team, 2017) with type of management and period as fixed effects.

Results and discussion

Table 1 shows the results of feeding take, milk production and composition. Total dry matter intake was slightly higher in treatments that included grazing (P<0.1). Milk yield and fat content were not affected by the different treatments, although with grazing a tendency to greater milk production was observed (30.1 kg d^{-1} vs 28.2 kg d^{-1} with and without grazing respectively, P<0.1). However, protein and urea contents decreased with grazing (P<0.001). Likewise, with the inclusion of legume silage the concentration of protein decreased (P<0.01) and the urea increased (P<0.001) in milk, especially in housed animals.

The content of fat-soluble antioxidants according to feeding system is shown in Table 2. No significant differences were observed in vitamin A due to type of feeding system. However, significant differences were observed in vitamin E due to effect of grazing. Milk from grazing cows had higher concentration of lutein and β-cryptoxanthin than cows feeding just silages (P<0.01). Although there were no differences in the concentration of carotenes among treatments, a higher proportion was observed in the grazing cows. Different studies have reported that the content of β-carotene and fat-soluble vitamins were as much as fourfold higher in grazing cows than in the milk of cows with TMR feeding or high proportions of concentrate (Butler *et al.*, 2008; Nozière *et al.*, 2006), and quality that remained constant throughout the study.

Conclusions

Grazing dairy cows tend to have a higher dry matter intake and a greater milk production, and they have lower protein concentration as well as lower urea content in milk than dairy cows eating indoors. Lutein and β-cryptoxanthin concentrations were higher in milk from grazing dairy cows without influence of silage type.

Table 1. Dry matter intake (DMI), milk yield and milk composition according to the feeding system.

	Non-grazing			Grazing			RSD	Statistical significance		
	RG	FB	P	RG	FB	P		S	G	S×G
DMI (kg day^{-1})	21.4	22.8	22.9	24.0	28.3	23.0	5.73	NS	●	NS
Milk (kgday^{-1})	28.3	28.5	27.9	30.2	30.1	30.1	4.16	NS	●	NS
Fat (g kg^{-1})	41.4	43.6	43.1	41.4	40.7	41.7	3.83	NS	NS	NS
Protein (g kg^{-1})	37.6^{a}	36.3^{ab}	36.1^{abc}	35.2^{bc}	34.6^{c}	35.4^{bc}	1.56	**	***	NS
Lactose (g kg^{-1})	47.5	47.4	47.8	47.8	48.3	47.6	1.42	NS	NS	NS
Solid non-fat (g kg^{-1})	93.2^{a}	91.6^{ab}	91.7^{ab}	90.7^{b}	90.6^{b}	90.7^{b}	1.76	*	**	NS
Urea (mg kg^{-1})	253^{bcd}	272^{bc}	325^{a}	234^{d}	238^{cd}	278^{b}	33.4	***	***	NS

Ryegrass (RG), Faba bean (FB), Pea (P), Diet (D), Grazing (G), relative standard deviation (RSD). Statistical significance: NS P>0.05; ● P<0.1; * P<0.05; ** P<0.01; *** P<0.001. Figures with different letters in the same row are significantly different.

Table 2. Fat-soluble antioxidants composition (μg l^{-1} milk) according to the feeding system.

	Non-grazing			Grazing			RSD	Statistical significance		
	RG	FB	PB	RG	FB	PB		S	G	S×G
Retinol	852	747	893	771	721	827	233.3	NS	NS	NS
α-Tocopherol	1,005	1,171	1,181	1,372	1,412	1,645	303	NS	*	NS
γ-Tocopherol	89.6^{bc}	99.0^{ab}	128.7^{a}	59.5^{d}	66.9^{cd}	61.4^{cd}	16.3	**	***	NS
Lutein	7.67^{b}	11.09^{b}	8.53^{b}	25.72^{a}	30.39^{a}	21.48^{ab}	7.449	*	***	NS
Zeaxanthin	0.71	0.76	0.57	1.84	1.67	1.76	0.795	NS	*	NS
β-Cryptoxanthin	1.83^{b}	1.51^{b}	1.39^{b}	3.09^{a}	3.55^{a}	3.33^{a}	0.583	**	***	NS
All-trans-β-Carotene	149	150	119	218	242	245	69.5	NS	*	NS
9-cis-β-Carotene	0.92^{b}	1.08^{b}	0.57^{b}	1.35^{ab}	2.03^{ab}	2.72^{a}	0.831	●	*	NS
13-cis-β-Carotene	3.30	3.90	2.38	6.22	6.75	7.55	2.635	NS	*	NS

Ryegrass (RG), Faba bean (FB), Pea (P), Diet (D), Grazing (G), relative standard deviation (RSD). Statistical significance: NS P>0.05; ● P<0.1; * P<0.05; ** P<0.01; *** P<0.001. Figures with different letters in the same row are significantly different.

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References

Agabriel C., Cornu A., Journal C., Sibra C., Grolier P. and Martin B. (2007) Tanker milk variability according to farm feeding practices: vitamins A and E, carotenoids, color, and terpenoids. *Journal of Dairy Science* 90, 4774-4896.

Butler G., Nielsen J.H., Slots T., Seal C., Eyre M.D., Sanderson R. and Leifert C. (2008) Fatty acid and fat soluble antioxidant concentrations in milk from high-and low-input conventional and organic systems: seasonal variation. *Journal of the Science of Food and Agriculture* 88, 1431-1441.

Elgersma A., Søegaard K. and Jensen S.K. (2013) Fatty acids, α-tocopherol, β-carotene, and lutein contents in forage legumes, forbs,and a grass-clover mixture. *Journal of Agricultural and Food Chemistry* 61, 11913-11920.

Elgersma A., Søegaard K. and Jensen S.K. (2015) Interrelations between herbage yield, α-tocopherol, β-carotene, lutein, protein, and fiber in non-leguminous forbs, forage legumes, and a grass-clover mixture as affected by harvest date. *Journal of Agricultural and Food Chemistry* 63, 406-414.

Grażyna C., Hanna C., Adam A. and Magdalena B.M. (2017) Natural antioxidants in milk and dairy products. *International Journal of Dairy Technology* 70, 165-178.

Gentili A., Caretti F., Bellante S., Ventura S., Canepari S. and Curini R. (2013) Comprehensive profiling of carotenoids and fat-soluble vitamins in milk from different animal species by LC-DAD-MS/MS hyphenation. *Journal of Agricultural and Food Chemistry* 61, 1628-1639.

Havemose M.S., Weisbjerg M.R., Bredie W.L.P. and Nielsen J.H. (2004) Influence of feeding different types of roughage on the oxidative stability of milk. *International Dairy Journal* 14, 563-570.

Nozière P., Graulet B., Lucas A., Martin B., Grolier P. and Doreau M. (2006) Carotenoids for ruminants: from forages to dairy products. *Animal Feed Science and Technology* 131, 418-450.

Macoon B., Sollenberger L.E., Moore J.E., Staples C.R., Fike J.H. and Portier K.M. (2003) Comparison of three techniques for estimating the forage intake of lactating dairy cows on pasture. *Journal of Animal Science* 81, 2357-2366.

R Core Team (2017) *R: a language and environment for statistical computing*. R Foundation for Statistical computing, Vienna, Austria.

Effect of cow type and feeding strategy on grazing time in simplified rotational grazing systems

Delaby L.[1], Launay F.[2], Toutain A.[2], Dodin P.[2] and Delagarde R.[1]
[1]*INRA, Agrocampus Ouest, UMR Pegase, 16 Le Clos 35590 Saint Gilles, France;* [2]*INRA, Domaine Expérimental du Pin, Borculo, Exmes, 61310 Gouffern en Auge, France*

Abstract

Grazing time (GT) is one component of grass intake and varies with animal and sward characteristics. The objective of this experiment was to describe the effect of breed (Holstein vs Normande) and parity (primiparous vs multiparous) on grazing time within simplified rotational grazing systems. Two groups of cows corresponding to two long-term feeding strategies grazed separately. One group (High – H) received 4 kg d^{-1} of concentrate while the other (Low – L) received no supplement. Daily grazing time was measured with Lifecorder technology on 30 dairy cows (15 H and 15 L) during 5 grazing sequences (2 H and 3 L) varying between 7 and 13 days of paddock residence depending on grass availability. On average, the daily paddock access time was 1,155 min and grazing time was 530 min. Parity had no significant effect on GT. Holstein cows grazed for an additional 27 min compared with Normande cows. Grazing time was reduced with concentrate allocation, by 10 min for each 1 kg of concentrate intake. Within a paddock, the GT varied with a regular increase during the residency period. This experiment confirms the large variation in GT between cows and also with the grazing conditions.

Keywords: dairy cow, grazing, grazing time

Introduction

The development of automatic devices capable of recording animal movements offers opportunities to better understand animal behaviour notably at grazing. Grazing time (GT) is one component of grass intake and varies with animal and sward characteristics (Bargo *et al.*, 2003). Grazing time is sometimes presented and debated as a potential indicator of good health, welfare quality, and also an opportunity to evaluate intake. The main objective of this paper is to describe the variation in GT between different types of dairy cows managed within a simplified rotational grazing system characterised by long-term residency and large variation in grass availability during the grazing sequence.

Materials and methods

The grazing time measurements were undertaken in spring 2019 within a long-term experiment named 'The cow for the system' at the INRA experimental farm of Le Pin-au-Haras, Normandy (48.44°N; 0.09° E). The objective of the long-term experiment is to compare the performance of different types of cows in two feeding systems (FS) with a compact calving period in winter. The different types of cows (n=70) balanced in the two FS are the consequence of breed (Holstein – Ho vs Normande – No), of parity (primiparous vs multiparous), and of genetic types (high genetic merit for milk yield or for fat and protein content).

During the grazing season, the low (L) FS was based on grass only with a global stocking rate (SR) of 1.8 cows ha^{-1} while in the high (H) FS, with a SR of 3.0 cows ha^{-1}, the cows received 4 kg of concentrate daily and forage supplements as necessary in summer. At grazing, the two groups (H and L FS) were managed on separate platforms applying the principle of a simplified rotational grazing system, as described by Hoden *et al.* (1991). This grazing system is based on three large paddocks (2.4 ha each) grazed in spring and extended to 5 or 6 paddocks in summer and autumn. The main characteristic of this grazing system is the long residence time in a paddock, varying between 8 to 14 days depending on pre grazing sward height (PreGH) and the paddock area. The pastures are composed of perennial ryegrass and white clover, with few other species. The annual level of N mineral fertilisation applied was fixed at 130 and 200 kg ha^{-1}, respectively, for the L and H systems.

At turnout (mid-March), 30 cows (15 Ho and 15 No with 5 and 10 primiparous and multiparous per breed) were selected in the both FS (15 H and 15 L) and equipped with Lifecorder devices (LCP, Suzuken Co. Ltd., Nagoya, Japan) fixed on a collar and placed around the cows neck. The Lifecorder technology is based on a uniaxial accelerometer that records physical activity level (range 1-9) for each 2 min period. Lifecorder has been successfully used and validated to record individual grazing time and nycthemeral pattern (Delagarde and Lamberton, 2015).

During the grazing season, GT was measured during 2 H and 3 L grazing rotations of duration between 7 to 13 days, in May, June and July. No forage supplement was fed to the H group during these grazing rotations. The two groups grazed day and night except during the two daily milking times. Individual milk yield (MY) was measured at every milking and cows were weighed once weekly (Table 1).

Finally, 704 records were validated. The mixed model applied on SAS 9.4 release software on GT and MY takes into account the parity (n=2), the breed (n=2), FS (n=2), the day in the paddock (n=7 to 13) and the grazing rotation (n=5) within FS. Cows were considered as random effect.

Results and discussion

The daily access time to grazing was on average 1,155 min per day and the mean grazing time was 530 min per day, with a minimum and maximum of, respectively, 242 and 722 min. On average GT represented 46% of the access time. Only 5% of the records were less than 400 min and 17% were greater than 600 min per day.

The GT did not differ between primiparous and multiparous cows (540 min, P=0.81) and was significantly lower by 24 min for the No (523 min) compared to the Ho cows (557 min, Table 2). The cows in the H FS, receiving 4 kg of concentrate per cow per day, grazed less per day (519 min) than those in the Low FS (561 min). As expected, the average milk yield produced during the experimental period was significantly greater for the multiparous cows (+6.9 kg), for the Ho breed (+4.3 kg) and in the H FS (+6.0 kg; Table 2). These results are consistent with the values reported in the literature (Pérez-Prieto

Table 1. Pasture characteristics during the 5 rotations of grazing time measurements.[1]

Feeding	High	High	Low	Low	Low
Date in 2019	16 to 28 May	8 to 17 June	15 to 26 May	9 to 18 June	8 to 14 July
PreGH (cm)	15.4	12.5	13. 2	11.6	10.0
PostGH (cm)	6.3	5.3	5.4	4.8	4.8

[1] PreGH = pre grazing sward height; PostGH = post grazing sward height.

Table 2. Adjusted means of grazing time and daily milk yield of different type of cows according to the breed, the parity or the feeding system.[1]

	Breed		Parity		Feeding system		RSE	Breed effect	Parity effect	FS effect
	Ho	No	Pp	Mp	High	Low				
Grazing time (min)	557	523	538	542	519	561	45.3	0.013	0.810	0.002
Milk yield (kg day^{-1})	23.0	18.7	17.4	24.3	23.8	17.8	1.75	0.001	0.001	0.001

[1] Ho = Holstein; No = Normande; Pp = primiparous; Mp = multiparous; FS = feeding system; RSE = residual standard error.

and Delagarde, 2013). Notably, the negative effect of concentrate on GT (Bargo *et al*, 2003; Sheahan *et al*, 2011) induces a reduction of 10 min GT per kg of concentrate. To better explain the variation in GT between cows, the individual MY and BW were introduced into the mixed model but were not significant (P>0.10).

Grazing time and milk yield are sensitive to the day in the paddock (Figure 1). This reflects the grass availability and quality declining during the residence time, as previously described by Hoden *et al.* (1991). The GT is lowest on the second day in the paddock (450 min) and increases each day thereafter to reach 550 min between days 6 to 10 and 600 min on days 12 and 13. The milk yield profile is a consequence of the daily decline in grass dry matter intake (DMI) despite GT increasing. The dairy cows try to compensate for the reduction in intake rate by increasing GT but this is insufficient to maintain grass DMI and milk yield drops (Delagarde *et al*, 2010).

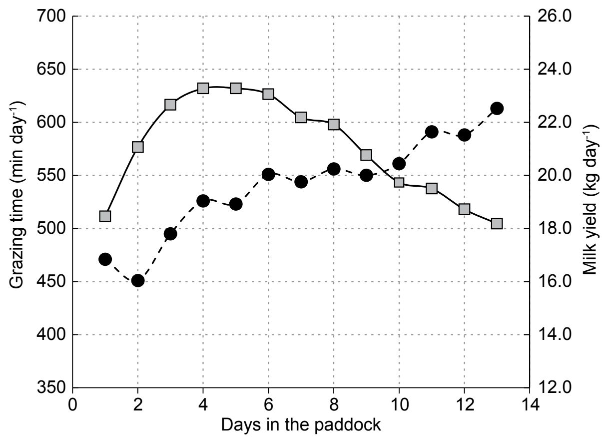

Figure 1. Daily variation of grazing time (●) and milk yield (■) during the grazing sequence in simplified rotational grazing systems.

Conclusions

Dairy cow GT was highly variable, partially dependent of the animal characteristics but also sensitive to the grazing system and the level of concentrate supplementation. The GT associated with the intake rate is one of the adaptation keys used by the animal to try to maintain the level of DM intake when the level of grass allowance declines as observed during the grazing residency in a paddock.

References

Bargo F., Muller L.D., Kolver E.S. and Delahoy J.E (2003) Production and digestion of supplemented dairy cows on pasture. *Journal of Dairy Science* 86, 1-42.

Delagarde R. and Lamberton P. (2015) Daily grazing time of dairy cows is recorded accurately using the Lifecorder Plus device. *Applied Animal Behaviour Science* 165, 25-32.

Delagarde R., Peyraud J.L. and Wade M. (2010) Daily pattern of feeding activities of dairy cows in an 8-d rotational grazing system. *Grassland Science in Europe* 15, 931-933.

Hoden A., Peyraud J.L., Muller A., Delaby L. and Faverdin P. (1991) Simplified rotational grazing management of dairy cows: effects of rates of stocking and concentrate. *Journal of Agricultural Science, Cambridge* 116, 417-428.

Pérez Prieto L.A and Delagarde R. (2015) Meta-analysis of the effect of pasture allowance on pasture intake, milk production, and grazing behavior of dairy cows grazing temperate grasslands. *Journal of Dairy Science* 96, 6671-6689.

SAS Institute Inc. (2013). Base SAS 9.4®. SAS User's Guide: Statistics. Cary, NC: SAS Institute Inc.

Sheahan A.J., Kolver E.S. and Roche J.R. (2011) Genetic strain and diet effects on grazing behavior, pasture intake, and milk production. *Journal of Dairy Science* 94, 3583-3591.

High soluble carbohydrate concentration may increase hay intake, digestibility and milk production in dairy cows

Deroche B.[1,2], Bouchon M.[3], Pomiès D.[1], Martin B.[1], Renaud J.P.[4], Aoun M.[2] and Baumont R.[1]
[1] *Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, 63122 Saint-Genès-Champanelle, France;* [2] *IDENA, 44880 Sautron, France;* [3] *INRAE, UE Herbipôle, 63122 Saint-Genès-Champanelle, France;* [4] *PHILICOT, 71150 Chagny, France*

Abstract

The total soluble carbohydrate (TSC) content in the diet may affect ruminant performance. In this study we aimed to investigate the effect of contrasted TSC content in hay on intake, diet digestibility and milk production in dairy cows. Two hays with contrasted TSC contents (H+: 168 vs H–: 113 g kg^{-1} DM) were fed *ad libitum*. In addition, regrowth hay was offered at a fixed amount of 3 kg day^{-1}. Two concentrates (rapidly degradable starch: RS, vs slowly degradable starch: SS) were used to supplement the hay diets with 5 to 7 kg day^{-1} depending on individual milk production level. The four experimental diets (H+ vs H– crossed with RS vs SS) were fed during 9 weeks to four groups of 14 dairy cows in mid-lactation. Cows fed with H+ hay ate more of the total diet (+0.6 kg day^{-1}; P=0.049) with a higher digestibility (+1.8 points of digestibility; P<0.001), allowing an increase in milk yield of +1.4 kg day^{-1} (P<0.001). Intake and production was not affected by the nature of concentrate and no significant interaction was observed between type of hay and concentrate. Hays with high TSC content have higher digestibility, improving intake and performance of dairy cows.

Keywords: soluble carbohydrates, milk yield, dry matter intake, digestibility, hay

Introduction

Depending on the chemical composition of the forage, a higher total soluble carbohydrate (TSC) content may increase the milk yield of dairy cows (Ellis *et al.*, 2011). Generally, TSC content is not used in feed value prediction models of forages, but its increase in forages could be linked to higher intake and digestibility (Fisher *et al.*, 1999). In this study, we aimed to investigate the effect of contrasted TSC contents in the forage part of the diet crossed with different nature of starch supplementation on intake, diet digestibility and milk yield of dairy cows.

Materials and methods

We used two hays, with contrasting TSC contents (H+: 168 g kg^{-1} dry matter (DM) vs H–: 113 g kg^{-1} DM; Table 1), harvested during the first vegetation cycle on two permanent grasslands, and field dried. The H+ hay was harvested in 2017 at heading stage on grassland composed of 90% grasses and 10% legumes and forbs. The H– hay was harvested in 2018 at flowering stage on grassland composed of 74% grasses, 15% forbs and 11% legumes. The two hays were fed *ad libitum* to dairy cows. In addition, cows had free access to a maximum amount of 3 kg day^{-1} of leguminous regrowth hay (crude protein (CP) = 114; neutral detergent fibre (NDF) = 545; acid detergent fibre (ADF) = 342 and TSC = 142 g kg^{-1} DM). Two concentrates, differing in the rate of starch degradability in the rumen (RS: rapidly degradable starch including 40% of wheat, vs SS: slowly degradable starch, including 40% of maize), were fed at between 5 and 7 kg day^{-1} depending on individual milk yield. The concentrates were formulated to be iso-proteic (CP = 260 g kg^{-1} DM) and iso-energetic (NDF = 157 and starch = 306 g kg^{-1} DM).

Fifty-six dairy cows, in mid-lactation (including 28% of primiparous), were allocated into four groups according to similar milk yield, dry matter intake, body condition score (1.66±0.35 on 0-5 scale) and body weight (665±59 kg). After a 3-week control period on a same diet (hay *ad libitum*, 3 kg d^{-1} of

Table 1. Chemical composition and predicted organic matter digestibility of the two hays.[1]

Feed measurements	H+	H–	SEM	*P*-value
Crude protein (g kg^{-1} dry matter (DM))	87.9	102	3.546	0.117
Neutral detergent fibre (g kg^{-1} DM)	588	628	8.653	0.080
Acid detergent fibre (g kg^{-1} DM)	303	346	5.287	0.016
Total soluble carbohydrate (g kg^{-1} DM)	168	113	3.915	0.002
Predicted organic matter digestibility (%)	63.3	58.8	1.398	0.017

[1] H+ = hay rich in total soluble carbohydrate; H– = hay poor in total soluble carbohydrate; DM = dry matter; SEM = standard error of the mean; Organic matter digestibility predicted according to INRA (2018).

regrowth hay, 6 kg d^{-1} of concentrate per cow) and a 2-week transition period, the four experimental diets (H+ vs H– crossed with RS vs SS) were fed during a 9-week experimental period to the four groups of 14 cows.

Milk yield and DM intake (DMI) of the hays and concentrates were measured individually during each week of the experimental period. According to Peyraud (1998), the digestibility of the whole diet was estimated by the nitrogen content measured in faeces collected from each cow during the 1st, 6th and 9th week of the experimental period. The results were analysed according to a Mixed model (SAS 5.1) with last week of the control period as a covariate, week of the experimental period as a repeated effect, and type of hay (H+ vs H–), type of concentrate (RS vs SS) and their interaction as fixed effects.

Results and discussion

The TSC content of the hay H+ was significantly higher than the hay H– (+55 g kg^{-1}; P=0.002), and this could explain the lower content of NDF (-40 g kg^{-1}; P=0.080) and ADF (-43 g kg^{-1}; P=0.016) (Table 1), by a substitution effect between soluble and structural carbohydrates (Fisher *et al.*, 1999).

Milk yield (+1.4 kg day^{-1}; P<0.001) and total dry matter intake (+0.6 kg day^{-1}; P=0.049) were significantly increased when cows were fed H+ diets, without any effect of the nature of concentrate (Table 2). The increase in DMI was due to a higher intake of H+ hay (+2.1 kg day^{-1}; P<0.001), even if cows fed H+ hay ate less regrowth hay than cows fed with H– hay (-1.43 kg day^{-1}; P<0.001). Cows fed H+ diets preferred to maximise the intake of H+ rather than of regrowth hay, although the latter had a higher CP and lower NDF content and a similar predicted organic matter digestibility (63.0%). This is in accordance with the preference shown by ruminants for forages richer in TSC (Fisher *et al.*, 1999). Finally, the digestibility of the H+ diets was higher (+1.8 points of digestibility; P<0.001), than that of

Table 2. Milk yield, intake and diet digestibility of dairy cows fed hay and concentrate.[1]

Animal measurements	H+		H–		SEM			*P*-value		
	SS	RS	SS	RS	Hay	C	Hay×C	Hay	C	Hay×C
Milk yield (kg day^{-1})	21.2	21.0	19.5	19.9	0.278	0.276	0.390	<0.001	0.778	0.380
Total DMI (kg day^{-1})	20.0	20.9	19.9	19.9	0.220	0.222	0.303	0.049	0.188	0.126
Hay DMI (kg day^{-1})	14.5	15.2	12.9	12.7	0.191	0.189	0.264	<0.001	0.289	0.072
Regrowth hay DMI (kg day^{-1})	0.79	0.75	2.12	2.25	0.052	0.053	0.074	<0.001	0.475	0.231
Concentrate DMI (kg day^{-1})	4.79	4.79	4.73	4.77	0.141	0.136	0.192	0.823	0.912	0.877
Diet digestibility (%)	75.3	75.1	73.6	73.1	0.240	0.239	0.337	<0.001	0.080	0.375

[1] H+ = hay rich in total soluble carbohydrate; H– = hay poor in total soluble carbohydrate; SEM = standard error of the mean; SS = slowly degradable starch concentrate; RS = rapidly degradable starch concentrate; C = concentrate; DMI = dry matter intake; estimated by nitrogen content in faeces.

H– diets. According to the INRA feeding system for ruminants (INRA, 2018), it can be estimated that cows fed with H+ diets received 1964 kcal day^{-1} more net energy than the cows fed H- diets, which can explain the difference in milk yield.

Conclusions

This study has provided evidence to show that high content of TSC in hay increases the organic matter digestibility and animals' preference for the forage. It resulted in higher voluntary intake and higher digestibility of the diet, and led to an increase in the milk yield of dairy cows.

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References

Ellis J.L., Dijkstra J., Bannink A., Parsons A.J., Rasmussen S., Edwards G.R., Kebreab E. and France J. (2011) The effect of high-sugar grass on predicted nitrogen excretion and milk yield simulated using a dynamic model. *Journal of Dairy Science* 94, 3105-3118.

Fisher D.S., Mayland H.F. and Burns J.C. (1999) Variation in ruminants' preference for tall fescue hays cut either at sundown or at sunup. *Journal of Animal Science* 77, 762-768.

INRA (2018) *INRA feeding system for ruminants*. Wageningen Academic Publishers, Wageningen, the Netherlands, 640 pp.

Peyraud J.L. (1998) Techniques for measuring faecal flow, digestibility and intake of herbage in grazing ruminants. In: *Proceedings of the IXth European Intake Workshop*, North Wyke, UK., pp. 39-43.

Effect of post-grazing sward height, finishing diet and sire breed on performance of suckler steers

Doyle P.[1,2], McGee M.[1], Moloney A.P.[1], Kelly A.K.[2] and O'Riordan E.G.[1]
[1]*Teagasc, Animal & Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland;*
[2]*University College Dublin, Belfield, Dublin 4, Ireland*

Abstract

The effects of sire breed maturity (early- (EM) or late- (LM)), post-grazing sward height (PGSH, 4 or 6 cm) and indoor finishing diet (grass silage only (SO) or grass silage + 3.8 kg concentrate dry matter head^{-1} daily (SC)) on performance of weaned, spring-born suckler-bred steers (n=72) slaughtered at 24 months of age were examined. After the 'first' indoor-winter, steers were rotationally grazed in 12 groups of 6 for 196 days; pre-grazing herbage mass offered was similar for each treatment. Steers were then finished indoors for 131 days. There were no interactions between treatments. At pasture, EM had higher (P<0.01) live-weight gain (LWG) than LM and steers grazing to 6 cm had higher (P<0.01) LWG than those grazing to 4 cm. During finishing, LWG was greater (P=0.08) for 4 than 6 cm PGSH and greater (P<0.001) for SC than SO. Carcass weight did not differ between breed maturities, was heavier for 6 than 4 cm PGSH (P=0.07), and for SC than SO (P<0.001). In conclusion, grazing to a higher PGSH and consuming concentrates during the finishing period enhanced growth of both EM and LM steers.

Keywords: beef cattle, breed maturity, concentrate supplementation, grass-fed beef, grazing, residual sward height

Introduction

In temperate climates grazed pasture tends to be the cheapest feed source for ruminants (Finneran *et al.*, 2012). Increasing liveweight gain (LWG) at pasture can thus reduce production costs within grass-based beef systems. The effect of post-grazing sward height (PGSH) on LWG of beef cattle at pasture is unclear; cattle grazing to higher PGSH had greater (5.0 vs 3.5 cm; O'Riordan *et al.*, 2011) or similar (5.6 vs 4.4 cm; Minchin and McGee, 2010) LWG under rotational stocking systems. Additionally, there is limited information on the residual effects of PGSH on animal performance following housing at the end of the grazing season. Compared to late-maturing (LM) breed types, early-maturing (EM) genotypes may be more suitable to grass-based systems due to their propensity for greater fat deposition at a younger age, and indeed may be more suitable to 'finishing' on forage-only diets (Regan *et al.,* 2018). There is increasing interest in omitting concentrates from the diet of cattle because of the perceived superior healthiness and environmental acceptability of 100% grass-fed beef products (Monahan *et al.*, 2018). Within this context, the objective of this study was to evaluate the performance, growth and carcass characteristics of suckler-bred EM and LM steers on contrasting PGSH during their second grazing season, followed by a finishing period during the second winter of grass silage only (SO) or grass silage + 3.8 kg concentrate dry matter (DM) head^{-1} daily (SC).

Materials and methods

This study was conducted at Teagasc, Grange Research Centre between October 2017 and March 2019. Spring born, EM (Aberdeen Angus and Hereford, n=36) and LM (Limousin and Charolais, n=36) sired, weaned suckler-bred bulls out of LM cross dams were sourced at ca. 8 months of age from commercial farms and castrated 3 weeks post-arrival. Within sire breed and descending live-weight, steers were assigned to a two (sire breed maturity: EM and LM) × two (PGSH: 4 or 6 cm) factorial arrangement of treatments. During the 'first' winter steers were penned in groups of 6 in a concrete slatted floor shed and offered grass silage *ad libitum* (*in vitro* DM digestibility (DMD), 739 g kg^{-1}). Steers were turned out to pasture on 19 April. Within each PGSH, there were 3 groups of steers per sire breed maturity, resulting in 12 grazing groups, each containing 6 cattle; grazing group was the experimental unit. Steers rotationally grazed the same pre-grazing herbage mass on *Lolium perenne*-dominant swards to their assigned PGSH in breed-specific, replicate grazing groups, for 196 days. At the end of the grazing season (1 November), steers were housed for the 'second' winter. Within grazing group, steers were assigned at random to two winter finishing diets (grass silage – 756 g kg^{-1} DMD – *ad libitum* or grass silage *ad libitum* supplemented with 3.8 kg DM of a barley-based concentrate) offered individually using a Calan gate feeding system, in accordance with a split-plot design. Animals were slaughtered in a commercial abattoir after 131 days. Liveweight was measured pre- and post- turn-out to pasture, pre- and post-housing, at slaughter and every 2 weeks in between. At pasture, pre- and post-grazing sward height (rising plate meter), herbage mass (lawnmower cuts) and estimated herbage DM intake using the disappearance method were measured, and representative samples of herbage were collected for DM determination and herbage nutritive analysis as described by Lawrence *et al.* (2012). Pre-grazing herbage mass was 2,157 and 2,081 kg DM ha^{-1} for 4 and 6 cm, respectively. Post-slaughter carcasses were graded mechanically for conformation and fat scores (EU beef carcass classification) on a continuous 15-point scale.

Data from the grazing season were statistically analysed using the GLM procedure of Statistical Analysis Software (SAS). The statistical model contained fixed effects for sire breed maturity, PGSH and their interactions. Data pertaining to the 'second' winter and post-slaughter were analysed using the MIXED procedure of SAS. The experimental unit was the sub-group within the previous grazing group. The statistical model contained sire breed maturity, PGSH and finishing diet and their interactions as fixed effects, and the interaction between grazing group, sire breed maturity and PGSH as a random effect. Differences between means were tested for significance using the PDIFF statement and adjusted for Tukey, as appropriate. Data were considered statistically significant when P<0.05 and considered a tendency towards statistical significance when P<0.10.

Results and discussion

Mean (standard deviation) (n=36) PGSH during the grazing season was 4.15 (0.47) and 5.80 (0.55) cm for 4 and 6 cm PGSH, respectively. Corresponding herbage organic matter digestibility (above the assigned PGSH) was 754 and 773 g kg^{-1} DM. There were no 3-way interactions for breed maturity, PGSH and finishing diet for feed intake, animal growth and carcass traits (Table 1); however, there were breed maturity × PGSH interactions for slaughter weight and fat score (Table 1). The EM steers had a higher intake at pasture (P<0.05) and LWG (P<0.01) over the grazing season and were heavier (P=0.10) than LM steers at housing prior to the second winter. The 6 cm PGSH steers had a higher (P<0.05) intake at pasture, and in accord with the findings of O'Riordan *et al.* (2011) had higher (P<0.01) pasture LWG and higher (P<0.001) housing weight at the end of the grazing season compared to the 4 cm PGSH steers. During the 'second' winter, total DM intake was higher for EM compared to LM (P<0.001) and for SC compared to SO (P<0.001) with no difference in DM intake between PGSH. Liveweight gain during the 'second' winter was higher (P=0.08) for 4 than 6 cm PGSH suggesting compensatory growth, and for SC than SO (P<0.001) with no difference between sire breed maturity. Feed conversion ratio was better for 4 than 6 cm PGSH (P=0.08) and for SC than SO (P<0.05) with no difference between sire breed maturity. Carcass weight was 11 kg heavier (P=0.07) for 6 than 4 cm PGSH, and 37 kg heavier (P<0.001) for SC than SO, and in contrast to previous findings (Regan *et al.,* 2018) did not differ (P>0.05) between sire breed maturity. Fat score and conformation score did not differ (P>0.05) between PGSH. Consistent with Regan *et al.* (2018) both fat and conformation score were significantly higher for SC compared to SO, and fat score was higher and conformation score was lower for EM than LM.

Table 1. Effect of sire breed maturity (SBM – early- (EM) and late- (LM)), post-grazing sward height (PGSH) and finishing diet (FD – grass silage only (SO) and grass silage supplemented with 3.8 kg dry matter (DM) concentrates (SC)) on average daily gain (ADG), slaughter weight and carcass traits of suckler-bred steers.[1]

	SBM		PGSH		FD		SEM	Significance[3]		
	EM	LM	4 cm	6 cm	SO	SC		SBM	PGSH	FD
Daily DM intake (kg head^{-1})										
Grazed herbage	6.3	5.9	6.0	6.3			0.08	*	*	
Silage, 2nd winter	7.5	7.0	7.3	7.2	8.1	6.4	0.07	***	NS	***
Total, 2nd winter	9.4	8.9	9.2	9.1	8.1	10.2	0.07	***	NS	***
ADG: Grazing season (kg)	0.95	0.85	0.85	0.95			0.020	**	**	
ADG: 2nd winter (kg)	0.92	0.85	0.92	0.84	0.73	1.04	0.028	NS	0.08	***
FCR	10.8	11.1	10.5	11.3	11.7	10.1	0.29	NS	0.08	*
Housing weight (kg)	561	553	545	569	555	559	3.3	0.10	***	***
Slaughter weight (kg)[2]	670	653	655	669	641	682	5.5	*	*	***
Carcass weight (kg)	367	376	366	377	353	390	3.4	NS	0.07	***
Kill-out proportion (g kg^{-1})	548	577	560	564	552	573	2.7	***	NS	***
Conformation, 1-15	7.1	8.6	7.9	7.8	7.2	8.5	0.20	***	NS	**
Fat[2], 1-15	8.5	6.8	7.6	7.7	6.8	8.5	0.21	***	NS	***

[1] FCR = feed conversion ratio (kg DM intake kg^{-1} live-weight gain); SEM = standard error of the mean; DM = dry matter.

[2] SBM × PGSH interactions for slaughter weight ($P<0.05$) and fat score ($P<0.01$), where there was no difference in slaughter weight and fat score between EM 6 cm and LM 6 cm, but EM 4 cm had a greater slaughter weight and fat score ($P<0.001$) than LM 4 cm.

[3] NS = not significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Conclusions

Grazing to the lower PGSH restricted growth of both EM and LM steers at pasture. Despite EM having a superior LWG at pasture, this did not result in a heavier carcass compared to LM. Consuming concentrates during the finishing period enhanced growth of both genotypes.

References

Finneran E., Crosson P., O'Kiely P., Shalloo L., Forristal D. and Wallace M. (2012) Stochastic simulation of the cost of home-produced feeds for ruminant livestock systems. *The Journal of Agricultural Science* 150, 123-139.

Lawrence P., Kenny D.A., Earley B. and McGee M. (2012) Grazed grass herbage intake and performance of beef heifers with predetermined phenotypic residual feed intake classification. *Animal* 6, 1648-1661.

Monahan F.J., Schmidt O. and Moloney A.P. (2018) Meat provenance: Authentication of geographical origin and dietary background of meat. *Meat Science* 144, 2-14.

Minchin W. and McGee M. (2010) Effect of post-grazing sward height on grass production and performance of four beef heifer genotypes. *Advances in Animal Biosciences* April 2010, 324.

O'Riordan E.G. Keane M.G. and McGee M. (2011) Effects of turn-out date to pasture in spring and post-grazing sward height on performance of dairy crossbred steers during the grazing season. In: *Agricultural Research Forum*, Tullamore, Ireland, p. 86.

Regan M., McGee M., Moloney A.P., Kelly A.K. and O'Riordan E.G. (2018) Grass-based suckler steer weanling-to-beef production systems: effect of breed and slaughter age. *Grassland Science in Europe* 23, 488-490.

Pre-grazing herbage mass: grass production and quality in a rotational stocking system with beef cattle

Doyle P.[1,2], McGee M.[1], Moloney A.P.[1], Kelly A.K.[2] and O'Riordan E.G.[1]
[1] *Teagasc, Animal & Grassland Research Centre and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland;* [2] *University College Dublin, Belfield, Dublin 4, Ireland*

Abstract

The effect of pre-grazing herbage mass (PGHM) on herbage nutritive value and grass production in a rotational stocking system was examined over 203 days. Early- and late-maturing suckler-bred steers were assigned to a PGHM above 4 cm of either 1,500 (LHM) or 2,000 (HHM) kg dry matter (DM) ha^{-1} commencing on 11 April. Steers grazed *Lolium perenne*-dominant swards in three breed specific groups of nine animals per PGHM. Herbage in excess of grazing requirements was removed as silage. From April to August steers grazed 13.68 ha on the 'initial' grazing area following which an additional 4.56 ha was 'incorporated' to maintain pasture supply in autumn. Mean PGHM on the 'initial' grazing area was 1,495 and 2,164 kg DM ha^{-1}. Corresponding values for the 'incorporated' area were 1,552 and 1,758 kg DM ha^{-1}. Pastures with LHM tended to have higher organic matter digestibility (P=0.09) and had higher crude protein concentration (P<0.01) than HHM pastures. Daily pasture growth (kg DM ha^{-1}) did not differ between the two PGHM treatments; however, herbage accumulation was greater (P<0.01) for HHM than LHM on the 'initial' grazing area but there was no difference between PGHM on the 'incorporated' area.

Keywords: grazing, herbage accumulation, herbage nutritive value, pasture growth, regrowth interval, steers

Introduction

Achieving a balance between pasture quantity and 'quality' is a key economic driver in grass-based production systems. Pre-grazing herbage mass (PGHM) impacts grass production, utilisation, sward morphology, herbage nutritive value, grazing behaviour (Wims *et al.,* 2014), rumen digestion (Owens *et al.,* 2008) and animal live-weight gain (Doyle *et al.,* 2019). Increasing PGHM in a rotational stocking system can increase pasture growth; O'Riordan (2000) reported that increasing regrowth interval from 3 to 4 weeks increased herbage production equivalent to the application of 150 kg nitrogen fertiliser annum^{-1}. The effect of PGHM on herbage organic matter digestibility is inconsistent, with no effects (at treatments 1,150 vs 1,400 vs 2,000 kg DM ha^{-1}; Wims *et al.*, 2014) or decreases (at treatments 1,600 vs 2,400 kg DM ha^{-1}; Curran *et al.,* 2010) reported. The objective of this study is to investigate the effect of PGHM on grass production and nutritive value in a rotational stocking system with beef cattle.

Materials and methods

This study was conducted at Teagasc, Grange Research Centre, between March and November 2017. Spring-born, early-maturing (EM – Aberdeen Angus and Hereford sired, n=54) (303 (standard deviation (SD) 35.1) kg live weight) and late-maturing (LM – Limousin and Charolais sired, n=54) (334 (SD 20.9) kg live weight) weaned suckler-bred steers were randomly assigned within sire breed and descending live-weight, to a two (sire breed maturity: EM or LM) × two (PGHM (>4 cm): 1,500 or 2,000 kg DM ha^{-1}) factorial arrangement of treatments. The 'initial' grazing area from April to August was 13.68 ha, divided into four equal-sized farmlets that comprised nine paddocks per treatment; subsequently, an additional 4.56 ha in total (three paddocks per treatment) was 'incorporated' to the experimental area to maintain pasture supply in autumn. Each paddock was divided into three sub-paddocks to facilitate grazing three replicates of each PGHM in breed-specific groups, resulting in twelve grazing groups, each containing nine cattle. Steers were turned out to *Lolium perenne*-dominant pasture on 13 March 2017 to graze the experimental area and establish the target PGHM. Starting mean PGHM 'covers' (1,098 and 1,448 kg DM ha^{-1} for LHM and HHM, respectively) were established on 11 April (commencement of the experiment) and steers were rotationally grazed for 203 days. All pastures were grazed to a targeted post-grazing compressed sward height of 4 cm. Differences in PGHM were maintained throughout the grazing season mainly through a combination of variances in paddock residency time and number of grazing rotations. Herbage in excess of grazing requirements was removed as silage and its yield determined. Stocking rate increased from 2724 kg live-weight ha^{-1} at turn-out in spring and peaked at 3,947 kg live-weight ha^{-1} in August before the additional land was incorporated into the grazing rotation. Steers were housed on 31 October 2017.

Pasture growth rate was calculated as the difference between PGHM and the post-grazing herbage mass of the previous grazing, divided by the number of days between the two yield estimates. Herbage accumulation was calculated as the differences between pre- and post- herbage mass of the same rotation summed over the year for each paddock. Herbage accumulation was further sub-divided into what was grazed (excluding silage removed) for each paddock. Herbage accumulation included an adjustment factor for pasture growth while animals were resident in the paddock. Sward height and herbage mass (>4 cm) were measured in each sub-paddock pre- and post-grazing and at silage removal, by recording fifty heights using a Jenquip rising platemeter and a Honda rotary lawnmower; cutting 2 strips (0.53×5 m), respectively. Representative samples of herbage were collected for DM determination and herbage nutritive analysis as described by Lawrence *et al.* (2012).

All data were analysed using the MIXED procedure of Statistical Analysis Software (SAS). The experimental unit was paddock. Data pertaining to herbage production and rotation length were analysed using a statistical model which contained the fixed effects of PGHM, breed maturity, grazing area (i.e. 'initial' and 'incorporated' grazing areas) and their interactions; data were weighted for frequency of grazing (i.e. the number of times the paddock was defoliated). Differences between means were tested for significance using the PDIFF statement and adjusted for Tukey. Data for herbage nutritive value was analysed using the same model, except that grazing area was excluded; means presented include both grazing areas combined.

Results and discussion

Mean PGHM was 1,495 and 2,164 kg DM ha^{-1} for the 'initial' grazing area (P<0.001) and 1,552 and 1,758 kg DM ha^{-1} for the 'incorporated' area (P>0.05) for LHM and HHM, respectively. Breed maturity was not significant (P>0.05) and there were no interactions (P>0.05) for any parameters measured. Mean rotation length was greater for HHM than LHM, both on the 'initial' (44 vs 33 days; P<0.001) and 'incorporated' (40 vs 33 days; P=0.07) grazing areas, respectively. Mean rest days were greater (P<0.001) for HHM than LHM on the 'initial' grazing area (40 vs 30 days) with no difference (P>0.05) between HHM and LHM on the 'incorporated' area (36 vs 30 days). The LHM pastures had higher organic matter digestibility (P=0.09) and crude protein concentration (P<0.01), and lower (P<0.05) neutral detergent fibre and acid detergent fibre concentrations than HHM, with no difference (P>0.05) between PGHM in crude ash (Table 1). Although organic matter digestibility was lower for HHM in this study, live-weight gain of steers grazing HHM was higher than those grazing LHM (0.89 vs 0.84 kg/day; Doyle *et al.,* 2019). Furthermore, the lower crude protein concentration for HHM pastures can potentially reduce N excretion to the environment (O'Connor *et al.*, 2019). There was no difference (P>0.05) in daily pasture growth between HHM and LHM for the 'initial' (48 vs 44 kg DM ha^{-1} day^{-1}, respectively) or 'incorporated' (44 vs 40 kg DM ha^{-1} day^{-1}, respectively) grazing areas. However, total herbage accumulation was greater (P<0.01) for HHM than LHM on the 'initial' grazing area (9,665 vs 8,651 kg DM ha^{-1}) with no difference (P>0.05) on the 'incorporated' area (4,748 vs 4,308 kg DM ha^{-1}

Table 1. The effect of low (1,500 kg DM ha^{-1}) and high (2,000 kg DM ha^{-1}) pre-grazing herbage mass (PGHM) on herbage accumulation and nutritive value.[1]

		PGHM		SEM	Significance[2]
		1,500	2,000		
Herbage accumulation:	'Initial' grazing area (kg DM ha^{-1})	8,651	9,665	174.7	**
	'Incorporated' area (kg DM ha^{-1})	4,308	4,748	403.9	NS
Organic matter digestibility (g kg^{-1})		776	769	2.6	0.09
Crude protein (g kg^{-1} DM)		225	210	3.6	**
Neutral detergent fibre (g kg^{-1} DM)		423	432	2.7	*
Acid detergent fibre (g kg^{-1} DM)		232	239	2.5	*
Crude ash (g kg^{-1} DM)		103	100	1.7	NS

[1] DM = dry matter; SEM = standard error of the mean.
[2] NS = not significant; * P<0.05; ** P<0.01.

for HHM and LHM, respectively) (Table 1). There was no difference (P>0.05) in the total quantity of herbage consumed between HHM and LHM both for the 'initial' (8,173 vs 7,432 kg DM ha^{-1}) and 'incorporated' (4,748 vs 3,675 kg DM ha^{-1}) grazing areas. Although not statistically different, the proportionately 0.10 higher grazed herbage accumulation for HHM compared to LHM was consistent with the statistically significant difference (0.097) reported by Wims *et al.* (2014).

Conclusions

Maintaining a lower PGHM increased herbage crude protein concentration, which can potentially increase N excretion by grazing cattle, and reduced total herbage accumulation in a rotational stocking system.

References

Curran J., Delaby L., Kennedy E., Murphy J.P., Boland T.M. and O'Donovan M. (2010) Sward characteristics, grass dry matter intake and milk production performance are affected by pre-grazing herbage mass and pasture allowance. *Livestock Science* 127, 144-154.

Doyle P., Kelly A.K., McGee M., Moloney A.P. and O'Riordan E.G. (2019) Effect of pre-grazing herbage mass on performance of early- and late-maturing suckler bred steers. *Advances in Animal Biosciences* 10, 64. Lawrence P., Kenny D.A., Earley B. and McGee M. (2012) Grazed grass herbage intake and performance of beef heifers with predetermined phenotypic residual feed intake classification. *Animal*, 6, 1648-1661.

O'Connor A., Moloney A.P., O'Kiely P., Boland T. and McGee M. (2019) Effects of fertiliser nitrogen rate to spring grass on apparent digestibility, nitrogen balance, ruminal fermentation and microbial nitrogen production in beef cattle and *in vitro* rumen fermentation and methane output. *Animal Feed Science and Technology* 254, 114-198.

O'Riordan E.G. (2000) Effect of regrowth interval and nitrogen application rate on herbage production. In: *Grazing management: the principles and practice of grazing, for profit and environmental gain, within temperate grassland systems.* Proceedings of the British Grassland Society Conference held at the Cairn Hotel, Harrogate, 29 February-2 March 2000, pp. 113-114.

Owens D., McGee M. and Boland T. (2008) Effect of grass regrowth interval on intake, rumen digestion and nutrient flow to the omasum in beef cattle. *Animal Feed Science and Technology* 146, 21-41.

Wims C.M., Delaby L., Boland T.M. and O'Donovan M. (2014) Effect of pre-grazing herbage mass on dairy cow performance, grass dry matter production and output from perennial ryegrass (*Lolium perenne* L.) pastures. *Animal* 8, 141-151.

Effect of autumn herbage mass on pasture productivity and animal performance in spring-calving grass-based dairy systems

Evers S.H.[1,2], Delaby L.[3], Pierce K.M.[2] and Horan B.[1]

[1]*Animal & Grassland Research & Innovation Centre, Teagasc Moorepark, Fermoy, Co. Cork, Ireland;* [2]*INRA, AgroCampus Ouest, UMR 1348, Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Elevage, 35590 Saint-Gilles, France;* [3]*School of Agriculture, Food Science & Veterinary Medicine, UCD, Belfield, Dublin 4, Ireland*

Abstract

The objective of this study was to evaluate the impact of 3 available herbage mass strategies (HM) and 2 whole grazing intensities on the performance of spring calving dairy cows during late lactation. The 3×2 factorial design experiment was conducted in autumn over 3 years (2017, 2018 and 2019) and consisted of: Low Herbage Mass (LHM), Medium Herbage Mass (MHM) and High Herbage Mass availability (HHM) and 2 grazing intensities (GI; Medium Intensity (MI; 2.75 cows ha^{-1}) and High Intensity (HI; 3.25 cows ha^{-1}). Autumn mean pre-grazing biomass yield was 1,280, 1,731 and 2,080 kg dry matter (DM) ha^{-1} for LHM, MHM and HHM, respectively resulting in post-grazing residual heights of 34.3, 36.8 and 37.3 mm, respectively. There was no difference in milk and fat plus protein yield between the 3 HM treatments during autumn (1 and 1.52 kg cow^{-1} d^{-1}, respectively). Grazing intensity had no effect on pre-grazing measurements, with the exception of daily herbage allowance which was higher (*P*<0.001) for MI compared with HI (+2.2 kg DM cow^{-1} d^{-1}). The combination of increased GI and increased concentrate supplementation for HI GI resulted in a reduced post-grazing sward height (-3 mm) and an increased fat plus protein yield (0.04 kg cow^{-1} d$^{-1)}$. These results highlight the potential for high intensity grazing systems to maintain an extended grazing season by increasing HM during autumn without detriment to individual animal performance.

Keywords: dairy system, milk production, grassland management

Introduction

The overall resilience and long term sustainability of the pasture-based dairy industries is dependent on increased productivity and improved efficiency of conversion of grazed pasture to animal products (Delaby and Horan, 2017). Stocking rate (SR), defined as the number of animals per unit area of land used during a specified defined period of time (cows hectare^{-1}) is widely acknowledged as the main driver of productivity within grazing systems, (Macdonald *et al.*, 2008; McCarthy *et al.*, 2013). SR has been increasing on Irish dairy farms since milk quota abolition in 2015 (Hanrahan *et al.*, 2017). Increasing SR places added feed demands on the available land area and can result in an increased feed supplementation requirement and a shortening of the grazing season length unless grazing management practices are adapted (Neal and Roche, 2018). One possibility to maintain a high reliance on grazing at higher system intensity is to alter autumn grazing management practice by extending rotation length and increasing herbage mass (Claffey *et al.*, 2019). At the same time, previous studies have also indicated that increasing herbage mass strategies (HM) during autumn can result in increased sward senescence and reduced nutritive value and thereby inadvertently reducing animal intake and performance (Beecher *et al.* 2015; Tuñon *et al.*, 2014).

The objective of this study was to evaluate the potential for adapted grazing management strategies during autumn to continue to deliver the biological and economic advantages of extended grazing while also maintaining sward quality and subsequent animal performance.

Materials and methods

The experiment was undertaken at the Animal and Grassland Research and Innovation Center, Teagasc Moorepark, Ireland for 11 weeks from September to November over 3 years (2017-2019. It was set up as a randomized block design with a 3×2 factorial arrangement of treatments. A total of 144 spring-calving dairy cows were randomly allocated to one of three whole-farm HM strategies; Low Herbage Mass (LHM; 350 kg dry matter (DM) ha^{-1} available at housing (week 11)), Medium Herbage Mass (MHM; 600 kg DM ha^{-1} available at housing) and High Herbage Mass (HHM; 850 kg DM ha^{-1} available at housing). The 3 HM treatments were established by extending rotation length from late summer. All treatments were balanced for breed (Holstein-Friesian (HF) and Jersey Holstein-Friesian (JFX)), parity, genetic merit, bodyweight and body condition score. Within each HM, the experimental animals were further divided into two grazing intensities (GI): a medium grazing intensity (MI; 2.75 cows ha^{-1}, target post grazing intensity of 40 mm, representing 90% of the pasture diet) and a high grazing intensity (HI; 3.25 cows ha^{-1}, target post grazing intensity of 35 mm, representing 90% of the pasture diet), receiving an additional +1.8 kg DM concentrate cow^{-1} d^{-1}, compared to MI. Milk yield was recorded daily and milk constituents weekly, while grazing measurements were undertaken on each paddock before grazing according to the methods of Delaby and Peyraud (1998). Least squares means for HM and GI were estimated using linear mixed models (PROC MIXED; SAS, 2010).

Results and discussion

Both HM and GI had a significant impact on autumn pasture and animal performance characteristics (Table 1). Pre-grazing sward height, biomass yield and daily herbage allowance (DHA) were highest for the HHM, intermediate for MHM and least for LHM. Due to the large differences in pre-grazing sward characteristics, the resultant post-grazing sward height for HHM and MHM (37.3 and 36.8 mm, respectively) were higher ($P<0.001$) than LHM (34.3 mm) while grazing efficiency was lower for both MHM and HHM (99%) compared to LHM (106%). There was no difference in pre-grazing sward height and biomass yield between GI (89.0 mm and 1,697 kg DM ha^{-1}, respectively). However, the higher SR of HI resulted in a lower ($P<0.001$) DHA and post-grazing sward height (12.0 kg DM and 34.6 mm, respectively) compared to MI (14.2 kg DM and 37.6 mm, respectively) and increased grazing efficiency (98 vs 105%). Despite large differences in pre-grazing sward characteristics between HM treatments, there was no significant impact on daily milk yield during autumn (15.7 kg cow^{-1}). The effect of HM on

Table 1. The effect of herbage mass (HM) and grazing intensity (GI) on pasture characteristics and daily cow performance during autumn.[1]

	Herbage mass[2]			Significance HM		Grazing intensity[3]		Significance GI	
	LHM	MHM	HHM	SE	*P*-value	MI	HI	SE	*P*-value
Pre-grazing sward height (mm)	76.6^a	90.4^b	100.0^c	0.12	0.001	89.2	88.9	0.11	0.74
Pre-grazing biomass (kg DM ha^{-1})	1,280^a	1,731^b	2,080^c	39.6	0.001	1,698	1,696	32.2	0.96
DHA (kg DM cow^{-1} d^{-1})	12.3^a	13.2^b	13.8^b	0.31	0.001	14.2^a	12.0^b	0.26	0.001
Post-grazing height (mm)	34.3^a	36.8^b	37.3^b	0.04	0.001	37.6	34.6	0.04	0.001
Grazing efficiency (%)	106^a	99^b	99^b	1.1	0.001	98	105	0.8	0.001
Milk yield (kg cow^{-1} d^{-1})	15.9	15.6	15.5	0.21	0.34	15.4	15.9	0.17	0.05
Fat + protein yield (kg cow^{-1} d^{-1})	1.53	1.54	1.49	0.018	0.11	1.50	1.54	0.015	0.05
Milk fat content (g kg^{-1})	57.4^a	58.8^a	57.1^b	0.04	0.01	57.7	57.9	0.34	0.63
Milk protein content (g kg^{-1})	42.8	43.3	42.9	0.19	0.15	43.1	42.9	0.16	0.50
Milk lactose content (g kg^{-1})	46.6	45.6	46.12	0.44	0.28	45.9	46.4	0.37	0.29

[1] DHA = daily herbage allowance; DM = dry matter; SE = standard error.

[2] LHM = low herbage mass; MHM = medium herbage mass; HHM = high herbage mass.

[3] MI = medium intensity; HI = high intensity.

daily fat plus protein yield approached significance (P=0.1) due to lower yield of HHM (1.49 kg cow^{-1} d^{-1}) compared to MHM (1.54 kg cow^{-1} d^{-1}) while LHM was intermediate (1.53 kg cow^{-1} d^{-1}). HM treatment had no significant effect on milk protein and lactose content (43.0 and 46.1 g kg^{-1}, respectively) while milk fat content was highest (P<0.01) for MHM (58.8 g kg^{-1}), least for HHM (57.1 g kg^{-1}) and intermediate for LHM (57.4 g kg^{-1}). Despite receiving a lower DHA, the additional supplementation of 1.8 kg DM cow^{-1} to HI GI resulted in higher (P=0.05) daily milk (+0.5 kg cow^{-1}) and fat plus protein yield (+0.04 kg cow^{-1}) compared to MI with no significant impacts on fat and protein composition.

Conclusions

The results of the current study indicate that increasing overall herbage mass during autumn can be an effective grazing management strategy to increase spring grass availability while having no effect on late lactation milk and fat plus protein yield. Equally, the combination of reduced post-grazing residuals and increased concentrate supplementation can be an effective strategy to increase animal performance and maintain grazing efficiency within higher intensity grazing systems during autumn.

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References

Beecher M., Hennessy D., Boland T., McEvoy M., O'Donovan M. and Lewis E. (2015) The variation in morphology of perennial ryegrass cultivars throughout the grazing season and effects on organic matter digestibility. *Grass and Forage Science* 70, 19-29.

Claffey A., Delaby L., Boland T. and Egan M. (2019) Implications of adapting autumn grazing management on spring herbage production–the effect on late lactation milk production and the subsequent response in early lactation animal performance. *Livestock Science* 103870.

Delaby L. and Horan B. (2017) Improved efficiency in temperate grass based dairy systems. Paper presented at the 54. Annual Meeting of the Brazilian Society of Animal Science.

Delaby L., Peyraud J.-L., Bouttier A. and Peccatte J.-R. (1998) Effet d'une réduction simultanée de la fertilisation azotée et du chargement sur les performances des vaches laitières et la valorisation du pâturage.

Hanrahan L., Geoghegan A., O'Donovan M., Griffith V., Ruelle E., Wallace M. and Shalloo L. (2017) PastureBase Ireland: A grassland decision support system and national database. *Computers and Electronics in Agriculture* 136, 193-201.

Macdonald K., Penno J., Lancaster J. and Roche J. (2008) Effect of stocking rate on pasture production, milk production, and reproduction of dairy cows in pasture-based systems. *Journal of Dairy Science* 91, 2151-2163.

Mccarthy B., Delaby L., Pierce K., Brennan A. and Horan B. (2013) The effect of stocking rate and calving date on milk production of Holstein–Friesian dairy cows. *Livestock Science* 153, 123-134.

Neal M. and Roche J. (2018) Profitable and resilient pasture-based dairy farm businesses in New Zealand. Paper presented at the Proc. of the Australasian Dairy Science Symposium.

Tuñon G., Kennedy E., Horan B., Hennessy D., Lopez-Villalobos N., Kemp P. and O'Donovan M. (2014) Effect of grazing severity on perennial ryegrass herbage production and sward structural characteristics throughout an entire grazing season. *Grass and Forage Science* 69, 104-118.

Prediction of butyric spores with butyric acid and anaerobic spores in silages

Fabri F.B.[1], Van Vliet P.C.J.[1], Abbink G.W.[2], Klein Koerkamp D.D.A.B.[1] and Van Oostrum M.J.[1]
[1]*Eurofins Agro International Agro Competence Center, Binnenhaven 5, 6709 PD Wageningen, the Netherlands;* [2]*Groeikracht BV, Groenloseweg 114, 7104 GA Winterswijk Meddo, the Netherlands*

Abstract

The aim of this study was to investigate the relationship between butyric acid in silage and the amount of anaerobic spores present in the silage. We hypothesised that a high concentration of butyric acid correlates with a high amount of *Clostridium tyrobutyricum* spores in the silage. 293 silages were analysed for composition and butyric acid, and for anaerobic spores. No correlation between butyric acid and anaerobic spores was found. We concluded that silages with high butyric acid content do not always contain a large number of anaerobic spores (>4 log kve g kg^{-1} DM). As no correlation was found between butyric acid and anaerobic spores, *C. tyrobutyricum* spores cannot be predicted from normal silage analysis.

Keywords: anaerobic spores, silage, *Clostridium,* butyric acid, prediction

Introduction

During the conservation process of silages, the pH drops due to production of primarily lactic and acetic acids (Muck, 1988). During the initial phase of silage fermentation, *Clostridium tyrobutyricum* increase in numbers by multiplication (Woolford, 1984). This bacterium ferments lactic acid to acetic and butyric acid at low pH. However, if the pH has decreased significantly below 4.2, in addition with enough organic acids, the growth of *C. tyrobutyricum* is inhibited (Jonsson, 1991). However, any existing *Clostridium* spores can survive under these low pH (<4.2) circumstances.

When the silage is fed to cattle, any still existing *C. tyrobutyricum* bacteria are broken down in the rumen of the cow. The spores are not broken down and are excreted in the manure. If hygienic conditions are insufficient, these spores can contaminate the milk. During the heating process of the milk, *C tyrobutyricum* bacteria hatched from spores will be killed, but the unhatched spores (anaerobic spores) will survive the heating process.

The remaining spores of *C. tyrobutyricum* in the milk can hatch during the final stage of cheese making, which can result in considerable losses in the production of semi-hard cheeses, such as Gouda (Klijn *et al.*, 1995). The bacteria cause a deviant smell and taste which makes the cheese unsalable (van Schooten *et al.*, 2005).

Monitoring the amount of spores in the milk is therefore crucial for cheese production. It has been found that a number of anaerobic spores above log 4 (10,000 kve g kg dry matter (DM)) is critical (Vissers *et al.*, 2007), because spores can cause a deviant taste and smell, since these spores can hatch into bacteria.

Butyric acid is often used as an indicator of a possibly large presence of *C. tyrobutyricum*. Also, butyric acid is often more detected in silages containing a combination of two or more of the following characteristics: high pH (>4.2), normal to low dry matter content (<40%), high ash content (>105 g kg DM^{-1}) and high protein content (in grass >180 g kg^{-1} DM) (Eurofins Agro, 2019).

The aim of this study was to investigate the relationship between the amount of butyric acid measured in silage and the number of anaerobic spores present in the silage. We hypothesise that a high concentration of butyric acid correlates with a high amount of *C. tyrobutyricum* spores in the silage.

Materials and methods

A total of 293 silages were analysed by Eurofins Agro (Wageningen, NL) for composition and butyric acid (Table 1). These silages were also analysed for anaerobic spores. After arriving at the laboratory all samples were split; half were weighed and dried to determine the DM content, the other half was used to determine the butyric acid and anaerobic spores content. Butyric acid was analysed according to the ANA-A-MKZ1 protocol, an internal document of Eurofins Agro. The sample was first injected with water and then frozen. After thawing the sample the extract was filtered and diluted. Samples were analysed through liquid chromatography.

Samples for anaerobic spores were measured according to the ANAL-10400 protocol (based on NEN6813:2014) by Nutricontrol Veghel in the Netherlands. The samples were placed on ice water after making a suitable dilution. The dilution was pasteurised and then cooled to ca. 20 °C. After pasteurization, the final solution was spread on agar plates, and the number of colonies was counted.

Results and discussion

The relation between anaerobic spores and the content of butyric acid is shown in Figure 1. There was no correlation found for maize (R^2=0.01) and a small one for grass (R^2=0.24).

Table 1. Characteristics of the samples used in this study. Median and the 10% (0.1p) and 90% (0.9p) percentile of dry matter in g kg^{-1} and pH are shown.

Silage	n	Dry matter (g kg^{-1})			pH		
		Median	0.1p	0.9p	Median	0.1p	0.9p
Grass	58	388	247	607	4.5	4.2	6.0
Maize	235	388	337	469	3.9	3.8	4.3

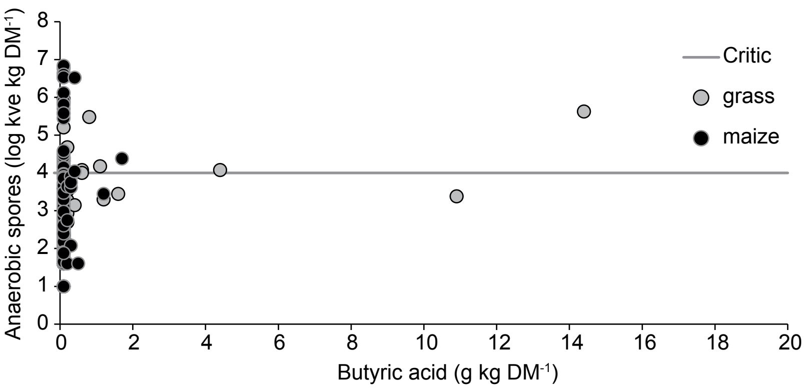

Figure 1. Relationship between butyric acid contents (X-axis) and anaerobic spores (Y-axis). The line represents the critical value (log 4) above which the quality of cheese making is negatively affected.

Within the data, 12% (36 samples) were high in anaerobic spores but had low butyric acid concentrations. The following types of silages were part of these 36 silages:

- 6 grass silages; 4 of these had a high dry matter (DM) content (>60% DM), high pH (>5.5) and a high protein content (>18% crude protein);
- 30 maize silages; 50% of these silages have a DM content >45% DM.

In total, 14% of the samples had a log kve kg^{-1} DM of 4 or higher; however, only 1% (2 samples) had a high butyric acid (>3 g kg^{-1} DM) and a log kve kg^{-1} DM of >4. One sample had a log kve kg^{-1} DM <4 but a very high butyric acid (10.9 g kg^{-1} DM).

In this study we were looking for a relationship between *C. tyrobutyricum* spores and butyric acid in silages. The spores were measured using a general method to determine anaerobic spores. Within the 293 analysed samples, 263 samples (222 maize and 41 grass) were analysed at the detection level of 0.1 g of butyric acid kg^{-1} DM, although the anaerobic spores varied between 1 and 6.8 log kve g kg^{-1} DM.

Several studies have looked at different types of spores in raw milk. Martin (1974) found 11 different types of anaerobic spores in raw milk. Clostridium was present at 4.7%. Another study found 12 different anaerobic spores in milk of which 10.2% of the total spores were not identified (Miller *et al.*, 2015). We therefore expect that the anaerobic spores measured in our study probably consisted of several different species, resulting in the absence of a correlation between *C. tyrobutyricum* and butyric acid. As we do not know what percentage of the anaerobic spores consisted of *C. tyrobutyricum*, we could not predict the presence of butyric acid using the applied method for determining anaerobic spores.

Conclusions

Within this dataset no correlation between butyric acid and anaerobic spores was found. It can be concluded that silages with high butyric acid content do not always contain a large number of anaerobic spores (above 4 log kve g kg^{-1} DM). However, literature data show that at a butyric acid content higher than 3 g kg^{-1} DM, it is likely to have a high number of anaerobic spores (above log 4). Because no correlation was found between butyric acid and anaerobic spores, the spores of *C. tyrobutyricum* cannot be predicted from the normal silage analysis.

References

Eurofins Agro (2019) Laag droge-stofgehalte, groot risico op boterzuur. Available at: https://www.eurofins-agro.com/nl-nl/laag-droge-stofgehalte-groot-risico-op-boterzuur.

Jonsson A. (1991) Growth of *Clostridium tyrobutyricum* during fermentation and aerobic deterioration of grass silage. *Journal of the Science of Food and Agriculture*, 54, 557-568.

Klijn K. *et al.* (1995) Identification of *Clostridium tyrobutyricum* as the causative agent of late blowing in cheese by species-specific PCR amplification. *Applied and Environmental Microbiology*, 61, 2919-2924.

Martin J. (1974) Significance of bacterial spores in milk. *Journal of Milk and Food Technology*, 37, 94-98.

Miller R. *et al.* (2015) Spore populations among bulk tank raw milk and dairy powders are significantly different. *Journal of Dairy Science*, 98, 8492-8504.

Muck, R. (1988) Factors influencing silage quality and their implications for management. *Journal of Dairy Science*, 71, 2992-3002.

NEN 6813:2014, Melk en melkproducten – bepaling van het gehalte aan sporen van mesofiele aerobe sporenvormende bacteriën.

Van Schooten H.A., Slaghuis B.A., Wemmenhove H. and Vissers M. (2005) Sporen van boterzuurbacteriën; plaaggeest van kuil tot kaas. Animal Sciences Group, Wageningen, the Netherlands.

Vissers M. *et al.* (2007) Concentrations of butyric acid bacteria spores in silage and relationships with aerobic deterioration. *Journal of Dairy Science*, 90, 928-936.

Woolford M. (1984) *The Silage Fermentation*. New York: Marcel Dekker, Inc.

Increasing the supply of herbage mass during autumn in pasture-based dairy systems

Fenger F.[1,2], Casey I.A.[1] and Humphreys J.[2]
[1]*Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Co. Cork, Ireland;*
[2]*Department of Chemical and Life Sciences, Waterford Institute of Technology, Waterford, Ireland*

Abstract

The effects of increasing the supply of herbage mass (HM) available in a pasture-based dairy system during autumn (1 Aug to end of the grazing season [closing]) on late lactation milk production and other key performance indicators have not been evaluated. Multiple regression models were fitted to a dataset containing 63 grazing system experiments conducted with spring-calving dairy herds between 2001 and 2018 at Solohead Research Farm, Ireland (52°30'N, 08°12'W). The supply of HM per system was measured as average herbage cover (AHC; HM >4 cm; average of all paddocks) and pre-grazing HM. Increasing AHC and pre-grazing HM had no effect (P>0.05) on late lactation milk production. On average over the 17 years, each increase in peak AHC (highest AHC during autumn) of 100 kg dry matter (DM) ha^{-1} increased the length of the grazing season in autumn by 1.9±0.46 days (P<0.001, partial R^2=0.43) and increased closing AHC by 47±7.2 kg DM ha^{-1} (P<0.001, partial R^2=0.38). Opening AHC in February increased with closing AHC (P<0.001, R^2=0.37). Therefore, increasing AHC during autumn allows for extended grazing while not compromising milk production.

Keywords: autumn, extended grazing, grazing management, pasture-based milk production

Introduction

The length of the grazing season, the period when herbage growth rate (GR) meets the demand for herbage mass (HM) by grazing cattle, is limited in temperate latitudes. Several studies reported that a long grazing season improves profitability of pasture-based dairy systems by maximizing low cost grazed grass in the diet of dairy cows and thereby reducing costs of production (Hanrahan *et al.*, 2018). Extending the total length of the grazing season requires making HM available for grazing at all times of low herbage GR, particularly before and after pastures are closed for the winter. In late summer and early autumn the GR generally exceeds demand by grazing cows. This surplus HM can be harvested for grass silage or used to accumulate HM. By increasing the rotation interval, the accumulated HM is transferred *in situ* to later in the grazing season. The supply of HM is quantified as average herbage cover (AHC; HM >4 cm; average of all paddocks) at system level and as pre-grazing herbage mass (PGHM) at paddock level. However, accumulating high AHC and PGHM during the autumn to extend the grazing season could negatively affect the nutritive value of the sward and, hence, milk yield.

Materials and methods

A dataset was created for the purpose of this study from grazing system experiments conducted at Solohead Research Farm in Ireland (52°30'N, 08°12'W) between 2001 and 2018. They were all system studies of spring-calving pasture-based dairy herds over an entire grazing season (n=63 systems). Assignment of cows to herds and paddocks to systems, grazing management and recording of days at pasture was described by Humphreys *et al.* (2008, 2009), Phelan *et al.* (2013) and Tuohy *et al.* (2014). Data for this study encompassing the 'end of season management period' were from 1 Aug to 31 Dec of each year. Autumn was defined as the timeframe from 1 Aug to closing, which demarks the end of grazing and the beginning of the 'closed' period during winter when all cows were housed, generally during December and January (of the following year). 'Early spring' turnout in February was denoted opening in this study. Average herbage cover (AHC) was measured once per week on each system using a rising plate meter (measuring compressed sward height (cm) which was converted into herbage mass (kg dry matter (DM) ha^{-1}) with a sward density of 240 kg DM cm^{-1} ha^{-1}). Peak AHC was the highest AHC recorded in each system during autumn. AHC at closing and at opening where recorded accordingly. Some systems were missing data for either closing AHC or opening AHC as defined above and were removed from the dataset for the corresponding analysis. PGHM (kg DM ha^{-1}) was determined before each grazing on every paddock by harvesting a strip of grass to a cutting height of 40 mm above ground level. A subsample was dried for DM determination. Mean GR in autumn was the mean of GR of each system determined at each grazing by dividing PGHM by the rotation interval. Soil moisture deficit (SMD) (Schulte *et al.*, 2005) during autumn was modelled assuming a poorly drained soil. The number of days where SMD was 0 mm or above (threshold for trafficability with bovine livestock (Herbin *et al.*, 2011)) was recorded. Mean daily milk yield per cow and daily yield of fat and protein per cow (milk solids yield) were recorded in late lactation from 1 Aug to dry off (mean date 3 Dec) and are presented as the mean yield of all cows in a herd in a system. The dataset was analysed for factors associated with days at pasture during autumn, closing AHC, opening AHC and daily milk yield and daily milk solids yield in late lactation using multiple regression analysis in the GLMSELECT procedure in SAS 9.4. A stepwise selection process was applied with a 5% significance level for inclusion and exclusion of factors into the model. The dataset was tested for interactions between independent variables and quadratic relationships. Results are presented as means ± standard error.

Results and discussion

The dataset created for this study represents a wide range of grazing management practices and grazing conditions as well as varying weather and grass growing conditions. Peak AHC (ranging between 634 and 1,800 kg DM ha^{-1}), closing AHC (ranging between 36 and 855 kg DM ha^{-1}) and opening AHC (ranging between 118 and 1,191 kg DM ha^{-1}) encompassed a range of HM supply scenarios that have not been captured by previous studies. As peak AHC was highly correlated with mean AHC during autumn (Pearson's r=0.96, P<0.001) and with mean PGHM during autumn (r=0.81, P<0.001), peak AHC was used as the main indicator of the supply of HM in this study. Over the 17 years, there was no detectable impact (P>0.05) of peak AHC, PGHM or days at pasture on daily milk yield (16.31±0.26 kg cow^{-1} day^{-1}) or daily milk solids yield (1.39±0.02 kg cow^{-1} day^{-1}) in late lactation. That means neither higher PGHM nor a change in the proportion of grazed grass vs grass silage in the diet impacted milk production in late lactation, similar to that found by O'Neill *et al.* (2013) and Claffey *et al.* (2020). Of the variables associated with days at pasture during autumn (Table 1) only peak AHC and SR were factors controlled by grazing management. On average, each increase in peak AHC of 100 kg DM ha^{-1} increased the length of the grazing season in autumn by 1.9±0.46 days (P<0.001). Variations in peak AHC also explained the largest part of the variation in closing AHC (partial R^2=0.38). Opening AHC was positively associated with closing AHC (P<0.001, R^2=0.37). Claffey *et al.* (2020) proposed that earlier closing dates in autumn increase both closing AHC and opening AHC for early spring grazing. Accumulating a high AHC as presented in this study combines the benefits of a long grazing season in autumn with an increased supply of HM in early spring and, hence, higher milk production associated with early spring grazing. As the increase in HM per system is usually generated by utilising surplus GR in late summer, there is no extra cost associated with creating higher peak AHC in autumn.

Conclusions

Increasing the supply of HM available in a pasture-based dairy system during autumn allows for extended grazing in late autumn and early spring while not compromising milk production. This strategy has potential to increase the total annual length of the grazing season and hence, increase profitability on pasture-based dairy farms.

Table 1. Summary of the stepwise selection process from multiple regression analyses of factors associated with days at pasture in autumn (1 Aug to closing), AHC at closing and AHC at opening (February) in a multi-year dataset (2001 to 2018).[1]

Dependent variable	Step	Independent variable	Estimate (SE)	Partial R^2	Model R^2
Days at pasture	0	Intercept	11.5 (18.33)		0.72 (n=63)
(days per cow)[2]	1	Peak AHC[3,4] (kg DM ha^{-1})	1.9 (0.46) ***	0.43	
	2	Stocking rate (cows ha^{-1})	-11.5 (2.43) ***	0.14	
	3	SMD >0 mm (days)	0.15 (0.04) ***	0.06	
	4	Growth rate[2] (kg DM ha^{-1} day^{-1})	0.46 (0.13) ***	0.04	
	5	Date of peak AHC (day in year)	0.22 (0.07) **	0.06	
Closing AHC	0	Intercept	-446 (255.2)		0.56 (n=57)
(kg DM ha^{-1})	1	Peak AHC[3,4] (kg DM ha^{-1})	46.6 (7.21) ***	0.38	
	2	Days at pasture[2] (days per cow)	-7.8 (1.83) ***	0.08	
	3	Date of peak AHC (day in year)	4.1 (1.16) ***	0.10	
Opening AHC	0	Intercept	314 (62.7) ***		0.37 (n=53)
(kg DM ha^{-1})	1	Closing AHC[4] (kg DM ha^{-1})	71.5 (13.18) ***	-	

[1] SE = standard error; DM = dry matter; AHC = average herbage cover; SMD = soil moisture deficit; ** = $P<0.01$; *** = $P<0.001$.
[2] During autumn.
[3] Highest AHC during autumn.
[4] Increase of 100 kg DM ha^{-1}.

References

Claffey A., Delaby L., Boland T.M. and Egan M. (2020) Implications of adapting autumn grazing management on spring herbage production – the effect on late lactation milk production and the subsequent response in early lactation animal performance. *Livestock Science* 231, 103870.

Hanrahan L., McHugh N., Hennessy T., Moran B., Kearney R., Wallace M. and Shalloo L. (2018) Factors associated with profitability in pasture-based systems of milk production. *Journal of Dairy Science* 101, 5474-5485.

Herbin T., Hennessy D., Richards K.G., Piwowarczyk A., Murphy J.J. and Holden N.M. (2011) The effects of dairy cow weight on selected soil physical properties indicative of compaction. *Soil Use and Management* 27, 36-44.

Humphreys J., Casey I.A., Darmody P., O'Connell K., Fenton O. and Watson C.J. (2008) Quantities of mineral N in soil and concentrations of nitrate-N in groundwater in four grassland-based systems of dairy production on a clay-loam soil in a moist temperate climate. *Grass and Forage Science* 63, 481-494.

Humphreys J., Casey I.A. and Laidlaw A.S. (2009) Comparison of milk production from clover-based and fertilizer-N-based grassland on a clay-loam soil under moist temperate climatic conditions. *Irish Journal of Agricultural and Food Research* 48, 189-207.

O'Neill B., Lewis E., O'Donovan M., Shalloo L., Galvin N., Mulligan F., Boland T.M. and Delagarde R. (2013) Predicting grass dry matter intake, milk yield and milk fat and protein yield of spring calving grazing dairy cows during the grazing season. *Animal* 7, 1379-1389.

Phelan P., Casey I.A. and Humphreys J. (2013) The effect of target postgrazing height on sward clover content, herbage yield, and dairy production from grass-white clover pasture. *Journal of Dairy Science* 96, 1598-1611.

Schulte R. P., Diamond J., Finkele K., Holden N. M. & Brereton A. J. (2005) Predicting the soil moisture conditions of Irish grasslands. *Irish Journal of Agricultural and Food Research 44*, 95-110

Tuohy P., Fenton O., Holden N. M. & Humphreys J. (2014) The effects of treading by two breeds of dairy cow with different live weights on soil physical properties, poaching damage and herbage production on a poorly drained clay-loam soil. *Journal of Agricultural Science 153*, 1424-1436

Demonstrating the impact of genetic merit on ewe performance in a grass-based system

Fetherstone N.[1,2], McHugh N.[1], Boland T.M.[2] and McGovern F.M.[1]
[1]*Animal and Bioscience centre, Teagasc, Mellows Campus, Athenry, Co. Galway, Ireland;* [2]*School of Agriculture & Food Science, University College Dublin, Dublin 4, Ireland*

Abstract

Records from 108 pedigree Suffolk and Texel ewes from three treatment groups: New Zealand ewes of high genetic merit (High NZ), Irish ewes of high genetic merit (High Irish) and Irish ewes of low genetic merit (Low Irish) were analysed in order to investigate lamb live-weight production and ewe conversion efficiency for animals of varying genetic merit and origin. Lamb live-weight (kg) was recorded at birth and at six weeks. Ewe live-weight (kg) and body condition score (BCS) were measured at lambing, six weeks post-lambing and at weaning. Change in live-weight (kg) and BCS from birth to six weeks post-lambing and from birth to weaning was calculated. Estimated dry matter intake (DMI; kg dry matter (DM) day^{-1}) was recorded at six weeks post-lambing. Litter live-weight gain from birth until six weeks post-lambing, kg lamb reared per kg ewe live-weight at six weeks post-lambing, lamb live-weight gain per kg estimated ewe DMI at six weeks post-lambing were calculated (kg day^{-1}). The *n-alkane* DMI technique was carried out. Ewe live-weight and BCS did not differ significantly at any time point between the three treatment groups, nor did the change between any two time points. Compared to Low Irish ewes, at six weeks post-lambing, high genetic merit ewes (High NZ & Irish) had a greater litter live-weight gain, produced a greater amount of lamb live-weight per kg of ewe live-weight and had a more efficient DMI to lamb live-weight conversion, regardless of number of lambs reared per ewe. To conclude, high genetic merit ewes, regardless of origin, are more efficient in terms of lamb live-weight production and ewe conversion efficiency than low genetic merit ewes.

Keywords: sheep, genetic merit, live-weight, dry matter intake, grass-based production

Introduction

With challenges of food security, sustainable production and land availability at the forefront of debate within the agri-industry, the importance of efficiency becomes increasingly more pertinent. Within the sheep industry, it has been demonstrated that milk yield is a driver of superior lamb growth in early lactation (McGovern *et al.*, 2015). Increased availability of milk will improve weaning weight, which in turn, reduces days to slaughter and therefore, improves overall efficiency of the ewe (Santos *et al.,* 2015). The overall aim was to investigate whether, through the use of high genetic merit animals sourced from domestic or foreign genetics, it would be possible to increase lamb live-weight production or ewe conversion efficiency, through increasing output per ewe in a grass based system.

Materials and methods

Data were collected as part of a genetic merit validation study, conducted at Teagasc, Athenry, Co. Galway, Ireland operated on a grass based system, over a two-year period (2016 and 2017).Three treatment groups were established, containing high genetic merit New Zealand animals (High NZ), high genetic merit Irish animals (High Irish) and low genetic merit Irish animals (Low Irish), as selected from New Zealand's Maternal Worth Index and Ireland's €uro-star Replacement Index, respectively. Ewes of high genetic merit are ranked within the top 20% of animals while ewes of low genetic merit are within the bottom 20%. Ewes were artificially inseminated to a synchronized oestrus within genetic strain and pregnancy scanned. The mean lambing date was 8 March. Eighteen ewes were sampled for daily dry matter intake from each treatment group each year. They were balanced for rearing litter size and breed, each containing

8 single and 10 twin rearing Suffolk and Texel ewes (50:50 split). Lamb live-weight (kg) was recorded at birth and at six weeks post-lambing and used to assess the genetic capacity of lamb growth. Ewes were weighed (kg) and body condition scored (BCS; using a 1 to 5 scale) at lambing, six weeks post-lambing and weaning. Number of lambs scanned and weaned per ewe was recorded. Total litter live-weight reared per ewe at birth and at six weeks post-lambing was calculated on a per ewe basis. To assess the importance of ewe milk yield in early lactation, litter live-weight gain from birth to six weeks post-lambing and kg of lamb reared per kg ewe live-weight at six weeks post-lambing were calculated.

At turnout post-lambing, all groups of ewes grazed a perennial ryegrass (*Lolium perenne*) sward to 3.5 cm for the first rotation and to 4.0 cm thereafter, as part of a rotational grazing system with 11 kg N ewe^{-1} year^{-1} applied. Each of the opening spring and average first rotation herbage covers (kg ha^{-1}) were similar across the three different grazing groups. Dry matter intake (DMI; kg dry matter (DM) day^{-1}) was estimated using the *n-alkane* technique (Dove and Mayes, 1996) at six weeks post-lambing. This involved administering a daily *n-alkane* bolus to each ewe for 11 days. Faecal collection commenced on day 6 for the next six days. Herbage samples, representative of the herbage available to the ewes were collected from day 6 to 11. Faecal samples were pooled by ewe and dried at 40 °C for 48 hours. Herbage samples were bowl chopped and freeze dried. The ratio of herbage ^{33}C alkane (tritriacontane) to dosed ^{32}C alkane was used to estimate DMI. Lamb live-weight (kg) produced per kg of estimated ewe DMI at six weeks post-lambing was calculated, by dividing the average daily gain (kg day^{-1}) of the litter by the estimated daily DMI of the ewe at six weeks (kg DM day^{-1}).

Data were analysed using a linear mixed model in PROC MIXED (SAS 9.4) where genetic strain (breed and genetic merit), ewe age, number of lambs scanned or weaned and year were included as fixed effects. Ewe age and lamb's sire within genetic strain were included as repeated and random effects, respectively.

Results and discussion

Live-weight and BCS of High NZ, High Irish and Low Irish ewes were similar at lambing, at six weeks post-lambing and at weaning ($P>0.05$; Table 1). Demonstrating the mobilization of ewe body fat during early lactation, there was a change in live-weight and BCS between lambing and six weeks post-lambing for Low Irish ewes that gained considerably more than High Irish and High NZ ewes, which lost live-weight or either lost or retained BCS during the same time period. Low Irish ewes gained more BCS than High Irish and High NZ but neither live-weight and BCS were statistically significant ($P>0.05$). Litter live-weight per ewe (kg) was similar at birth but High Irish (3.26 kg) and High NZ (3.36 kg) ewes produced more lamb live-weight at six weeks post-lambing compared with the Low Irish ewes ($P<0.05$). High genetic merit ewes (NZ and Irish) had a greater litter live-weight gain from birth until six weeks post-lambing compared to the Low Irish ewes ($P<0.05$).

Ewe efficiency results proved the ability of high genetic merit ewes to produce a greater amount of lamb live-weight per kg of ewe live-weight at six weeks post-lambing, regardless of origin ($P<0.05$). Results showed that High Irish ewes were able to produce an extra 47 g of lamb per kg of ewe live-weight at six weeks post-lambing, compared with the Low Irish ewes ($P<0.05$). This suggests that a 75 kg High Irish ewe had the potential to produce an extra 3.525 kg in total litter live-weight when the lambs reached six weeks old compared with a Low Irish ewe. High NZ ewes had a superior ability to convert DMI into lamb live-weight gain over the High and Low Irish ewes, thereby promoting greater lamb daily live-weight gain per kg DMI at six weeks post-lambing ($P<0.05$).

Table 1. Live-weight and conversion efficiency of High NZ, High Irish and Low Irish ewes.[1]

	New Zealand	High Irish	Low Irish	SEM	*P*-value
Ewe live-weight, kg					
Lambing	82.27	82.95	81.55	3.824	NS
Six weeks post-lambing	83.11	82.63	85.19	3.614	NS
Weaning	78.26[a]	73.43[b]	76.53[ab]	2.682	<0.05
Ewe BCS, 1-5 scale					
Lambing	3.16	3.05	3.02	0.172	NS
Six weeks post-lambing	3.14	3.06	3.08	0.148	NS
Weaning	3.31	3.28	3.20	0.096	NS
Litter live-weight, kg					
Birth	7.07	7.05	6.80	0.661	NS
Six weeks post-lambing	27.36[ab]	27.26[a]	24.00[b]	2.091	<0.05
Gain from birth to six weeks post-lambing	20.23[a]	20.14[a]	17.06[b]	1.788	<0.05
Ewe conversion efficiency					
Dry matter intake, kg DM day^{-1}	2.20	2.43	2.35	0.211	NS
Lamb live-weight produced per kg ewe live-weight at six weeks post-lambing, kg	0.260[a]	0.260[a]	0.213[b]	0.0262	<0.05
Lamb live-weight gained per kg ewe dry matter intake at six weeks, kg	0.244[a]	0.206[b]	0.181[b]	0.0224	<0.05

[1] SEM = standard error of the mean; DM = dry matter; BCS = body condition score; NS = not significant.

Conclusions

Ewes of high genetic merit have a greater litter live-weight gain from birth to six weeks post-lambing, can produce more lamb live-weight relative to the ewe's own live-weight and require less DMI to do so, all regardless of country of origin. This demonstrates the potential benefit of widespread use of high genetic merit animals in order to increase the overall productivity and efficiency of the current Irish and ultimately, global sheep industry.

References

Dove H. and Mayes R.W. (1996) Plant wax components: a new approach to estimating intake and diet composition in herbivores. *Journal of Nutrition* 126, 13-26.

McGovern F.M., Campion F.P., Sweeney T., Fair S., Lott S. and Boland T.M. (2015) Altering ewe nutrition in late gestation: ii. the impact on fetal development and offspring performance. *Journal of Animal Science* 93, 4873-4882.

Santos B.F.S., McHugh N., Byrne T.J., Berry D.P. and Amer P.R. (2015) Comparison of breeding objectives across countries with application to sheep indexes in New Zealand and Ireland. *Journal of Animal Breeding and Genetics* 132, 144-154.

Challenges in evaluating mycotoxins in grass silages

Franco M.[1], Manni K.[1], Detmann E.[2], Rämö S.[1], Huuskonen A.[1] and Rinne M.[1]
[1]Natural Resources Institute Finland (Luke), Finland; [2]Federal University of Viçosa (UFV), Brazil

Abstract

Filamentous fungi (moulds) are found all over the world and in different climates. Some fungal species produce toxic substances (mycotoxins) as a defence under environmental stress or competition for substrate. Correct knowledge of type and quantity of mycotoxins in feeds is important to avoid harmful effects on animals, caretakers and product quality. The objective of this study was to evaluate the ability of different laboratories across Europe in converging similar results for mycotoxins in grass silages produced in Finland. Ten samples comprising different hygienic qualities were sent to four laboratories in Europe. Results of mycotoxin analyses showed no consensus among laboratories. This indicates a serious challenge in controlling the potential hazards of mycotoxin contamination in grass silages. Emphasis should be given to develop analytical systems to be more reliable and in line with each other.

Keywords: collaborative study, feed contamination, laboratory analysis, methodology

Introduction

Mycotoxins are secondary metabolites of filamentous fungi (moulds) that cause detrimental effects on crops, animals and humans, causing health problems and economic losses. These toxic substances can be found in feeds used in animal feeding that can be contaminated at various stages in the feeding chain from soil, plant production, harvesting, and preservation until the feeding phase. Typically, mycotoxin-producing fungi can be divided to those produced on field while plants are growing (*Fusarium, Cladosporium* and *Alternaria*) and to those formed during the storage period (*Aspergillus* and *Penicillium*). Presence of mycotoxins in feedstock can cause detrimental effects on animal and human health and massive economic losses worldwide in agricultural trade. Ruminant animals such as goats, sheep, cattle or even deer are known to be less sensitive to negative effects of mycotoxins than non-ruminants. Nevertheless, milk and meat production, reproduction and growth can be reduced when ruminants ingest feed contaminated with mycotoxins during extended periods (Hussein and Brasel, 2001). A serious problem that may arise in dairy production system is the transformation of aflatoxins into hydroxylated metabolites, which can be found in milk and dairy products obtained from livestock that have ingested contaminated feed (Boudra *et al.*, 2007). According to Zain (2011), some of these compounds ingested by humans may present carcinogenic properties. Accurate knowledge of type and amount of mycotoxins in feeds is important in identifying the weaknesses of feed production system. It is also possible to use binders (Nazarizadeh and Pourreza, 2019) under conditions where mycotoxin contamination is unavoidable, but to do that efficiently, the type of mycotoxin and level of feed contamination should be known. Currently there is no consensus among laboratories on methods for measuring mycotoxins in animal feeds. On this basis, there is a real need for further exploration to develop methodologies of mycotoxin analysis particularly for forages, as many of the methods are sensitive to the matrix. So far, more emphasis has been put on cereals, but since forages form the major part of cattle diets this information is also needed. The objective of this collaborative study was to evaluate the inter-laboratory variation between four different laboratories across Europe with regard to mycotoxin analysis in fresh grass and grass silages produced in Finland.

Materials and methods

An exploratory ring-test study was carried out to evaluate the mycotoxin contents in a set of forages. A total of ten samples (two fresh grasses and eight silages) were compiled from Luke's samples base (Table 1) comprising a wide range of hygienic quality matrixes (Franco *et al.*, 2019a,b). All samples were

Table 1. Experimental samples used for the ring-test.

Sample description	Abbreviation	Sample description	Abbreviation
Timothy grass	Tim	Red clover grass	Rc
Tim ensiled untreated with additive	TimUT	Rc ensiled untreated with additive	RcUT
Tim ensiled with formic acid	TimFA	Rc ensiled with formic acid	RcFA
TimUT contaminated with soil	TimUTsoil	RcUT contaminated with soil	RcUTsoil
TimFA contaminated with soil	TimFAsoil	RcFA contaminated with soil	RcFAsoil

divided into four equal amounts, packed into plastic bags with proper identification known only by the researchers, but blind for the laboratories. Samples were sent to four laboratories in Europe, comprising Austria, Estonia, Finland and Poland. Key questions for the elucidation of the main parameters and analytical procedures employed were sent to each laboratory. Laboratories were asked to analyse the samples using their current protocol and they used different quantification methods, such as LC-MS/MS API4000 by Sciex (Lab 1), EN 17280 using LC-MS/MS (Lab 2), enzyme immunoassay for the quantitative determination of deoxynivalenol and zearalenone (Lab 3) and QuEChERs extraction and dSPE clean-up with identification and quantification by UPLC-MS/MS with MRM technique (Lab 4). For ethical reasons, the laboratory names will not be disclosed. Data were compiled for every laboratory, processed, presented and described as descriptive data.

Results and discussion

Each laboratory provided the results with different amounts of replicates per sample. Lab 1 supplied four replicates, Labs 2 and 4 provided two replicates each, and Lab 3 used only one replicate. There were no substantial differences between replicates within each laboratory. The final compilation of data comprised 17 different types of mycotoxins, although not all laboratories analysed all mycotoxins. For instance, Labs 3 and 1 supplied only two and six mycotoxin types, respectively. Deoxynivalenol (DON) was analysed only by Labs 1 and 3 (Table 2). In Lab 1 DON was detected only in TimUTsoil (2.8 μg kg^{-1}) and RcFA (9.1 μg kg^{-1}), however Lab 3 detected DON for all ten samples with concentrations varying from 293 to 1157 μg kg^{-1}. Zearalenone (ZEA) was analysed by all laboratories. Labs 1 and 3 detected it in all samples, Lab 2 reported ZEA in only half of the samples while it was not detected in any sample in Lab 4. The ZEA variation was 0.5 to 18.4, 7.7 to 19.0 and 189 to 791 μg kg^{-1} for the Labs 1, 2 and 3, respectively, showing a serious discrepancy between detected quantities between the laboratories. Nivalenol (1.3 μg kg^{-1}) was detected only in RcFA in Lab 1. The HT2 toxin was detected in six samples by Lab 1 and varied from 0.2 to 3.5 μg kg^{-1}. Beauvericin (BE), enniatin A1 (EA), enniatin B, enniatin B1 (EB1), moniliformin and sterigmatocystin were detected by Lab 2 resulting in averages of 4.3, 16.0, 200.7, 16.8 and 3.2 μg kg^{-1}, respectively. Lab 4 also detected BE, EA and EB1, however below limit of quantification (LOQ; <25, <10 and <10 μg kg^{-1}, respectively).

The Rc was the only sample where ergocornine, ergocorninine and ergosine were detected and only by Lab 2, and the averages were 7.44, 12.23 and 5.88 μg kg^{-1}, respectively. Moniliformin (222 μg kg^{-1}) was analysed only by Lab 2 and detected only in RcFAsoil. Roquefortine C (ROC) was detected in RcUT by Labs 2 and 4 (120.5 and 38.5 μg kg^{-1}). The ROC was also detected in 3 other samples by Lab 4, however lower than LOQ (<25 μg kg^{-1}). Mycophenolic acid was detected only by Lab 4 in RcUT, however lower than the LOQ (<30 μg kg^{-1}). Mycotoxins such as diacetoxyscirpenol and T2 toxin were not detected in any of the samples or laboratories involved in this collaborative study.

Table 2. Descriptive data of mycotoxins detected in different laboratories.[1]

	Lab 1 (n=4)	Lab 2 (n=2)	Lab 3 (n=1)	Lab 4 (n=2)
Tim	HT2, ZEA	ZEA, BE, EA, EB, EB1, STE	DON, ZEA	BE
Rc	HT2, ZEA	BE, EA, EB, EB1, ERC, ERN, ERG	DON, ZEA	BE, EA, EB1
TimUT	ZEA	BE, EA, STE	DON, ZEA	BE, ROC
TimFA	HT2, ZEA	ZEA, EA, EB, EB1, STE	DON, ZEA	BE, EA, EB1
TimUTsoil	HT2, ZEA, DON	-	DON, ZEA	BE
TimFAsoil	ZEA	ZEA, EB1, STE	DON, ZEA	BE, ROC
RcUT	HT2, ZEA	ZEA, BE, EA, EB, EB1, ROC	DON, ZEA	BE, EA, EB1, ROC, MA
RcFA	HT2, ZEA, DON, NIV	ZEA, BE, EA, EB, EB1	DON, ZEA	BE, EA, EB1, ROC
RcUTsoil	ZEA	BE, EA, EB, EB1	DON, ZEA	BE, EA, EB1
RcFAsoil	ZEA	BE, EA, EB, EB1, MO	DON, ZEA	BE, EA, EB1

[1] n = number of replicates per laboratory; BE = beauvericin; DON = deoxynivalenol; EA = enniatin A1; EB = enniatin B; EB1 = enniatin B1; ERC = ergocornine; ERN = ergocorninine; ERG = ergosine; HT2 = HT2 toxin; MA = mycophenol acid; MO = moniliformin; NIV = nivalenol; ROC = roquefortine C; STE = sterigmatocystin; ZEA = zearalenone.

Conclusions

According to this collaborative study to investigate mycotoxins in grass silages produced in Finland, there was no consensus among laboratories for the results of mycotoxin analyses. To be able to cope with the potential risks of mycotoxins in grass silages, emphasis should be given to develop the analytical systems that are more reliable and in line with each other.

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References

Boudra H., Barnouin J., Dragacci S. and Morgavi D.P. (2007) Aflatoxin M_1 and ochratoxin A in raw bulk milk from French dairy herds. *Journal of Dairy Science* 90, 3197-3201.

Franco M., Stefanski T., Jalava T., Huuskonen A. and Rinne M. (2019a) Manipulation of mixed red clover and grass silage quality through compaction, soil contamination and use of additives. In: Udén P., Eriksson T., Spörndly R., Rustas B-O. and Karlsson J. (eds.) *Proceedings of the 10th Nordic Feed Science Conference*. Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, pp. 143-149.

Franco M., Stefanski T., Jalava T., Huuskonen A. and Rinne M. (2019b) Effect of compaction, soil contamination and additive treatments on grass silage quality. In: Jambor V. (ed.) *Proceedings of the 18th International Symposium Forage Conservation*. Mendel University, Brno, Czech Republic, pp. 108-109.

Hussein H.S. and Brasel J.M. (2001) Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology* 167, 101-134.

Nazarizadeh H. and Pourreza J. (2019) Evaluation of three mycotoxin binders to prevent the adverse effects of aflatoxin B_1 in growing broilers. *Journal of Applied Animal Research* 47, 135-139.

Zain M.E. (2011) Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society* 15, 129-144.

Type of protein supplementation on dairy cow performance on grass silage-based diet

Halmemies-Beauchet-Filleau A., Jaakkola S., Kokkonen T. and Vanhatalo A.
Department of Agricultural Sciences, University of Helsinki, Finland

Abstract

We studied if the milk production response to faba bean (*Vicia faba*: FB) can be improved by rumen-protected methionine, to a comparable level with rapeseed meal. Twelve Nordic Red cows averaging 96 d in milk were used in three 4×4 Latin squares with 21-d periods. Treatments were total mixed rations (TMR) including unsupplemented control and isonitrogenously given rapeseed meal, FB meal or FB meal with rumen-protected methionine (15 g d^{-1}). Concentrates (500 g kg^{-1} TMR dry matter (DM), crude protein (CP) 130 or 190 g kg^{-1} concentrate DM) contained also barley, oats and molassed sugarbeet pulp. Forage was regrowth grass silage (10.5 MJ ME kg^{-1} DM, 175 g CP kg^{-1} DM). Protein supplementation increased DM intake, but there was no significant difference in milk yield between the diets. Protein supplementation or protein source did not alter milk fat or lactose concentration. Milk protein concentration was increased by protein supplementation, and methionine supplementation of FB diet (34.8 vs 36.6 g kg^{-1}). Milk urea was increased by protein supplementation. The protein supplementation of grass silage-based diet did not improve milk yield and there was no difference in milk production responses between rapeseed and FB. However, methionine supplementation of FB increased milk protein concentration.

Keywords: grass silage, faba bean, rapeseed, methionine

Introduction

European animal production is largely dependent on imported soya bean (*Glycine max*). Therefore, finding alternative domestic protein feeds is of interest. The milk production response of rapeseed (*Brassica napus*) meal is superior to that of soya bean meal on grass silage-based diets of dairy cows (Huhtanen *et al.,* 2011). Faba bean (*Vicia faba*) is an ancient, underexploited grain legume with N-fixing capacity and crop rotational benefits. The protein in faba beans is, however, low in methionine, which is often the limiting amino acid for the lactation performance of dairy cows (Halmemies-Beauchet-Filleau *et al.*, 2018). The aim of this study was to compare the milk production response of rapeseed meal and faba bean protein on grass silage-based diets typical of Finnish dairy cow feeding regimen. We hypothesised that (1) protein supplementation improves milk production, and (2) the milk production response of faba bean protein is inferior to that of rapeseed meal, but (3) can be improved by rumen-protected methionine supplement.

Materials and methods

The dairy cow study was conducted at the University of Helsinki Viikki Research Farm in Finland during spring 2019. Twelve multiparous Nordic Red cows averaging 96 d in milk were used in 4×4 Latin squares with 21-d periods. Treatments were total mixed rations including unsupplemented control with no protein feed and isonitrogenously given rapeseed meal, faba bean meal, or faba bean meal supplemented with rumen-protected methionine (15 g d^{-1}). Concentrates (500 g kg^{-1} total mixed ratio dry matter, crude protein 130 or 190 g kg^{-1} concentrate dry matter) contained also barley, oats and molassed sugarbeet pulp. Forage was regrowth grass silage (10.5 MJ ME kg^{-1} dry matter, 175 g crude protein kg^{-1} dry matter) from timothy-meadow fescue (*Phleum pratense-Schedonorus pratensis*) ley. Total mixed rations were fed *ad libitum*. In addition, all animals were given molassed sugarbeet pulp 1 kg d^{-1} during milking. The rumen-protected methionine was mixed with molassed sugarbeet pulp. The cows were housed in tie-stalls and milked twice daily. Feed, milk and plasma samples were analysed by standard methods as described previously by Lamminen *et al.* (2019). The data were analysed by ANOVA with a model that included the fixed effects of treatment, square and period within a square, and random effect of cow within a square (SAS 9.4.). The contrasts were (1) protein supplementation, (2) rapeseed meal *versus* faba bean diets and (3) the use of rumen-protected methionine on faba bean diet.

Results and discussion

Protein supplementation increased dry matter intake (Table 1, P<0.001), but there was no significant difference in milk yield between the diets. This was unexpected as protein supplementation in general improves milk yield (Huhtanen *et al.*, 2011) and the milk production response of faba bean protein is in general inferior to that of rapeseed (Lamminen *et al.*, 2019; Puhakka *et al.*, 2016). In this experiment, the basal grass silage contained high amount of crude protein. This together with protein in cereals was enough to fulfil the nitrogen requirements of rumen microbes and further mammary demand for circulating essential amino acids that are principally of microbial and feed origin to synthesize milk protein. Plasma methionine concentration was numerically the lowest for faba bean diet (Figure 1), but it was almost doubled by rumen-protected methionine supplementation (P<0.001). Protein supplementation or protein source did not alter milk fat or lactose concentration, but affected milk protein. Milk protein concentration was increased both by protein supplementation (P=0.004) and methionine supplementation of faba bean diet (P<0.001) in particular. For methionine supplementation, part of this increase may be due to concentration effect as methionine supplementation of faba bean protein tended to decrease milk yield (P=0.084). The possible reason for this tendency is, however, unclear. It

Table 1. Type of protein supplementation on dairy cow performance on grass silage-based diet.

	Treatment				SEM	Significance		
	Control	Rapeseed	Faba bean	Faba bean + Met		Control vs protein feeds	Rapeseed vs faba beans	Met
Intake, kg dry matter d^{-1}	22.5	24.0	23.6	23.4	0.65	<0.001	0.088	0.421
Yield, kg d^{-1}								
Milk	30.7	31.2	32.2	30.5	1.65	0.446	0.849	0.084
Energy corrected milk	31.1	32.1	32.7	31.6	1.97	0.178	0.931	0.224
Content, g kg^{-1}								
Fat	41.0	42.1	41.8	42.1	1.20	0.290	0.865	0.836
Protein	35.0	35.5	34.8	36.6	0.69	0.004	0.272	<0.001
Lactose	43.8	43.7	43.8	43.9	0.61	0.943	0.683	0.765
Urea, mg dl^{-1}	22.0	32.0	33.0	33.6	1.49	<0.001	0.120	0.562
Milk N to feed N, g kg^{-1}	315	255	264	267	10.8	<0.001	0.070	0.669

[1] SEM = standard error of the mean.

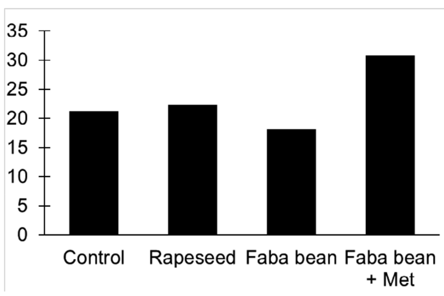

Figure 1. Plasma methionine concentrations (μmol l^{-1}).

seems that the low methionine content of faba bean protein was not a limiting factor for milk protein synthesis in the current study as milk protein yield was unaffected by methionine supplementation (data not shown). Protein supplementation increased milk urea and decreased the conversion of feed protein to milk protein (P<0.001) that are typical responses for extra protein in the diet (e.g. Puhakka *et al.,* 2016). In the control diet, the milk urea content of 22 mg dl^{-1} supports the adequacy of nitrogen relative to energy available for rumen microbes.

Conclusions

The protein supplementation increased feed intake as expected. However, the protein supplementation of grass silage of relatively high crude protein content and low energy content did not improve milk yield and decreased the conversion of feed protein to milk protein. There was no difference in milk production response between rapeseed and faba bean protein. However, methionine supplementation of faba bean improved milk protein concentration, but not milk protein yield.

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References

Halmemies-Beauchet-Filleau A., Rinne M., Lamminen M., Mapato C., Ampapon T., Wanapat M. and Vanhatalo A. (2018) Alternative and novel feeds for ruminants: nutritive value, product quality and environmental aspects. *Animal* 12(s2), s295-s309.

Huhtanen P., Hetta M. and Swensson C. (2011) Evaluation of canola meal as a protein supplement for dairy cows: a review and a meta-analysis. *Canadian Journal of Animal Science* 91, 529-543.

Lamminen M., Halmemies-Beauchet-Filleau A., Kokkonen T., Jaakkola S. and Vanhatalo A. (2019) Different microalgae species as a substitutive protein feed for soya bean meal in grass silage based dairy cow diets. *Animal Feed Science and Technology* 247, 112-126.

Puhakka L., Jaakkola S., Simpura I., Kokkonen T. and Vanhatalo A. (2016) Effects of replacing rapeseed meal with fava bean at 2 concentrate crude protein levels on feed intake, nutrient digestion, and milk production in cows fed grass silage-based diets. *Journal of Dairy Science* 99, 7993-8006.

Effect of regrowth period for perennial ryegrass on yield and nutritive value of grass

Hansen N.P.[1], Kristensen T.[2], Johansen M.[1] and Weisbjerg M.R.[1]
[1]*Department of Animal Science, Aarhus University, Foulum, Denmark;* [2]*Department of Agroecology, Aarhus University, Foulum, Denmark*

Abstract

During an eight-week period, perennial ryegrass was harvested at early and late maturity stage, corresponding to three and five weeks of regrowth. Each week, growth rate and stem proportion were determined, and chemical composition was analysed. During the experimental period, longer regrowth period resulted in increased growth rate and a lower nutritive quality of the grass. Irrespective of regrowth period, there was a large variation in growth rate and chemical composition, and thereby also in nutritive quality throughout the experiment. When optimising cutting strategy, these relations should be assessed in conjunction with the effects on feed intake and milk performance when grass is fed to dairy cows.

Keywords: perennial ryegrass, growth rate, organic matter digestibility, stem proportion

Introduction

Barn feeding with fresh cut grass has gained increasing interest for dairy cow management in Denmark. However, both the quality and quantity of grass change throughout the growing season and knowledge about field yield and nutritive value of the grass is a prerequisite for the farmer to optimise both grassland yields and utilisation of the grass by the cows. With longer regrowth period, field yield might increase, but digestibility of organic matter will decrease and dairy cows might respond by reducing feed intake (Johansen *et al.*, 2017). The objective of this experiment was to study the effects of a constant regrowth period of perennial ryegrass on growth characteristics and chemical composition. The presented results were obtained during a larger study, where the effects of harvesting grass with different lengths of period for regrowth on feed intake and milk performance in dairy cows were investigated also, but these results are not included in this paper.

Materials and methods

An experiment was conducted from mid-May to start-July 2019 at Aarhus University, Foulum, Denmark. The experiment started in week 20 and lasted for 8 weeks. In the experiment, fresh grass was harvested at early (3 weeks of regrowth; EG) and late (5 weeks of regrowth; LG) maturity stage. A field of perennial ryegrass (*Lolium perenne*) was divided into two blocks that were further divided into three and five plots for EG and LG, respectively. During the experiment, one plot from each of EG and LG were harvested at a time over a one-week period in order to supply feed for the feeding trial. By the end of the week, the remaining grass in the respective plots was harvested and removed. Each day, weight and dry matter (DM) content of harvested grass was measured to determine DM yield, and the sum within plot was used to calculate average growth rate during the 3 or 5 weeks of growth for EG and LG, respectively. All plots were fertilised with 78 kg N ha^{-1} on 2 March, and 78 kg N ha^{-1} on 28 May (week 22) or 4 June (the two plots that were harvested during week 22). Before the experiment started, plots within EG and LG were managed to obtain three and five weeks of regrowth. For EG, grass from all three plots were harvested and removed three weeks prior to harvest for each plot (Monday week 17, 18, and 19, respectively). For LG, grass in the first plot remained untouched, grass in the second plot was mown (Monday week 16), and grass in the remaining three plots were harvested and removed five weeks prior to harvest for each plot (Monday week 17, 18, and 19, respectively). After all plots had been harvested the first time during the experiment (starting from week 20), plots were harvested a second time in the same order, and for

EG, two plots were harvested a third time (week 26 and 27). The grass was harvested with a direct cut and loader wagon (Grass Tech Grazer, Borris, Co. Carlow, Ireland). Thursday each week, a subsample (approx. 130 g) of EG and LG, respectively, was divided into leaves (leaf blade) and stems (leave sheath, stem, and flower) by hand prior to DM determination, to determine leaf-to-stem ratio on DM basis. Within EG and LG, representative samples from harvested grass were pooled across three days weekly from week 22 to 27 for chemical analysis of neutral detergent fibre (NDF), acid detergent fibre (ADF), *in vitro* organic matter (OM) digestibility, and crude protein (CP). Statistical analyses were done in R (version 3.5.2) and the following model was used to analyse data for stem proportion and chemical composition:

$$Y_{mw} = \mu + \alpha_m + \beta_w + E_{mw}$$

where Y is the dependent variable, μ is the overall mean, α is the fixed effect of maturity stage (m = EG, LG), β is the fixed effect of week (w = 20 to 27 for stem proportion and w = 22 to 27 for chemical composition), and E is the random residual error assumed to be independent and normal distributed.

Results and discussion

Growth rate (kg DM ha^{-1} day^{-1}) varied throughout the experiment for both EG and LG (Figure 1A). EG was almost equal to LG in week 20 and 21, probably due to a very cold period in the beginning of May. In addition, grass harvested in week 21 had been mown five weeks earlier during cold weather, possibly hampering the regrowth in the beginning of that period. A limited amount of rain was recorded until week 24, when 34% of the total rainfall during the experimental period was recorded. The combination of rainfall and an increasing temperature (mean daily temperature exceeded 15 °C during week 23) probably caused the increased growth rate (Buxton, 1996) for week 24. As the temperature increased, the stem proportion and the concentration of NDF and ADF also increased (Figure 1B, C, and D, respectively) for both EG and LG. Across all weeks, stem proportion, NDF and ADF concentration were 14.4%-units, 46 and 34 g kg DM^{-1} higher in LG than EG (P<0.01). Stem proportion peaked in week 23 and 24 for EG and LG, respectively. The subsequent decrease in stem proportion in weeks corresponding to the second cut reflected that stem proportion in the second cut in general decreases when the first cut is delayed (Soegaard *et al.*, 2011). The change in stem proportion was reflected in the concentration of NDF and ADF, which peaked in week 24 and declined for the rest of the experiment. The increase in stem proportion is well reflected in a decreasing OM digestibility (Figure 1E), whereas OM digestibility starts to increase as stem proportion decreases. Across all weeks, OM digestibility was 3.6%-units higher in EG compared to LG (P<0.01). The difference in CP concentration between EG and LG was relatively constant from week 22 to 25 (Figure 1F). Until week 25, all plots had been fertilised twice; 50% before the experiment started and 50% after and during the first harvest for EG and LG, respectively. In week 26 and 27, grass from the plots in EG was harvested for the third time without having received further N fertiliser after the second harvest. The low concentration of CP in EG during week 26 and 27 might therefore have been caused by a depletion of N in the soil.

Conclusions

Longer regrowth period resulted in increased growth rate but lower nutritive quality of perennial ryegrass from mid-May until the beginning of July. Generally, there was large variation over time irrespective of regrowth period. The variation was related to weather conditions and nutrient accessibility. Difference on DM yield and nutritive quality will be assessed in conjunction with the effects on feed intake and milk performance, when subsequently feeding the grass to dairy cows.

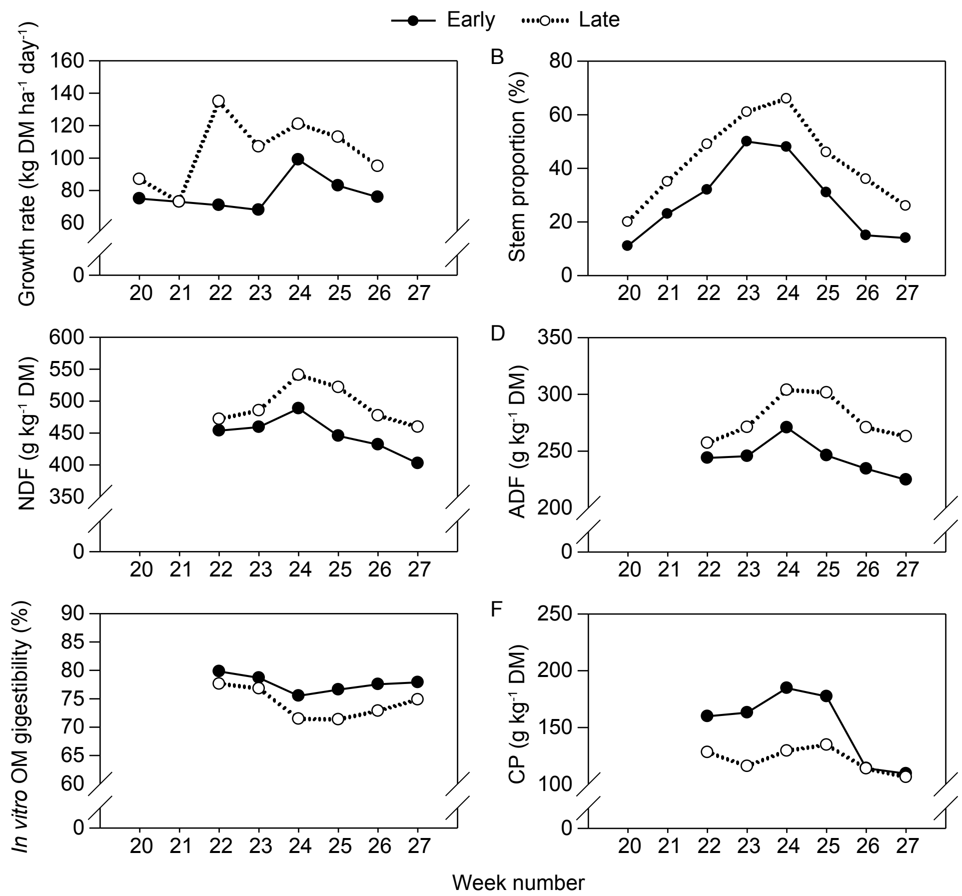

Figure 1. Development in growth rate (A), stem proportion (B), neutral detergent fibre (NDF) concentration (C), acid detergent fibre (ADF) concentration (D), *in vitro* organic matter (OM) digestibility (E), and crude protein (CP) concentration (F) in early (3 weeks regrowth) and late (5 weeks regrowth) cut grass during the experiment.

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References

Buxton D.R. (1996) Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science and Technology* 59, 37-49.

Johansen M., Søegaard K., Lund P. and Weisbjerg M.R. (2017) Digestibility and clover proportion determine milk production when silages of different grass and clover species are fed to dairy cows. *Journal of Dairy Science* 100, 8861-8880.

Soegaard K. (2011) Slætstrategi. Optimering af protein- og energiproduktion i rødkløverbaserede blandinger. *Oversigt over Landsforsøg 2011*. Landbrug og Fødevarer, Aarhus, Denmark. pp 350-355.

Beef production with dairy and dairy × beef breed steers based on forage and semi-natural pastures

Hessle A. and Arvidsson Segerkvist K.
Department of Animal Environment and Health, Swedish University of Agricultural Sciences, P.O. Box 234, 532 23 Skara, Sweden

Abstract

This study compared animal performance and carcass characteristics in steers born to a dairy breed dam and a dairy or beef breed sire allocated to one of two indoor feed intensities, both including grazing semi-natural pastures during summer. Groups comprising 16 purebred dairy (D) steers and 16 dairy × Charolais crossbreeds (C) were allocated to high indoor feed intensity with slaughter at 21 months of age (H), while two groups of 16 D and 16 C animals were allocated to low indoor feed intensity, slaughtered at 28 months of age (L). The animals were mainly fed grass-clover silage while housed. The H steers grazed semi-natural pastures for one summer, whereas the L steers grazed semi-natural pastures for two summers. From weaning to slaughter, live weight gain was 0.94 and 0.77 kg day^{-1} for H and L steers, respectively, with no breed effect on weight gain. However, C carcasses had higher weight, conformation score, and proportion of high-valued retail cuts than D carcasses. In conclusion, breed had no effect on the performance of living animals, but dairy × beef crossbred steers produced heavier and higher-quality carcasses than purebred dairy steers.

Keywords: animal performance, carcass characteristics, feed intensity, grazing, semi-natural pasture, crossbreeding

Introduction

Semi-natural pastures have been used to feed livestock for centuries. These landscapes are characterized by a specific native flora and fauna and are dependent on human management to be maintained (Emanuelsson, 2009). These areas cannot deliver human food except through grazing livestock and are therefore valuable for global food supply (Godfray *et al.*, 2010). Almost 60% of Swedish beef production is based on dairy cattle. Swedish male calves of dairy breeds are most often raised as bulls in intensive indoor systems. Only 22% are castrated and raised as steers, often on semi-natural pastures. A disadvantage with dairy steers is their low carcass conformation score (Gård and Djurhälsan, 2019). If carcass quality can be increased using beef breed sires, then rearing male calves born in dairy herds as steers in grazing systems can result in increased income in beef production. The objective of this study was to compare animal performance and carcass characteristics in steers born to a dairy cow and a dairy or a beef breed sire in two different production systems, both including grazing of semi-natural pastures.

Materials and methods

The experiment was conducted at SLU Götala Beef and Lamb Research, Skara, Sweden, and included 64 steers. Animals were pure dairy breed (D; 12 Swedish Red and 20 Swedish Holstein), and dairy × beef crossbreed (C; 12 Swedish Red × Charolais and 20 Swedish Holstein × Charolais). The D steers and the C steers were split into two sub-groups allocated to one of the two production systems differing in indoor feed intensity, the number of grazing periods and slaughter age. Spring-born calves were allocated to a moderately high feed intensity strategy with slaughter at 21 months of age (H) and turned out to pasture at 10-12 months of age. During the following indoor period they were fed an early-cut forage (379 g dry matter (DM) kg^{-1}, 10.2 MJ kg^{-1} DM), and slaughtered at the end of their second indoor period. Autumn-born calves were allocated to a low feed intensity with slaughter at 28 months (L) and turned out to pasture at 6-9 months of age. During the indoor period, they were fed late-cut forage (473 g DM kg^{-1}, 8.57 MJ kg^{-1} DM), allowing only low weight gain. They were then kept on grass for another grazing period and finally finished indoors with an early-cut forage (460 g DM kg^{-1}, 10.5 MJ kg^{-1} DM). The average feed intake was calculated weekly and the steers weighed every fortnight. For more details, see Hessle *et al.* (2019).

After slaughter, carcass conformation and fat cover were graded according to the EUROP classification system. Carcasses were then split into the fore- and hindquarters between the 10th and 11th ribs. The marbling score of *M. longissimus dorsi* was evaluated visually by using a 10-point scale based on the USDA standard (USDA, 2020). The right hindquarter from each animal was weighed, as were seven high-value retail cuts. The Mixed procedure in SAS was used for statistical evaluation and differences were considered significant if $P<0.05$.

Results and discussion

The C steers were heavier than D steers throughout their lifetime, and they had a higher feed intake expressed as kg DM but no differences in live weight gain or feed efficiency were detected (Table 1). The moderate dietary energy concentration was most likely too low for the C steers to utilise their higher genetic potential for growth. Further, H steers were heavier than L steers at the start of indoor period 2 (Table 1), and they had a higher feed efficiency than the latter. Feeding the H steers with earlier-harvested grass-clover silage than the L steers during the indoor period 2 contributed to an even greater difference (Table 1). Despite the modest breed effect on live weight gain, after slaughter, the superior traits of the crossbreeds were revealed (Table 2). Carcasses of CH steers were 32 kg heavier than those of DH steers, and when the rearing period was prolonged from 21 to 28 months of age the difference was even greater, where carcasses from CL steers weighed 50 kg more than carcasses from DL steers. Hence, comparison of live weight gain is not sufficient when evaluating the effects of beef breed crosses. The heavier carcass of the C steers was accompanied by a higher dressing percentage and a higher conformation score (Table

Table 1. Daily feed intake and daily live weight gain of purebred dairy (D) and crossbred dairy × beef (C) steers in two production systems (Prod. sys) with higher (H) or lower (L) feed intensity, slaughtered at 21 (H) or at 28 (L) months of age.[1]

	Item	Breed		Prod.sys.		SE	Significance	
		D	C	H	L		Breed	Prod.sys.
Indoor period 1	Initial live weight, kg	115	140	121	133	7.78	0.051	ns
	Dry matter intake, kg	5.43	5.63	5.85	5.21	0.12	0.036	0.026
	Feed efficiency, ME MJ g $gain^{-1}$	58.7	59.4	60.1	58.1	0.78	ns	0.095
	Live weight gain, kg	1.05	1.06	1.08	1.02	0.03	ns	ns
Grazing period 1	Initial live weight, kg	304	331	372	263	0.95	0.008	<0.0001
	Live weight gain, kg	0.51	0.55	0.46	0.60	0.03	ns	0.005
Indoor period 2	Initial live weight, kg	377	409	438	348	6.80	0.001	<0.0001
	Dry matter intake, kg	10.5	11.0	11.8	9.6	0.24	ns	0.004
	Feed efficiency, ME MJ g $gain^{-1}$	114	116	108	122	2.52	ns	0.022
	Live weight gain, kg	0.89	0.94	1.14	0.68	0.03	ns	<0.0001
Grazing period 2	Initial live weight, kg	466	517	-	491	12.8	0.020	-
	Live weight gain, kg	0.53	0.73	-	0.63	0.13	ns	-
Indoor period 3	Initial live weight, kg	548	602	-	575	12.5	0.006	-
	Dry matter intake, kg	13.5	14.3	-	13.9	0.20	0.058	-
	Feed efficiency, ME MJ g $gain^{-1}$	127	122	-	125	15.0	ns	-
	Live weight gain, kg	1.21	1.34	-	1.28	0.11	0.058	-
Weaning to slaughter	Live weight gain, kg	0.84	0.86	0.94	0.77	0.02	ns	<0.0001

[1] SE = standard error; ME = metabolizable energy; ns = not significant.

Table 2. Carcass characteristics of purebred dairy (D) and crossbreed dairy × beef (C) steers in two production systems (Prod.sys) with higher (H) or lower (L) feed intensity, slaughtered at 21 (H) or at 28 (L) months of age.[1]

Item	Breed		Prod.sys.		SE	Significance	
	D	C	H	L		Breed	Prod.sys.
Live weight, kg	640	682	628	695	10.4	0.003	0.0002
Carcass weight, kg	294	335	299	329	4.9	<0.0001	0.0002
Dressing, g kg^{-1}	45.8	49.1	47.6	47.3	0.36	0.0001	ns
Conformation[2]	4.0	5.7	4.6	5.1	0.20	0.0001	0.084
Fatness[3]	7.2	7.4	7.9	6.8	0.24	ns	ns
Marbling4[3]	2.0	1.5	1.7	1.8	0.17	0.054	ns
HQ, kg	74.6	85.7	77.3	83.1	1.21	<0.0001	0.003
Retail cuts[5], g kg^{-1} of HQ	36.7	38.3	37.1	37.9	0.31	0.004	0.086

[1] HQ = right hindquarter; SE = standard error.

[2] EUROP system: 4 = 0−, 5 = 0, 6 = 0+.

[3] EUROP system: 6 = 2+, 7 = 3−, 8 = 3.

[4] Visually determined on a scale 1 = lean and 5 = well-marbled.

[5] High-value retail cuts; strip loin, fillet, topside, outside round, eye of round, top rump, and rump steak.

2). Increased feed intensity tended to improve conformation score but effects on fatness or marbling score (Table 2) were not found. The C steers had a heavier hindquarter and higher proportions of valuable retail cuts compared to the D steers (Table 2). Comparisons between the production systems revealed that the hindquarter was heavier in L animals, but there were only minor differences in the proportion of valuable retail cuts between L and H.

Conclusions

Compared with purebred dairy steers, dairy × beef breed crosses had higher carcass weight, higher conformation score and higher proportion of valuable retail cuts, which are all traits of economic value. The breed difference in conformation was greater for extensively raised steers than for more intensively raised steers. Hence, using steers with dairy × beef crosses in beef production based on forage and semi-natural pastures results in more commercially valuable carcasses.

Acknowledgements

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References

Emanuelsson U. (2009) *The rural landscapes of Europe – How man has shaped European nature*, 1st ed., Formas Research Council, Stockholm, Sweden, 384 pp.

Gård and Djurhälsan (2019) *Carcass quality outcome for whole year 2018.* Available at: https://www.gardochdjurhalsan.se/wp-content/uploads/2019/06/kvalitetsutfall_helar_2018.pdf (in Swedish).

Godfray H.C.J., Beddington J.R., Crute I.R., Haddad L., Lawrence D., Muir J.F., Pretty J., Robinson S., Thomas S.M. and Toulmin C. (2010) Food security: the challenge of feeding 9 billion people. *Science* 327, 812-818.

Hessle A., Therkildsen M. and Arvidsson Segerkvist K. (2019) Beef production systems with steers of dairy and dairy × beef breeds based on forage and semi-natural pastures. *Animal* 9, 1064.

USDA (2020) USDA Grading Shields. Available at: https://www.ams.usda.gov/grades-standards/beef/shields-and-marbling-pictures.

Zero-grazing versus conventional grazing in early lactation autumn-calving dairy cows in Ireland

Holohan C.[1], Mulligan F.J.[2], Somers J.[3], Pierce K.M.[1] and Lynch M.B.[1]
[1]School of Agriculture and Food Science, University College Dublin, Lyons Farm, Lyons Estate, Celbridge, Naas, Co. Kildare, Ireland; [2]School of Veterinary Medicine, University College Dublin, Veterinary Science Centre, Belfield Dublin 4, Ireland; [3]Glanbia Ireland, Co. Kilkenny, Ireland

Abstract

'Zero-grazing' (the mechanical harvesting and feeding of fresh grass) is increasingly used in grass-based milk production systems alongside conventional grazing to supply freshly cut grass from outside land blocks to cows during periods of grass shortages on the main grazing block and where farm fragmentation limits grazing opportunities. This study aimed to determine the effect of zero-grazing versus conventional grazing in early lactation on the performance of autumn-calved dairy cows. Twenty-four Holstein-Friesian cows were blocked and assigned to one of two treatments in a randomized complete block design (n=12) for a 35d experimental period. The two treatments were: zero-grazing (ZG), and grazing (G). The ZG group were housed full-time and fed zero-grazed grass, while the G group grazed outdoors at pasture. Both treatment groups were offered *ad-libitum* quantities of perennial ryegrass (*Lolium perenne* L.) from within the same paddock. Average milk yield in the ZG treatment did not significantly differ from the G treatment (P>0.05). Likewise, zero-grazing did not have an effect on milk composition, somatic cell count, BCS, locomotion score, or rumen pH (P>0.05). Results suggest that zero-grazing did not affect cow performance and could be used as a means of utilising grass in the diet where conventional grazing is not possible.

Keywords: zero-grazing, cow performance, early lactation

Introduction

Zero-grazing (also known as 'cut and carry' or 'green chop') is an alternative feeding system where fresh grass is cut and fed directly to housed cows. The fresh grass is typically cut standing by one machine which transports the grass from the field to the housed cows. This is commonplace in some parts of mainland Europe (Meul *et al.*, 2012), however, conventional paddock grazing predominates in the temperate grass-based dairy production systems typically operated in Ireland. Although well-managed grazed grass is the most economical feed available for dairy cows in Ireland (Hanrahan *et al.*, 2018) there is growing interest in the role of ZG and its potential to compliment grazing systems (Agrisearch, 2018). Farm fragmentation and land availability are major barriers to expansion on Irish dairy farms and are considered the main driver in the uptake of ZG to date as it allows farmers to utilise outer land blocks as part of their grazing rotation. The objective of this study was to establish the effect on cow performance of using zero-grazing in the autumn period on early lactation autumn-calving dairy cows.

Materials and methods

The 7-week study was conducted between 1 October to 18 November 2018 at University College Dublin's Lyons Farm, Kildare, Republic of Ireland. Twenty-four autumn-calving Holstein-Friesian dairy cows in early lactation were blocked on days in milk (28±13), parity, predicted 305-day yield, body condition score (BCS) and body weight (BW), and assigned to one of two treatments in a randomised complete block design (n=12). Cows were offered the experimental diets for a 14-day dietary acclimatization period. Following this, cows remained on their treatments for a further 35-day experimental period. The two treatments were: zero-grazing (ZG), and grazing (G). The ZG group were housed full-time and fed zero-grazed grass, while the G group grazed outdoors at pasture. Both treatment groups were offered *ad-libitum* quantities of perennial ryegrass (*Lolium perenne* L.) and grazing and zero-grazing was carried out within the same paddock to ensure uniformity of grass quality offered to both groups. Average pre-cutting /pre-grazing yield over the experimental period was 2,024 kg dry matter (DM) ha^{-1} (±557). Post-cutting sward height for ZG was 4 cm, while post-grazing sward height for G was 5 cm. Both treatments received a daily buffer feed consisting of 2.5 kg DM grass silage and 1.8 kg DM maize meal directly before milking and 7.2 kg DM concentrates in-parlour. Grass for ZG treatment was harvested at 10:00 h daily using a specialised ZG machine (Zero-Grazer, Oldcastle, Ireland). Mean total distance walked per day between paddock and milking parlour for G group was 1.64 km (±0.37). Individual daily milk yield and weekly milk composition and quality were recorded. Rumen fluid samples were collected weekly via Flora Rumen scoop oral oesophageal sampler (Prof-Products, Guelph, ON, Canada) and samples were analysed immediately for pH (EC-25 pH/Conductivity Portable Meter, Phoenix Instrument, Garbsen, Germany). Locomotion scoring was performed on a bi-weekly basis using a 5-level ordinal scale (1 = healthy; 5 = severely lame). BCS was measured bi-weekly and determined using a 5-point scale (1 = emaciated; 5 = obese) with quarter-point increments and evaluated by the same trained researcher. Analysis of data was conducted using PROC MIXED of SAS (SAS Institute Inc.). The model included tests for the fixed effects of treatment, week, and their interactions. Statistical significance was assumed at $P<0.05$.

Results and discussion

In summary, zero-grazing did not have an effect on cow performance under the parameters measured in this study (Table 1). Average milk yield in the ZG treatment did not significantly differ from the G treatment ($P>0.05$). Likewise, ZG did not have an effect on milk fat or protein percentage. It is likely that *ad-libitum* feeding of pasture in the G treatment positively influenced milk production in that group. Where pasture feed budgeting is applied to grazing cows (as is commercially practised in grass-based milk production systems), lower milk production levels may be expected when compared to zero-grazed cows which are fed *ad-libitum* grass.

In terms of udder health and mastitis it has been previously reported that confined cows on average had higher somatic cell counts (SCC) and higher levels of clinical mastitis compared to those within grazing systems, possibly reflective of a greater general pathogen load and reduced hind-limb cleanliness within housed environments (Arnott *et al.*, 2015). However, zero-grazing did not have an effect on SCC in this study, with similar SCC levels observed between both treatment groups. Arnott *et al.* (2015) also reported increased levels of lameness/poorer foot health in cows with limited or no access to pasture. In this study zero-grazing had no effect on locomotion score. This would indicate that good udder and hoof health can be achieved in ZG systems where best management practices for indoor housing are applied.

Table 1. Observed cow performance parameters for each of the two treatments.[1]

	Zero-grazing	Grazing	SEM	*P*-value
Milk yield (kg cow^{-1} day^{-1})	31.67	30.54	2.119	<0.709
Milk solids (kg cow^{-1} day^{-1})	2.32	2.38	0.149	<0.761
Milk fat %	4.11	4.11	0.123	<0.997
Milk protein %	3.50	3.48	0.083	<0.878
SCC (cells ml^{-1})	57,000	72,000	23,000	<0.655
Locomotion score	1.79	1.56	0.193	<0.405
BCS	2.97	2.90	0.082	<0.583
Rumen pH	6.37	6.36	0.058	<0.869
Rumen ammonia (mM l^{-1})	8.03	8.11	0.478	<0.865

[1] SCC = somatic cell counts; BCS = body condition score; SEM = standard error of the mean.

A longer study period would however be beneficial in determining the long-term effect of ZG on hoof and udder health.

The study also found no significant difference in BCS between ZG and G. It has been previously suggested that grazed cows may mobilise more body reserves than zero-grazed cows due to lower feed intake and higher energy expenditure (Dohme-Meier *et al.*, 2014); however, the BCS results in this study indicate that energy expenditure and/or feed intake may have been similar in both ZG and G. The *ad-libitum* pasture feeding in the G treatment may have influenced this. There was also no effect on rumen pH or ammonia.

Conclusions

Zero-grazing in this study did not have an impact on cow performance when compared to conventional grazing and could be used as a means of utilising grass in the diet where conventional grazing is restricted due to land fragmentation or where grass from outside land blocks is required to fill feed deficits on the grazing block. The financial viability of using such a system requires further study.

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References

Agrisearch (2018) *Zero-grazing – A best practice guide.* Hillsborough: Agrisearch.

Arnott G., Ferris C. and O'Connell N. (2015) *A comparison of confinement and grazing systems for dairy cows: What does the science say?* Belfast: AgriSearch.

Dohme-Meier F., Kaufmann L., Görs S., Junghans P., Metges C., van Dorland H., Bruckmaier R. and Münger A. (2014) Comparison of energy expenditure, eating pattern and physical activity of grazing and zero-grazing dairy cows at different time points during lactation. *Livestock Science*, 162, 86-96.

Hanrahan L., McHugh N., Hennessy T., Moran B., Kearney R., Wallace M. and Shalloo L. (2018) Factors associated with profitability in pasture-based systems of milk production. *Journal of Dairy Science*, 101, 5474-5485.

Meul M., Van Passel S., Fremaut D. and Haesaert G. (2012) Higher sustainability performance of intensive grazing versus zero-grazing dairy systems. *Agronomy for Sustainable Development,* 32, 629.638.

Ensiling ability of species-rich mountain swards with elevated contents of condensed tannins

Ineichen S.[1], Wyss U.[2], Seiler A.B.[2] and Reidy B.[1]
[1]*School of Agricultural, Forest and Food Sciences HAFL, Bern University of Applied Sciences, Zollikofen, Switzerland;* [2]*Agroscope, Ruminant Research Unit, Posieux, Switzerland*

Abstract

Ensiling forage from species-rich swards is challenging due to low contents of fermentable carbohydrates and a high buffering capacity due to potential protein degradation. Species-rich swards from two mountain meadows were investigated for their fermentation quality and effects of condensed tannins (CT) on protein degradation during ensiling. Swards were obtained from two long-term fertiliser experiments located in the Swiss Alps, cut two or three times during the season of 2017. Swards were either unfertilised or fertilised with PK or NPK. Forages were wilted and ensiled in laboratory silos. Composition of nutrients, protein and CT were determined in wilted and ensiled forage. The fermentation quality was analysed >65 d post ensiling. Silages were characterised by high pH (4.60-5.19) and butyric acid content (1.5-17.8 g kg^{-1} dry matter (DM)), while ammonia-N ranged from 35 to 62 g kg^{-1} of total N. Protein fraction A increased by 20.7% during ensiling. Contents of CT varied from 5.0 to 18.0 g kg^{-1} DM. Contents of CT were not consistently correlated to the increase in protein faction A when related to type of fertilisation or harvest.

Keywords: biodiverse sward, fertilisation, protein fraction A, proteolysis, silage quality

Introduction

Mountain grasslands are rich in plant species composition and provide the major source of forage in mountain areas. Moderate contents of fermentable carbohydrates present in species-rich swards limit the formation of lactic acid to yield well-conserved silages. Legumes and herbs further increase the buffering capacity of the silage due to high contents of proteins, minerals and organic acids (Isselstein and Daniel, 1996). When compared to fresh or dried forage, ensiling increases protein degradation and the formation of readily soluble N, as measured by protein fraction A (Licitra *et al.*, 1996). Condensed tannins (CT), when added as silage additives, were shown to reduce protein degradation during ensiling as well as the formation of butyric acid (Jayanegara *et al.*, 2019). Numerous herbs have been described for their CT contents (Jayanegara *et al.*, 2011) and feed value when ensiled (Weißbach, 1998). However, only few studies have focused on the ensiling ability of species-rich swards with varying proportions of herbs from mountain sites (Wyss *et al.*, 2016). Therefore, this study assessed the ensiling quality and investigated the role of CT for protein degradation during ensiling of species-rich forage harvested from two Swiss mountains sites.

Materials and methods

The forage ensiled was obtained from two long-term mineral fertilisation field experiments located at 930 m a.s.l. (Thomet and Koch, 1993) and 1340 m a.s.l. (Baumberger *et al.*, 1996). Swards were either unfertilised (O) or fertilised with PK or NPK and sampled in three plots at each site. Annual amounts of fertiliser per ha were 75 kg N, 34.9 kg P, 199 kg K (at 930 m a.s.l.) and 80 kg N, 26.2 kg P and 149 kg K (at 1,340 m a.s.l.). During 2017, forage was harvested on 1 June, 8 August and 4 October (930 m a.s.l.) and 16 June and 23 August (1,340 m a.s.l.). The fresh forage was wilted to 30% dry matter (DM) and mechanically chopped to 2 cm and ensiled in 1.5 l laboratory silos for >65 d. Gross nutrient composition of wilted and ensiled forage was determined using near-infrared spectroscopy. Contents of CT (Terrill *et al.*, 1992) and protein fraction A (Licitra *et al.*, 1996) were determined in lyophilised samples.

Fermentation acids and ethanol were determined by gas chromatography. Silage pH and ammonia-N were determined electrometrically. Correlation analysis was applied to assess the effect of CT on the increase in protein fraction A (difference of ensiled to wilted forage). Analysis of variance was conducted including 'harvest, *h*' and 'type of fertilisation, *f*' and their interaction ('$h \times f$') on silage quality using Sigmaplot (V12.5). Differences among arithmetic means were considered significant at $P<0.05$.

Results and discussion

Silage quality of biodiverse swards was affected differently by harvest and fertilisation at the two grassland sites, with an overall more pronounced effect of harvest rather than fertilisation (Table 1). Protein concentrations of wilted forage were low when compared to intensively managed ryegrass swards, but comparable to those from other mountain swards (Wyss *et al.*, 2016). The formation of lactic acid was limited in all silages and, consequently, silage pH was too high in relation to silage DM contents. Ethanol formation in silages was similar between both sites and within expected ranges (Wyss *et al.*, 2016). While the concentrations of acetic acid were lower in silages from the site at 930 m a.s.l. than those at 1,340 m a.s.l., for concentrations of butyric acid the opposite effect was found. The higher the contents of CT, the lower the increase in protein fraction A from wilted to ensiled forage (Figure 1). However, a significant correlation was only observed in silages from forage fertilised with NPK. Those swards were particularly rich in CT, likely due to the presence of *Geranium sylvaticum*, which was particularly abundant in these swards (Ineichen, 2018).

Conclusions

The silage quality of the mountain grassland swards subjected to either no, PK or NKP fertilisation was insufficient with respect to silage pH and butyric acid contents. The silage quality produced from swards harvested from the grassland located at the lower elevation mountain site were primarily affected by harvest rather than fertilisation, while those from the higher located site showed more of interactions of '$h \times f$'. Based on the correlation of the CT content and the increase in protein fraction A from wilted to ensiled forages (i.e. ΔA), the effect of harvest tends to be stronger than that of fertilisation. The seasonal occurrence of specific tanniferous forage species may primarily contribute to the harvest-related effect.

Table 1. Content of condensed tannins (CT) and crude protein (CP) of wilted forage and silage quality (g kg^{-1} dry matter (DM) unless otherwise stated) of forage harvested from species-rich swards of two long-term mineral fertilisation field experiments in the Swiss mountains.

	930 m a.s.l.							1,340 m a.s.l.						
	0	PK	NPK	SEM	*h*	*f*	$h \times f$	0	PK	NPK	SEM	*h*	*f*	$h \times f$
Wilted														
CT	10.3	12.9	12.5	0.53	**	*	ns	7.9	7.4	8.7	0.44	ns	ns	**
CP	131	130	115	2.9	*	*	ns	118	129	130	2.9	ns	*	**
Ensiled														
DM	351	394	347	14.4	**	**	ns	322	357	334	6.00	*	*	ns
pH [1]	4.60	5.19	4.88	0.059	**	**	ns	4.96	5.15	5.03	0.052	**	ns	*
Lactic acid	35.0	24.2	32.1	1.85	**	**	**	34.5	31.7	33.8	1.62	ns	ns	*
Acetic acid	3.29	2.29	2.95	0.340	*	ns	ns	9.45	3.89	7.45	1.069	**	**	**
Butyric acid	11.2	7.9	13.3	1.25	**	ns	ns	3.9	4.1	2.1	0.55	ns	ns	*
Ethanol	5.37	6.10	5.46	0.542	**	ns	ns	5.39	5.81	5.03	0.496	ns	ns	ns
NH_3-N [g kg N^{-1}]	45.2	41.9	36.7	3.25	**	ns	ns	44.2	52.5	62.5	2.77	ns	*	ns
Fraction A	54.5	48.1	36.6	3.82	**	*	ns	48.6	51.3	49.6	1.79	ns	ns	ns

[1] Type of fertilisation: unfertilised swards (0), PK or NPK; *h* = harvest; *f* = fertilisation; $h \times f$ = interaction of harvest and fertilisation; SEM = standard error of means; fraction A = protein fraction A (Licitra *et al.*, 1996); ns = not significant; * $P<0.05$; ** $P<0.01$.

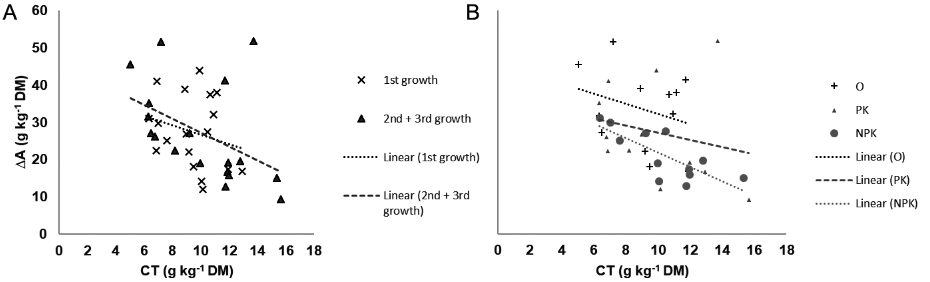

Figure 1. Relationship between condensed tannins (CT) content (g kg^{-1} dry matter (DM)) and the increase in protein fraction A from wilted to ensiled forage during the first ($y = -1.26x + 39.4$, $P>0.05$, $R^2=0.0582$) or following regrowth ($y = -1.87x + 45.9$, $P=0.05$, $R^2=0.2193$) (A) and in relation to type of fertilisation: 0: $y = -1.38x + 45.9$, $P>0.05$, $R^2=0.0876$; PK: $y = -0.98x + 36.9$, $P>0.05$, $R^2=0.0529$; NPK: $y = -1.93x + 41.3$, $P<0.05$, $R^2=0.5877$ (B).

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References

Baumberger C., Koch B., Thomet P., Christ H. and Gex P. (1996) Long-term effects of fertilization on the botanical composition of an alpine pasture. *Agrarforschung* 3, 275-278.

Ineichen S. (2018) *Suitability of forage stands with contrasting species richness to improve the nitrogen use efficiency and the milk fatty acid profile of dairy cows*. Dissertation, ETH Zurich, Switzerland.

Isselstein J. and Daniel P. (1996) The ensilability of grassland forbs. *Grassland Science in Europe* 1, 451-455.

Jayanegara A., Marquardt S., Kreuzer M. and Leiber F. (2011) Nutrient and energy content, *in vitro* ruminal fermentation characteristics and methanogenic potential of alpine forage plant species during early summer. *Journal of the Science of Food and Agriculture* 91, 1863-1870.

Jayanegara A., Sujarnoko T.U.P., Ridla M., Kondo M. and Kreuzer M. (2019) Silage quality as influenced by concentration and type of tannins present in the material ensiled: A meta-analysis. *Journal of Animal Physiology and Animal Nutrition* 103, 456-465.

Licitra G., Hernandez T.M. and Van Soest P.J. (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology* 57, 347-358.

Terill T.H., Rowan A.M., Douglas G.B. and Barry T. (1992) Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of the Science of Food and Agriculture* 58, 321-329.

Thomet P. and Koch B. (1993) Long-term effects of fertilization and cutting regime on a hay meadow. *Landwirtschaft Schweiz* 6, 107-114.

Weißbach F. (1998) Untersuchungen über die Beeinflussung des Gärungsverlaufes bei der Bereitung von Silage durch Wiesenkräuter verschiedener Spezies im Aufwuchs extensiv genutzter Wiesen. Landbauforschung Völkenrode. *Sonderheft* 185.

Wyss U., Dettling T. und Reidy B. (2016) Silagequalitäten im Berggebiet: eine Praxisuntersuchung. *Agrarforschung Schweiz* 7, 188-195.

Fermentation characteristics of grass-legume and whole crop barley ensiled with a mixed bacterial inoculant

Jatkauskas J.[1], Vrotniakiene V.[1], Witt K.L.[2] and Eisner I.[2]
[1]Institute of Animal Science of Lithuanian University of Health Sciences, R. Žebenkos 12, Baisogala, Radviliškis distr., Lithuania; [2]Chr-Hansen, Hoersholm, Denmark

Abstract

Grass-legume (red clover, lucerne and timothy 50:20:30) and whole-crop barley forage were ensiled into cylindrical big bales for 120 days with and without inoculation (SiloSolve FC containing *Lactococcus lactis* DSM 11037/1k2081 and *Lactobacillus buchneri* DSM 22501/1k20738 at 1.5×10^5 cfu g^{-1} forage). After 120 days of fermentation in the field, the big bales were opened, sampled and tested for fermentation parameters, yeast and mould count. Aerobic stability was measured in the field. Weight loss was recorded and dry matter loss was calculated. Visual scoring of fungal growth on the surface of bales was evaluated. Legume-grass and whole crop barley treated with SiloSolve FC relative to untreated control silage was associated with reduced dry matter loss, improvement of positive indicators of fermentation, including a reduction of pH and increased individual concentrations of lactic and acetic acids, and lowered negative indicators of fermentation, including reduced ammonia N, ethanol and butyric acid. Inoculation increased the count of lactic acid bacteria and reduced the counts of moulds and yeasts. Reduction in yeast and mould population during the anaerobic phase of silage conservation and during aerobic exposure appears to be the main reason for the improvement in aerobic stability of the inoculated silages.

Keywords: bales, lucerne, small cereals, silage, inoculation

Introduction

Grass, grass-legume and maize silage are the main crops used in dairy cows and fattening cattle diets in many countries. Recent weather conditions indicate there is potential to feed standing crops of small cereals to livestock as ensiled whole crop. The popularity of ensiling whole crop wheat or barley has increased in recent years as cereal whole-crops offer a good alternative or complement to traditional fermented forages. Whole-crop cereal silage is relatively easy to preserve; however, as it contains a higher starch and sugar content, it is vulnerable to aerobic spoilage. To improve the fermentation quality and decrease nutrient loss, lactic acid bacteria (LAB) are applied to promote adequate lactic acid production, reduce pH in the silage and improve safe feeding of livestock (Kung, 2014; Li *et al.*, 2016). Several previous studies have shown that inoculation with *Lactobacillus buchneri* and *Lactococcus lactis* is able to improve aerobic stability in various silages (Gallo *et al.*, 2018; Witt *et al.,* 2015) and can reduce detrimental effects of air during feed-out (Auerbach and Nadeau, 2019). This study aimed to compare fermentation characteristics, microbial population and aerobic stability of grass-legume and whole-crop barley forage ensiled in field conditions with or without a mixed lactic acid bacteria inoculant containing *L. lactis* and *L. buchneri.*

Materials and methods

This study was a randomized block design (2×2×10 reps.) to analyse the effect of using the silage inoculant SiloSolve FC (containing *L. lactis* DSM 11037/1k2081 and *L. buchneri* DSM 22501/1k20738) on two forages: (1) grass-legume (red clover, lucerne and timothy – *Trifolium pratense*, Nordi, *Medicago sativa*, Creno and *Phleum pratense,* Ulla. 50:20:30) and (2) whole-crop barley (*Hordeum vulgare*., Anakin). Homogenous plots of a grass-legume crop and whole crop barley forage were each divided into two blocks and mowed with a disc mower-conditioner harvester. Wilted herbage (up to 359.4 g dry matter (DM) kg^{-1} grass-legume) and unwilted whole crop barley (at 380.2 g DM kg^{-1}) were baled into 1.2 m wide ×

1.2 m diameter cylindrical bales wrapped with six layers of plastic film, weighed and labelled. Ten big bales for both SiloSolve FC treated forages were prepared as well as ten control big bales without additive. Inoculant was applied during the baling process, using a commercial pump, at rate of 4 litres suspension per ton of forage targeting a dosage of 1.5×10^5 cfu g^{-1} of fresh forage. All bales were individually weighed after wrapping and again after 120 days of storage to determine weight and DM losses. Five big bales from each treatment and each forage were chosen randomly and sampled after 120 days of storage for chemical and microbial analyses. Aerobic stability was evaluated by temperature method over 30 days of aeration and measured by inserting 70 cm long temperature sensors into the opened other five bales at 5 different points. All data were analysed as complete block design using the PROC GLM of SAS 9.4 (SAS Institute, Cary, NC, USA) with treatment as fixed effect.

Results and discussion

The results show that the use of the blend of *L. lactis* and *L. buchneri* resulted in a more efficient fermentation than in the untreated bales. This was reflected by composition of fermentation products in the silages and the lower (P<0.01; P<0.05) DM losses. Inoculant treatment restricted (P<0.01) formation of alcohols and resulted in lower proteolysis as indicated by the lower (P<0.01) ammonia-N content and had an effect (P<0.01) on microbial quality of the silage, as counts of LAB increased and counts of yeasts and moulds decreased compared with the control (Table1).

Combination of *Lactococcus lactis* and *Lactobacillus buchneri* was efficient in increasing (P<0.01) the aerobic stability of grass-legume and whole crop barley silage by suppressing growth of yeast and moulds (Table 2). These results are in agreement with several previous studies that showed inoculation with *Lactococcus lactis* and *Lactobacillus buchneri* improved aerobic stability in various silages (Gallo *et al.*, 2018; Witt *et al.*, 2015). The increase in aerobic stability was related to the large amount of acetic acid and lower yeast count, confirming results of Kleinschmit and Kung (2006). Lower pH value during aerobic aeration also indicates the resistance of the inoculated silage to aerobic deterioration. Fermentation data together with dry matter loss suggest that the inoculant has potential to reduce greenhouse gas emission during fermentation and feed-out periods.

Table 1. Chemical composition, fermentation products and microbial analyses of grass-legume and whole-crop barley silages produced in big bales.[1]

Items	Grass-legume silage				Whole crop barley silage			
	Control	SiloSolve	SE	*P*-value	Control	SiloSolve	SE	*P*-value
DM corrected, g kg^{-1}	328.5	344.2	1.375	**	357.2	363.6	2.657	ns
DM loss, g kg^{-1}	120.9	70.1	3.064	**	107.3	75.9	7.179	*
Crude protein, g kg^{-1}	139.4	168.2	3.628	**	88.7	100.2	1.992	**
pH	4.37	4.21	0.030	**	4.13	3.98	0.015	**
Ammonia-N, g kg^{-1} total N	60.08	39.31	1.831	**	115.68	65.90	5.102	**
Alcohols, g kg^{-1}	10.27	7.03	0.447	**	16.46	10.75	0.297	**
Lactic acid, g kg^{-1}	51.63	70.22	2.489	**	20.19	40.14	1.175	**
Acetic acid, g kg^{-1}	21.13	32.91	0.856	**	7.30	17.46	0.508	**
Butyric acid, g kg^{-1}	1.09	0.26	0.122	**	4.45	1.43	0.301	**
Propionic acid, g kg^{-1}	0.20	0.59	0.037	**	0.35	0.68	0.104	ns
LAB, log_{10} cfu g^{-1}	8.02	9.41	0.292	**	7.34	8.30	0.208	*
Yeasts, log_{10} cfu g^{-1}	2.33	1.00	0.049	**	2.42	1.00	0.037	**
Moulds, log_{10} cfu g^{-1}	2.44	1.22	0.080	**	1.70	1.51	0.068	ns

[1] Dry matter (DM), calculated dry matter losses and fermentation parameters are corrected for volatiles; LAB = lactic acid bacteria; cfu = colony forming units; SE = standard error; ns = not significant; * P<0.05; ** P<0.01.

Table 2. Aerobic stability characteristics of the silages under ambient field conditions.

Items[1]	Grass-legume silage				Whole crop barley silage			
	Control	SiloSolve	SE	*P*-value	Control	SiloSolve	SE	*P*-value
Fresh weight loss, %	3.66	1.64	0.311	**	5.18	2.37	0.378	**
pH	5.00	4.31	0.153	**	6.18	4.08	0.132	**
Yeasts, $\log_{10}$ cfu g^{-1}	5.60	1.12	0.209	**	4.06	1.88	0.159	**
Moulds, $\log_{10}$ cfu g^{-1}	6.87	1.40	0.237	**	5.69	2.06	0.173	**
Aerobic stability, h	346.8	720.0	65.978	**	258	720.0	14.325	**

[1] cfu = colony forming units; SE = standard error; ns = not significant; * $P<0.05$; ** $P<0.01$.

Conclusions

Inoculation affected the fermentation profile of both the grass-legume and whole crop barley silages according to their mode of action, and restricted growth of yeast and moulds during silage fermentation and aerobic exposure periods. Aerobic stability can be improved by inoculation, thereby maintaining good quality of the silage during feed-out.

References

Auerbach H. and Nadeau E. (2019) Biological and chemical additives maintain nutritive value of grass silage during air exposure. In: Gerlach K. and Sudekum K.-H. (eds.) *Proceedings of the XVIII International Silage Conference*, Bonn, Germany, pp. 220-221.

Gallo A., Bernardes T.F., Copani G., Fortunati P., Giuberti G., Bruschi S. and Masoero F. (2018) Effect of inoculation with *Lactobacillus buchneri* LB1819 and *Lactococcus lactis* O224 on fermentation and mycotoxin production in maize silage compacted at different densities. *Animal Feed Science and Technology* 246, 36-45.

Kleinschmit D. and Kung L. (2006) A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of maize and grass and small-grain silages. *Journal of Dairy Science* 89,4005-4013.

Kung L. (2014) Managing the aerobic stability of silages. In: Jambor V. *et al.* (eds.) *The 16th International Symposium of Forage Conservation*. Mendel University at Brno, Czech Republic. pp. 47-54.

Li P., Bai S., You M. and Shen Y. (2016) Effects of maturity stage and lactic acid bacteria on the fermentation quality and aerobic stability of Siberian wild rye silage. *Food Science and Nutrition* 4, 664-670.

Witt K.L., Jatkauskas J., Vrotniakiene V. and Voss M.T. (2015) Effects of a novel dual purpose silage additive on aerobic stability and fermentation characteristics of whole crop maize silage after a short time of ensiling. In: Nussio L.G, De Sousa D.O., Gritti V.C., De Souza Salvati G.G., Dos Santos W.P. and Salvo P.A.R. (eds.) *Proceedings of the XVII International Silage Conference*, Piracicaba, Sao Paulo, Brazil, pp. 82-83.

Relationship between L-lactate and DL-lactate in different silage types

Johansen M.[1], Weisbjerg M.R.[1], Novoa-Garrido M.[2], Kristensen N.B.[1,3] and Larsen M.[1]
[1]*Department of Animal Science, Aarhus University – Foulum, Tjele, Denmark;* [2]*Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway;* [3]*Current address: SEGES, Landbrug & Fødevarer, Aarhus, Denmark*

Abstract

The aim of this study was to examine the relationship between L-lactate and total lactate (DL-lactate) in a range of silage types. In total, 263 silage samples including pure grass silages, grass-clover silages, pure clover silages, whole crop maize silages, sugar beet silages, and seaweed silages were analysed. Across all silages, L:DL-lactate ratio was 0.495 indicating a racemic lactate production. However, in some types of seaweed samples, L:DL-lactate ratio was 0.09 and in silages with restricted fermentation the ratio was 0.94. In naturally fermented sugar beet silages, grass-clover silages and maize silages, the L:DL-lactate ratio was 0.448, 0.484 and 0.527, respectively. Inoculation with heterofermentative bacteria reduced L:DL-lactate ratio in both grass-clover and maize silages, whereas the L:DL-lactate ratio in silages fermented with a homofermentative inoculant did not differ from that in naturally fermented silages. The observed variation in L:DL-lactate ratios indicates that racemic lactate production is not valid for all types of silages.

Keywords: preservation, grass, clover, maize, seaweed, sugar beet

Introduction

Production of lactate is essential to obtain rapid and sufficient decreases in pH in order to produce a well-preserved silage. Lactic acid bacteria are responsible for the production of lactate. Lactic acid bacteria belong to the *Lactobacillales* order and include several genera such as *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus* and *Lactococcus*. All species of lactic acid bacteria in all genera produce lactate in both D- and L- isoforms, but the ratio of isomers is highly dependent of species, as some species mainly produce L-lactate (e.g. *Enterococcus* and *Lactococcus*), some species mainly produce D-lactate (e.g. *Leuconostoc*) and other species have a racemic lactate production (e.g. *Pediococcus* and many *Lactobacillus*) (Manome *et al.*, 1998, Pahlow *et al.*, 2003). It will be an advantage if L-lactate concentration in silages can be used as predictor for total lactate concentration, as the analytical method to determine L-lactate is easier and faster than the method to determine total lactate. The aim of the current study was to examine the relationship between L-lactate and total lactate (DL-lactate) in a range of different silages. Due to a mix of bacteria and many species with a racemic lactate production, a L:DL-lactate ratio of 0.5 was hypothesised.

Materials and methods

In total, 263 observations of L- and DL-lactate from individual silage samples from previous experiments were compiled in the dataset. The dataset consisted of: 121 maize silage samples from 39 Danish dairy farms collected three times during the year, where one third was naturally fermented, one third was fermented with a homofermentative inoculant and one third was fermented with a heterofermentative inoculant (Kristensen *et al.*, 2010a); 62 grass-clover silage samples of the spring growth from 31 Danish dairy farms collected two times during the year, where one third was naturally fermented, one third was fermented with a homofermentative inoculant and one third was fermented with a inoculant containing both homo- and heterofermentative bacteria (Kristensen *et al.*, 2010b); 36 seaweed silage samples from a 2×3×2 factorial ensiling experiment investigating the effect of species (*Saccharina latissima* or *Porphyra* *umbilicalis*), ensiling method (unwashed, washed or washed with formic acid addition) and drying (undried or pre-dried) (M. Novoa-Garrido *et al.*, unpublished); 24 sugar beet silage samples from a dairy cow feeding experiment with naturally fermented sugar beet silage or sugar beet silage ensiled with formic and propionic acid (Hellwing *et al.*, 2017); 8 grass silage and 4 clover silage samples of the spring growth from a dairy cow feeding experiment (Johansen *et al.*, 2017a); and 8 grass-clover silage samples from the spring growth and first regrowth wilted to different dry matter concentration before ensiling produced by two Danish organic farmers (Johansen *et al.*, 2017b). Extracts of all silage samples were made by blending silage with demineralized water followed by centrifugation. In silage extracts stabilised with 5% meta-phosphoric acid, L-lactate was analysed with membrane-immobilised substrate specific oxidase using an YSI 2900 or YSI 7100 Biochemistry Analyzer (YSI Inc., Yellow Springs, OH, USA). In unstabilised silage extracts, DL-lactate was analysed by gas chromatography-mass spectrometry (GC-MS) (Kristensen *et al.* 2010a). Regression analysis of L-lactate on DL-lactate without intercept was performed in R (version 3.6.1) using RStudio software (version 1.2.1335). The intercept was removed from the equation, as no L-lactate, per definition would be present if DL-lactate is not present, and the regression coefficient was then equal to the L:DL-lactate ratio.

Results and discussion

The collected data had L-lactate and DL-lactate concentrations in the range 0 to 85.9 and 0 to 161 g kg^{-1} of dry matter (DM), respectively, with mean values (± standard deviation (SD)) of 24.3 (±16.1) and 48.9 (±32.1), respectively (Figure 1). Across all silage samples, the regression coefficient of L-lactate on DL-lactate was 0.495 (standard error (SE)=0.00377) and did not differ from the expected ratio of 0.5 (P=0.17). This illustrates that the assumption of a racemic lactate production in general is valid across silages. However, undried, both control and washed, *S. latissima* seaweed silage samples had an average L:DL-lactate ratio of 0.09, indicating that bacteria mainly producing D-lactate were responsible for the lactate production in this type of seaweed silage. Substances in the complex seaweed matrix might have inhibited the membrane oxidase used in L-lactate analysis causing the absence of L-lactate in *S. latissima*; however, spiking seaweed samples with known amounts of L-lactate showed complete recovery. By comparison, average L:DL-lactate ratio was 0.57 in undried *P. umbilicalis* seaweed silage samples. In silages where fermentation was restricted, either by addition of an acidic additive (sugar beet and seaweed) or by a high DM concentration (grass-clover and seaweed), and the resulting L-lactate concentration was below 2 g kg^{-1} of DM, the average L:DL-lactate ratio was 0.94. This indicates that an approximate racemic lactate production is only valid in silage samples, where a significant fermentation has happened. The regression within silage type resulted in regression coefficients of 0.442 (SE=0.0147), 0.480 (SE=0.00419) and 0.597 (SE=0.0114) for pure grass, grass-clover and pure clover silages, respectively. This indicates that different lactic acid bacteria are present on the plant of grass and clover and therefore affect the fermentation during ensiling. However, only few pure grass and clover silage samples were present in the current dataset, and more samples are needed to draw final conclusions. For naturally fermented sugar beet, grass-clover and maize silages, the regression coefficients were 0.448 (SE=0.0125), 0.484 (SE=0.00705) and 0.527 (SE=0.00856), respectively, indicating that different lactic acid bacteria were responsible for the lactate production, and that a racemic lactate production is not valid for individual silage types. In both grass-clover and maize silages, the regression coefficients were similar for silages naturally fermented or fermented with a homofermentative inoculant, but the regression coefficients decreased to 0.469 (SE=0.00764) and 0.483 (SE=0.0110) for grass-clover and maize silages, respectively, when an inoculant with heterofermentative bacteria was used. The bacterium *L. buchneri*, which produce L:DL-lactate in the ratio 0.468 (Manome *et al.* 1998) was the used heterofermentative bacterium in both experiments, which can explain the decreased ratios.

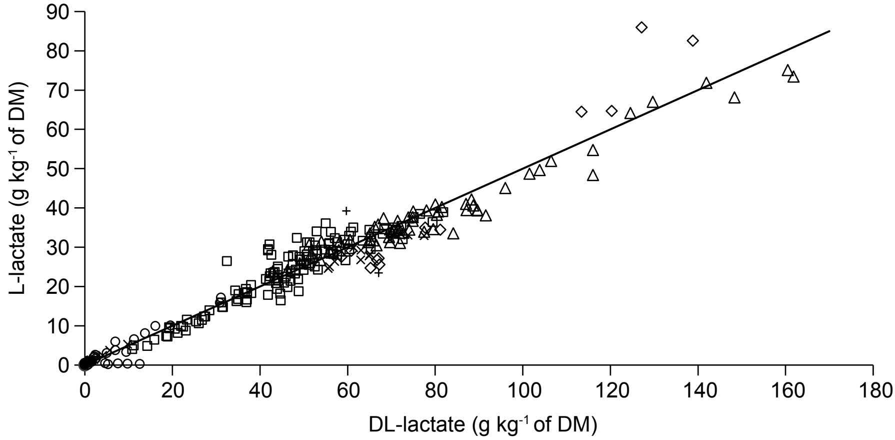

Figure 1. L-lactate as function of DL-lactate across maize silages (□, Kristensen *et al.*, 2010a), grass-clover silages (△, Kristensen *et al.*, 2010b; +, Johansen *et al.*, 2017b), seaweed silages (○, M. Novoa-Garrido *et al.*, unpublished), sugar beet silages (×, Hellwing *et al.*, 2017) and grass and clover silages (◇, Johansen *et al.*, 2017a) with the hypothesized L:DL-lactate ratio of 0.5 (–, y = 0.5x). DM = dry matter.

Conclusions

In general, racemic production of lactate seems valid across a large set of silage samples. However, the current results indicate that different types of silages and inoculants differ in L:DL-lactate ratio, whereby racemic lactate production was not always observed.

References

Hellwing A.L.F., Messerschmidt U., Larsen M. and Weisbjerg M.R. (2017) Effects of feeding sugar beets, ensiled with or without an additive, on the performance of dairy cows. *Livestock Science* 206, 37-44.

Johansen M., Søegaard K., Lund P. and Weisbjerg M.R. (2017a) Digestibility and clover proportion determine milk production when silages of different grass and clover species are fed to dairy cows. *Journal of Dairy Science* 100, 8861-8880.

Johansen M., Hellwing A.L.F., Lund P. and Weisbjerg M.R. (2017b) Metabolisable protein supply to lactating dairy cows increased with increasing dry matter concentration in grass-clover silage. *Animal Feed Science and Technology* 227, 95-106.

Kristensen N.B., Sloth K.H., Højbjerg O., Spliid N.H., Jensen C. and Thøgersen R. (2010a) Effects of microbial inoculants on corn silage fermentation, microbial contents, aerobic stability, and milk production under field conditions. *Journal of Dairy Science* 93, 3764-3774.

Kristensen N.B., Højbjerg O. and Thøgersen R. (2010b) Virkning af ensileringsmidler i græs – ensilering 2009 (in Danish). In: Kristensen N.B. (eds) *Ensilering af majs og græs*, Faculty of Agricultural Science, Foulum, Denmark, pp. 60-73.

Manome A., Okada S., Uchimura T. and Komagata K. (1998) The ratio of L-form to D-form of lactic acid as a criteria for the identification of lactic acid bacteria. *Journal of General and Applied Microbiology* 44, 371-374.

Pahlow G., Muck R.E., Driehuis F., Elferink S.J.W.H.O. and Spoelstra S.F. (2003) Microbiology of ensiling. In: Buxton D.R., Muck R.E. and Harrison J.H. (eds.) *Silage science and technology*, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI, USA, pp. 31-93.

Effects of malic or citric acid application on the fermentation of lucerne ensiled at two dry matter contents

Ke W.C.[1], Ding Z.T.[1], Franco M.[2], Li F.H.[1] and Guo X.S.[1]
[1]Probiotics and Biological Feed Research Centre, Lanzhou University, Lanzhou, China P.R.; [2]Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland

Abstract

The aim of this study was to evaluate the effects of malic or citric acids on the fermentation quality of ensiled lucerne at two different dry matter (DM) contents. Lucerne was harvested in the early flowering stage and wilted to DM contents of approximately 300 (moderately low) and 380 (normal) g kg^{-1} fresh weight of forage. Lucerne was treated with control (distilled water), 6 (g kg^{-1} fresh matter) DL-malic acid (MA) or 6 (g kg^{-1} fresh matter) citric acid (CA). After 60 d of ensiling, silages ensiled at a moderately low DM had lower pH and acetic acid concentration than in silage with a normal DM. Silages treated with MA or CA had higher lactic acid concentration but lower pH and acetic acid concentration than those in the control treatment. These effects were independent of silage DM. There were interactions for the effects of MA and CA on inhibiting lipolysis since the acids were more effective in treated silages with a normal DM than in the moderately low DM. Including malic and citric acids could effectively improve silage fermentation quality, limit proteolysis and lipolysis at two different DM contents.

Keywords: *Medicago sativa* L, organic acid, silage additive, lipolysis

Introduction

In order to achieve satisfactory silage fermentation, organic acids can be added to inhibit the growth of undesirable microorganisms and to preserve more nutrients. However, some acid additives are difficult to handle because they are corrosive to equipment. Malic (MA) and citric acids (CA) exist widely in nature and are confirmed as safe to use in the food industry. In contrast to the inhibition effects of traditional acid additives on microorganisms, MA and CA have been proven to accelerate the growth of lactic acid bacteria (Branen and Keenan, 1970; Passos *et al.*, 2003). Our previous work also showed that MA and CA could promote lactic acid fermentation and improve the fermentation quality of lucerne (Ke *et al.*, 2017). The profound effect of dry matter (DM) contents on silage fermentation has been observed in numerous previous experiments. Wilting forage to a higher DM content could result in greater polyunsaturated fatty acid (PUFA) loss (Van Ranst *et al.*, 2009). However, little is known about the effect of MA and CA on lucerne silage ensiled at different DM contents. We hypothesised that the application of MA or CA may lead to different responses of fermentation quality at different DM contents of forages and there might be interactions between the DM level and the acid treatments.

Materials and methods

Lucerne (*Medicago sativa* L.; 'Zhongmu 2#') was wilted to DM contents of approximately 300 (moderately low DM) and 380 (normal DM) g kg^{-1} fresh weight. The forage was chopped into lengths of 1 to 2 cm by using paper-cutters and treated with (1) distilled water (control treatment), (2) 6 (g kg^{-1} fresh matter) of MA, and (3) 6 (g kg^{-1} fresh matter) of CA. Each treatment was ensiled in four replicates in vacuum-sealed polyethylene plastic bags (500 g) and stored at ambient temperature ranging from 18 to 30 °C for 60 days. The chemical composition of silages was analysed as described by Ke *et al.* (2017). The results were analysed statistically using the GLM procedure of SAS 9.4. The treatments effects were separated using orthogonal contrasts to evaluate the effects of the DM content, additive treatments and their interactions.

Results and discussion

The fermentation characteristics of lucerne ensiled after 60 d are shown in Table 1. Similar to our previous work (Ke *et al.*, 2017), MA and CA application resulted in lower pH and acetic acid concentration but higher lactic acid concentration than in the control. In addition, silages at a moderately low DM content had higher lactic acid concentration and lower water-soluble carbohydrate (WSC) concentration than those in silages ensiled at a normal DM content. Because of additional fermentation substrates provided by the acid additives, application of MA increased the content of WSC in silage ensiled at a moderately low DM, compared to the control group. However, the difference was quite small and might not be meaningful in practice. Plant enzyme activities are associated with the conversion of protein nitrogen to non-protein nitrogen (NPN), and may reduce proteolysis with increasing DM contents, which was in accordance with the current work. Addition of MA and CA in lucerne silages resulted in lower concentrations of NH_3-N in silages at both DM contents. This result was more pronounced at the normal DM level, resulting in a significant additive × DM level interaction in line with Liu *et al.* (2011). Compared with MA-treated silage, CA-treated silage with a moderately low DM had lower pH, WSC and NH_3-N, but the differences were not significant in silages with a normal DM.

The majority of fatty acids in silages exist in the form of free fatty acids which could result in a high biohydrogenation rate (Van Ranst *et al.*, 2009). In this study, addition of MA and CA reduced lipolysis during fermentation as indicated by greater PUFA proportion and lower saturated fatty acids proportion in the acid-treated silages than in the control silage. Further, the response to acid application was greater with a normal DM, as indicated by the significant interaction.

Table 1. Fermentation characteristics of lucerne silages ensiled at two different dry matter contents after 60 d.[1]

Variable	Moderately low			Normal			SEM	*P*-value				
	C	MA	CA	C	MA	CA		DM	C vs ADD	MA vs CA	MA vs CA × DM	C vs ADD × DM
DM	317^{b}	329^{b}	317^{b}	383^{a}	374^{a}	378^{a}	1.2	<0.01	0.84	0.23	0.02	0.03
pH	4.88^{bc}	4.75^{c}	4.55^{d}	5.13^{a}	4.95^{b}	4.83^{bc}	0.013	<0.01	<0.01	<0.01	0.26	0.82
LA	40.7^{d}	53.6^{bc}	56.0^{ab}	40.8^{d}	51.2^{c}	56.2^{a}	0.22	0.12	<0.01	<0.01	0.03	0.19
AA	29.5^{a}	19.1^{c}	24.9^{b}	26.2^{b}	15.6^{d}	21.5^{c}	0.25	<0.01	<0.01	<0.01	0.94	0.90
WSC	10.2^{d}	13.7^{bc}	11.1^{d}	15.5^{ab}	16.8^{a}	16.9^{a}	0.24	<0.01	<0.01	0.02	0.01	0.32
NPN	754^{a}	682^{ab}	737^{ab}	685^{ab}	668^{ab}	649^{b}	7.6	0.01	0.06	0.37	0.09	0.60
NH_3-N	64.8^{b}	47.3^{c}	35.6^{d}	82.1^{a}	24.3^{e}	26.8^{e}	0.68	0.01	<0.01	0.03	<0.01	<0.01
SFA	37.7^{b}	32.8^{cd}	31.0^{d}	47.1^{a}	34.6^{c}	33.7^{c}	0.20	<0.01	<0.01	0.02	0.42	<0.01
MUFA	5.75^{ab}	5.28^{bc}	4.89^{cd}	5.98^{a}	4.74^{d}	4.58^{d}	0.042	0.03	<0.01	0.02	0.28	<0.01
PUFA	56.5^{c}	61.9^{ab}	64.1^{a}	47.0^{d}	60.7^{b}	61.7^{ab}	0.23	<0.01	<0.01	0.02	0.39	<0.01

[1] DM = dry matter, g kg^{-1} fresh matter; TN = total nitrogen; LA = lactic acid, g kg^{-1} DM; AA = acetic acid, g kg^{-1} DM; WSC = water soluble carbohydrates, g kg^{-1} DM; NPN = non-protein N, g kg^{-1} TN; NH_3-N = ammonia N, g kg^{-1} TN; SFA = saturated fatty acids, %; MUFA = monounsaturated fatty acid, %; PUFA = polyunsaturated fatty acid, %; SEM = standard error of the mean; C = control; MA = malic acid; CA = citric acid; ADD = acid additives. Values with same letter in a row are not significantly different at 5% Tukey test.

Conclusions

Including malic and citric acids could effectively improve silage fermentation quality, limit proteolysis and lipolysis at two different silage DM contents. The effect of malic and citric acids on lucerne fermentation was quite similar but addition of citric acid resulted in lower pH and NH_3-N in treated silages with a moderately low DM. Our findings suggest that the application of malic and citric acids might provide a viable alternative for low DM silages, but comparisons with other currently used biological and chemical additives should be conducted to allow evaluation of the efficiency and economic feasibility of their use.

Acknowledgements

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References

Branen A.L. and Keenan T.W. (1970) Growth stimulation of *Lactobacillus casei* by sodium citrate. *Journal of Dairy Science* 53, 593-597.

Ke W.C., Ding W.R., Xu D.M., Ding L.M., Zhang P., Li F.D. and Guo X.S. (2017) Effect of addition of malic or citric acids on fermentation quality and chemical characteristics of alfalfa silage. *Journal of Dairy Science* 100, 8958-8966.

Liu Q., Zhang J., Shi S. and Sun Q. (2011) The effects of wilting and storage temperatures on the fermentation quality and aerobic stability of stylo silage. *Animal Science Journal* 82, 549-553.

Passos F.V., Fleming H.P., Hassan H.M. and McFeeters R.F. (2003) Effect of malic acid on the growth kinetics of *Lactobacillus plantarum*. *Applied Microbiology and Biotechnology* 63, 207-211.

Van Ranst G., Fievez V., De Riek J. and Van Bockstaele E. (2009) Influence of ensiling forages at different dry matters and silage additives on lipid metabolism and fatty acid composition. *Animal Feed Science and Technology* 150, 62-74.

Early lactation once-a-day milking: the effects on dairy cow milk production

Kennedy E.[1], Delaby L.[2] and O'Donovan M.[1]
[1] *Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland;* [2] *INRA, Agro Campus Ouest, UMR 1348, Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Elevage, F-35590 Saint-Gilles, France*

Abstract

Seasonal pasture-based systems of milk production offer advantages such as the ability to match herd demand with grass growth and supply. Such a system can pose challenges such as increased labour input, particularly during the calving season. Once-a-day (OAD) milking, even for a short period of time at the start of lactation, may provide a solution. This experiment investigated the effects of twice-a-day (TAD) milking compared to OAD for four, six or eight-weeks at the start of lactation. Once each OAD period finished cows returned to TAD milking for the remainder of lactation. Sixty dairy-breed cows were randomised and assigned to treatments as they calved. Milk yield was recorded daily and milk composition weekly. During the first four weeks of lactation daily milk yield of TAD cows was 22% greater than the OAD herds (20.9 kg cow^{-1} day^{-1}). Following 35 weeks the six- and eight-week OAD herds had a lower cumulative milk yield (4,864 kg cow^{-1}) compared with the TAD cows (5,300 kg cow^{-1}); there were no differences between any of the other treatments. There was no significant difference in yield of milk fat + protein between treatments (401 kg cow^{-1}) at the end of lactation. The results of this study indicate that OAD milking for up to eight weeks, in early lactation does not reduce total lactation yield of milk fat plus milk protein.

Keywords: milking frequency, early lactation, dairy cows

Introduction

Herd size is increasing in Ireland following the abolition of milk quotas; since 2016 the number of dairy cows has increased by almost 6% (CSO, 2019). An increased herd size contributes to greater labour demands (Deming *et al.*, 2018), particularly during the calving and early lactation periods. Difficulties sourcing labour for a short-term period, such as a 12-week calving season, means dairy farmers need to identify alternative strategies to alleviate the peaks in labour requirement. Once-a-day (OAD) milking, even for a short period of time at the start of lactation, may provide a solution. While twice-a-day (TAD) milking is accepted as the standard milking frequency more dairy farmers are challenging this theory, particularly in systems where milk production per cow is not the focus and OAD milking may offer a viable alternative (Clark *et al.*, 2006; Stelwagen *et al.*, 2013). In a review paper Stelwagen *et al.* (2013) showed that OAD milking leads to reductions in milk production of approximately 22%, ranging from 7 to 40% in short-term experiments. The objective of this study was to investigate, in comparison with TAD milking, the effect of short-term OAD milking for four, six or eight weeks directly post-calving on immediate milk production and also on total lactation milk production and somatic cell count (SCC).

Materials and method

The experiment took place in Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland from February 13 to December 5, 2018. Sixty cows (15 primiparous and 45 in their second or greater lactation (multiparous)) were selected from the Teagasc Moorepark herd and randomly assigned pre-calving to a randomized block design experiment consisting of four treatments (n=15). The treatments were (1) milked twice-a-day for the entire lactation (TAD), (2) milked once-a-day (OAD) for the first four weeks of lactation then reverting back to twice a day for the remainder of lactation (OAD4), (3) milked OAD for the first six weeks of lactation then reverting back to twice a day for the remainder of lactation (OAD6), (4) milked OAD for the first eight weeks of lactation then reverting back to twice a day for the remainder of lactation (OAD8).

Cows (multiparous) were balanced based on the first 35 weeks milk yield, composition and somatic cell count from their previous lactation. In the case of primiparous animals they were balanced on the first 35 weeks of their dam's first lactation milk yield and composition. The mean calving date of the herd was 11 March 2018 (standard deviation (SD) 14.5 days). Previous lactation milk yield, previous lactation yield of fat + protein kg, previous lactation somatic cell count (SCC), and average parity number were 4,781 (SD 892.2) kg, 389 (SD 78.5) kg; 128,150 (SD 103,220.6) cells ml^{-1}, 2.9 (1.90) lactations, respectively. Within the herd (n=60) there were 46 Holstein Friesian (HF) cows and 14 HF × Jersey cross cows.

As cows calved, they were assigned to their respective treatments. TAD cows were milked at 07:00 and 16:00, OAD cows were milked in the morning only (07:00). All OAD cows were milked OAD until their respective date to commence TAD milking was reached. Cows were managed as two herds – one herd was milked TAD and the other herd was milked OAD; as cows moved from OAD to TAD they switched between herds. Yield of fat plus yield of protein (MSY) was calculated for all cows. Both herds grazed individually but adjacent to one another to ensure swards of similar pasture composition was offered. Herbage mass (HM; >3.5 cm) was measured twice weekly by cutting six strips (120 m^2) per grazing area. Pre and post grazing heights were measured daily. Equal quantities of fresh pasture were allocated to both herds twice daily. Once all cows were in a single herd, being milked TAD, fresh pasture was allocated once daily. Cows were fed an equal quantity of concentrate (875 kg cow^{-1}). Milking for all cows took place at 0700, the TAD cows were milked again at 16:00. Data were analysed using covariate analysis and mixed models in SAS v9.4. Terms for parity and treatment were included in the model. Pre-experimental values and days in milk were used as covariates in the model.

Results and discussion

After four weeks there was no difference in milk yield between any of the OAD herds (20.9 kg cow^{-1} day^{-1}), but they yielded significantly less (P<0.001) than the TAD cows (26.7 kg cow^{-1} day^{-1}; 22%). This was similar to the reductions reported in the review of Stelwagen *et al.* (2013). There was no effect of treatment on milk fat, protein and lactose concentration during the first four weeks (5.50, 3.38 and 4.55%, respectively). The TAD herd had a higher (P<0.001) milk fat, protein and lactose yield (+0.29, +0.17 and 0.28 kg cow^{-1} day^{-1}, respectively) than the three OAD herds, which were not significantly different to each other (1.19, 0.72 and 0.95 kg cow^{-1} day^{-1}, respectively), which was due to the higher volume of milk produced by the TAD herd. Consequently, the MSY of the TAD cows was also higher (P<0.001; +20%) than the three OAD herds (1.90 kg cow^{-1} day^{-1}).

Cumulative milk production over the first six weeks was greatest for the TAD cows (1,026 kg), the three OAD herds remained similar (815 kg). The cumulative six-week MSY of all the OAD herds was similar (71.3 kg cow^{-1}) but lower than the TAD cows (P<0.001; 87 kg cow^{-1}).

The TAD cows had significantly higher cumulative milk yield (P<0.001; 1415 kg cow^{-1}) after eight weeks compared to all other treatments. The cumulative milk yields of the OAD4 and OAD6 were similar (1,184 kg cow^{-1}) but the OAD4 herd had a higher yield than OAD8 (1,076 kg cow^{-1}); OAD6 and OAD8 were similar. The MSY at the end of eight weeks was similar for the OAD4 and OAD8 herds (100.2 kg cow^{-1}). All other treatments were different to each other.

Following the 35-week lactation the OAD6 (P<0.05; 4913 kg cow^{-1}) and OAD8 (P<0.01; 4815 kg cow^{-1}) treatments had a lower cumulative milk yield compared to the TAD cows (5,300 kg cow^{-1}; Table

1); there were no differences between any of the other treatments. There was a tendency (P=0.145) for the TAD to produce milk of a lower fat concentration (-0.24%) than all other three treatments (4.60%). The average milk protein concentration over the 35-week lactation was lower (P<0.05) for the TAD cows (-0.13%) compared to all other treatments which were similar (3.46%). There was no effect of treatment on milk lactose concentration (4.52%). Milk fat and protein yield after 35-weeks of lactation were not different between treatments (229 and 172 kg cow^{-1}, respectively). Milk lactose yield was greater for the TAD cows (240 kg cow^{-1}) compared to the OAD6 (P<0.01; 219 kg cow^{-1}) and OAD8 (P<0.001; 216 kg cow^{-1}) treatments, no other differences were observed between treatments. There was no significant difference in MSY between treatments (P=0.299; 401 kg cow^{-1}).

There was also no difference in SCC between any of the treatments at four, six, eight and 12 weeks of lactation (4.69, 4.69, 4.74 and 4.73, respectively).

Table 1. The effect of twice-a-day (TAD) or once-a-day (OAD) milking frequency for four, six or eight weeks in early lactation on milk production on 35-week milk yield and milk solids yield.[1]

	OAD4	OAD6	OAD8	TAD	SE	*P*-value
Cumulative 35-week milk yield (kg cow^{-1})	5,073^{ab}	4,913^{b}	4,815^{b}	5,300^{a}	128.1	0.035
Cumulative 35-week MSY (kg cow^{-1})	405	398	387	415	11.0	0.299

[1] Means within a row with different superscripts differ; SE = standard error.

Conclusions

The results of this study indicate that OAD milking for up to eight weeks, in early lactation, can be used as a strategy to alleviate labour demands without reducing total lactation yield of milk fat plus milk protein.

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References

Central Statistics Office (2019) Livestock survey 2018. Available at: https://www.cso.ie/en/releasesandpublications/er/lsd/livestocksurveydecember2018/.

Clark D.A., Phyn C.V.C., Tong M.J., Collis S.J. and Dalley D.E. (2006) A systems comparison of once- versus twice-daily milking of pastured dairy cows. *Journal of Dairy Science* 89, 1854-1862.

Deming J., Gleeson D., O'Dwyer T., Kinsella J. and O'Brien B. (2018) Measuring labor input on pasture-based dairy farms using a smartphone. *Journal of Dairy Science* 10, 9527-9543

Stelwagen, K., Phyn C.V.C., Davis S.R., Guinard-Flament J., Pomiès D., Roche J.R. and Kay J.K. (2013) Invited review: reduced milking frequency: milk production and management implications. *Journal of Dairy Science* 96, 3401-3413.

Interactive effects of three different compound feeds and two contrasting grass silages mixed at different proportions on *in vitro* gas production and fermentation kinetics

Kidane A., Hoøen A.J.H., Prestløkken E., Vhile S.G., Jensen R.B. and Mydland L.T.
Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Arboretveien 6, 1432 Aas, Norway

Abstract

In the Nordic countries a large proportion of the winter feed for dairy cattle comes from cultivated grassland, conserved as silage. We tested the effects of mixing two contrasting grass silages (early and late cut; ECS vs LCS) with three different compound feeds (soy-based, SBP; yeast-based, YBP; and barley-based, BBP) at varying ratios on total gas production and fermentation kinetics using ANKOM RF *in vitro* gas production system. The combinations resulted in 23 different hypothetical dairy cattle rations. In the mixed diets, increasing the proportion of compound feeds, but not type of compound feed, influenced total gas production. Increasing compound feed proportion reduced the half-time to asymptotic gas production and increased the fractional rate of gas production (R_{gp}) (P<0.05) with both silages. However, the increment with R_{gp} was higher in LCS than ECS (P<0.05). The predicted higher asymptotic gas production from the LCS relative to the ECS (P<0.05) in contrast to recorded gas volume would suggest that LCS might require more than 48 h fermentation to achieve asymptotic gas production. The observed results suggested that LCS would benefit more than ECS from increasing the proportion of compound feed for optimal rumen fermentation and *in vivo* dry matter digestibility in dairy cow diets.

Keywords: yeast protein, soybean, silage, *in vitro,* gas production

Introduction

Dairy cow diets in the Nordic countries comprise grass silage, as a roughage, and compound feeds. In the latter, considerable amounts of imported protein ingredients like soybean meal, rapeseed expellers and corn gluten meal are used. This is deemed not sustainable due to low self-sufficiency and negative environmental impact. Efficient use of local feed resources like grass silage would provide possibilities for reducing the proportion of compound feeds and the amount of imported protein ingredients in compound feeds, benefiting self-sufficiency and the environment. However, the quality of basal diet is often variable and, as such, determines the quality and quantity of accompanying compound feed and animal performance (Randby *et al.*, 2012).

The objective of this study was to evaluate the effects of three compound feeds at varying ratios on the *in vitro* fermentation characteristics of two contrasting silage qualities. The compound feeds and silage mixtures represented the theoretical range in dairy cow rations in addition to the pure silages and compound feeds. We hypothesised that the effects on fermentation characteristics of the compound feeds and their inclusion levels would differ depending on the basal silage quality.

Materials and methods

Two contrasting qualities of silages: early cut with relatively low neutral detergent fibre, NDF and high crude protein, CP (=ECS) vs late-cut silage with high NDF and low CP content (=LCS) were mixed with three compound feeds (soya-based protein, yeast-based protein and barley replacing both protein ingredients) in the ratio of 100:0, 75:25, 50:50, 25:75 and 0:100. The treatment combinations resulted in 23 dietary combinations. Mean chemical composition of the diets is presented in Table 1. The *in vitro*

Table 1. Mean chemical composition (g kg^{-1} DM) of the silages and compound feeds at varying ratios.[1]

Silage:compound feed ratio	Early cut grass silage				Late cut grass silage			
	NDF	CP	EE	Ash	NDF	CP	EE	Ash
100:00	532.5	182.4	46.3	73.9	575.1	97.4	42.5	67.4
75:25	446.2	174.1	45.1	72.1	478.2	110.5	42.2	67.2
50:50	359.9	165.9	43.9	70.2	381.2	123.5	42.0	67.0
25:75	273.6	157.7	42.6	68.4	284.3	136.5	41.7	66.7
00:100	187.4	149.5	41.4	66.5	187.4	149.5	41.4	66.5

[1] NDF = neutral detergent fibre, CP = crude protein, EE = ether extract. Values for the compound feeds are means over the 3 compound feed types.

rumen fermentation was carried out using approximately 1.0 g dry matter (DM) of the feeds incubated in triplicate and in two batches with 100 ml of buffered (Goering and Van Soest, 1970) rumen fluid (buffer:rumen fluid 2:1) for 48 h. The inocula were prepared from rumen fluid collected about 3 h post-morning feeding from two rumen-cannulated, non-lactating standard cows fed according to Norfor (2011) feeding standard. The *in vitro* residues were recovered by filtering through 12 µm nylon bags, washed using standard nylon bag technique and dried for determination of DM degradation according to Norfor (2011). The DM loss is reported here as apparent DM degraded after 48h *in vitro* incubation.

Gas production profiles were fitted for individual incubation flasks using the model of Groot *et al.* (1996). The model estimates asymptotic gas production (ml g^{-1} DM), time needed to produce half of the asymptotic gas volume, and shape parameter determining the sharpness of the switching characteristic of the profile. For this Proc NLIN in SAS was applied with MARQUARDT iterative method. Fractional rate of gas production (R_{gp}) was calculated assuming a fixed linear relationship between substrate fermentation and gas production (Groot *et al.,* 1996). Parameter estimates and apparent DM degraded were later analysed using SAS Proc Mixed.

Results and discussion

There was no interaction effect between the compound feed types and silage qualities for the gas production parameters. The pure compound feeds produced more gas and had higher R_{gp} than other dietary treatments, as expected. As a result, increasing the proportion of compound feed in the dietary mixtures increased total gas production and the R_{gp} (P<0.05) with both silages (Figure 1). The predicted asymptotic gas production from the LCS was higher than that of ECS (P<0.05) in contrast to recorded gas volume (data not shown). This suggested that LCS might require more than 48 h fermentation to achieve asymptotic gas production, with increased rate of gas production showing up later in the fermentation process. This is commonly observed with fibrous feeds which are high in fibre and hence, slowly fermentable.

Mean DM degradation increased with increasing proportion of compound feeds in the diets (Figure 2). This was supported by the observed level and rate of gas production patterns. Increasing the proportion of compound feed to the LCS had a larger effect on the *in vitro* DM degradation than on the ECS. The response of LCS with increasing proportion of compound feeds suggested that the LCS would benefit more for optimal rumen fermentation and *in vivo* DM digestibility in dairy cow diets at close to 50:50 or less of the basal diet.

Conclusions

The lack of interaction between silage quality and compound feed type on total gas production and parameter estimates suggested that the LCS and compound feed combinations did not create a limiting condition for microbial activity. The effects of increasing proportion of compound feeds on gas production and DM degradation suggested that the LCS would benefit more from higher inclusion of the compound feeds than the ECS.

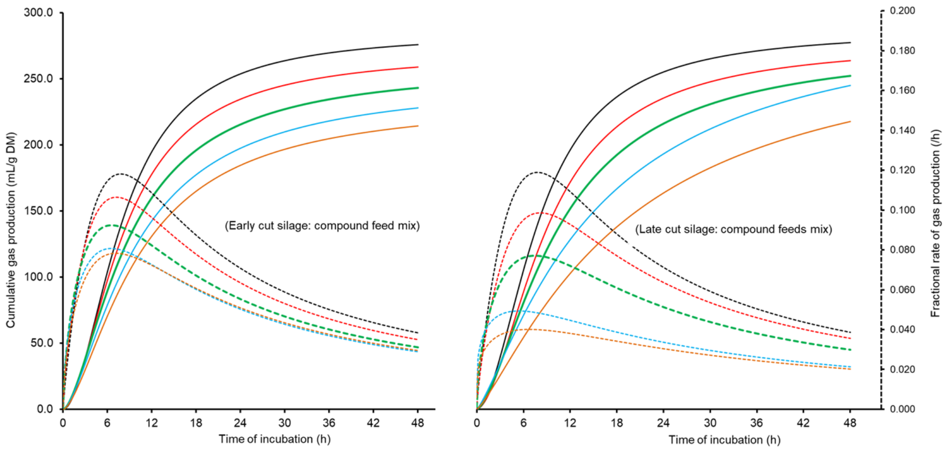

Figure 1. Cumulative gas production (GP; ml g^{-1} dry matter (DM), solid lines with left Y-axis) and fractional rate of gas production (R_{gp}; /h, broken lines with right Y-axis). Top to bottom in each graph: silage to compound feed ratio 0:100; 25:75; 50:50; 75:25 and 100:0 in respective order.

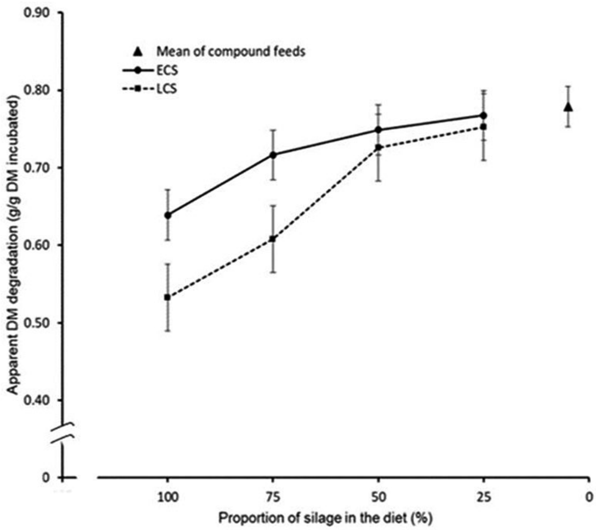

Figure 2. *In vitro* apparent dry matter (DM) degradation after 48 h.

Acknowledgements

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References

Goering H.K. and Van Soest P.J. (1970) Forage fiber analysis (apparatus, reagents, procedures and some applications). Agricultural Handbook No. 379 ARS-USDA, Washington, DC.

Groot J.C.J., Cone J.W., Williams B.A., Debersaques F.M.A. and Lantinga, E.A. (1996) Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feeds. *Animal Feed Science & Technology* 64, 77-89.

NorFor, (2011) The Nordic feed evaluation system. Volden H. (ed.). Wageningen Academic Publishers, Wageningen, the Netherlands.

Randby Å.T., Weisbjerg M.R., Nørgaard P. and Heringstad B. (2012) Early lactation feed intake and milk yield responses of dairy cows offered grass silages harvested at early maturity stages. *Journal of Dairy Science* 95, 304-317.

Lamb growth on pastures containing chicory (*Cichorium intybus*) under spring and summer grazing conditions

Kidane A.[1], Sørheim K.[2] and Steinshamn H.[3]
[1]*Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Arboretveien 6, 1432 Aas, Norway;* [2]*Norsk Senter for Økologisk Landbruk (NORSØK), Gunnars veg 6 NO-6630 Tingvoll, Norway;* [3]*Norwegian Institute of Bioeconomy Research (NIBIO), Division of Food Production and Society, Department of Grassland and Livestock, Tingvoll, Norway*

Abstract

Mountain grazing conditions represent a constraint on lamb growth performance for various reasons. One approach to counteract these effects is to graze lambs on improved pastures. We tested the effects of grazing ewes and/or their lambs on established grass-clover (GCM), chicory alone (CHA), and grass-clover-chicory (GCC) stands on spring (Exp.1) and summer (Exp.2) pastures on lamb performance. We hypothesized that CHA and GCC would sustain higher spring and autumn daily gain of lambs compared with GCM. In Exp.1, 12 twin-rearing ewes together with their 24 lambs were randomly allocated into three sward types replicated twice (n=2 ewes, 4 lambs/replicate) and monitored for 25 d before sending to mountain pasture. In Exp.2, 24 weaned lambs from the mountain pasture were again randomly allocated to one of the above pasture types and grazed for 28 d. We observed differences in chemical composition and estimated energy values between swards containing chicory and GCM swards. However, these did not affect lamb performance during both experiments, contrary to our previous findings, suggesting that grazing sheep either selected against chicory, or the observed differences in chemical composition among sward types were not strong enough to influence performance, or a combination of the two possible effects.

Keywords: chicory, lamb, growth, grazing

Introduction

Sheep comprise a substantial part of the livestock in Norway and grazed pastures contribute a large part of the feed that supports the sheep production. Most of the sheep population is kept on rangeland pastures from May to October. Prevalence of predators and parasites, restricted opportunities for direct and frequent monitoring, and requirement of a large grazing area to compensate for low forage quality are some of the constraints of rangeland pastures for sheep production. Established pastures close to farms provide buffer feed before sending and after collecting the flock from range pasture, along with concentrate supplementation. Therefore, there is a need to improve growth performance and feed-use efficiency of lambs by using alternative feed resources. In this regard, forage chicory (*Cichorium intybus*) has been shown to be high yielding and a good source of nutrients for grazing lambs either sown alone or in a mixed stand with conventional grass-clover (Golding *et al.,* 2011; Kidane *et al.,* 2009, 2014).

We hypothesized that chicory alone (CHA) or in mixture with the grass-clover stand (GCC) would sustain higher spring and summer daily gain of lambs compared with a grass-clover mixture (GCM). This was tested using performance of suckling-grazing (Exp. 1) and weaned (Exp. 2) lambs.

Materials and methods

The experiments were conducted with grazing ewes and lambs (Exp. 1; 24 lambs for 25 days) and weaned lambs (Exp. 2; 24 lambs for 28 days) on a second-year regrowth of pastures established as CHA, GCC and GCM. The experiments were conducted in Møre og Romsdal county (Tingvoll, Norway) and details on sward establishment and management are reported in Kidane *et al.* (2014). Lambs were randomly allocated into one of the three pasture sward types, replicated twice (2 plots; 4 lambs/replicate) after adjusting for initial body weight (BW). Lamb BW was monitored by weighing every other week. Herbage crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), non-fibre carbohydrate (NFC) and mineral composition were analysed as described in Kidane *et al.* (2014) on hand-cut samples collected every other week.

Data collected in the experiments were merged and analysed using repeated measurements ANOVA with SAS Mixed Models (SAS for Windows 9.4, SAS Institute Inc.; Cary, NC, USA) accounting for the fixed effects of the experiment. For BW, initial weights were used as covariates.

Results and discussion

Sowing chicory alone improved nutrient composition and energy values relative to the grass-clover stand, with increased CP, NFC, mineral composition and net energy values (Table 1). However, contrary to our hypothesis, lamb growth was not affected by the grazed forage type in any of the experiments (Figure 1.; $P>0.1$). It is possible that the nutrient requirements of lambs were not restricted in the GCM pasture, and effects of the observed differences in chemical composition were subtle.

Furthermore, previous reports of improved growth performance of lambs when grazing CHA or GCC compared with other swards were seen with relatively longer grazing periods (Kidane *et al.,* 2009, 2010, 2014; Komolong, 1994) than the 25 or 28 days used here. Lastly, it is difficult to imitate herbage samples to match that of the actual herbage dry matter as consumed by grazing lambs. Therefore, the nutrient

Table 1. Chemical composition (g kg^{-1} DM, unless otherwise mentioned) of herbage samples taken at three cutting points (start, middle and end of grazing) from grazed paddocks.[1]

Parameter	Forage type			Experiment		SE	*P*-value				
	CHA	GCC	GCM	Spring	Summer		*ft*	*exp*	*cd*	*ft*exp*	*ft*cd*
CP	165^a	146^b	134^b	126	170	6.9	**	**	**	ns	ns
NDF	420^b	528^a	528^a	494	497	12.7	**	ns	**	ns	ns
ADF	293^b	316^a	307^b	294	317	8.6	*	**	**	ns	ns
NFC	305^a	226^b	238^b	280	233	12.2	**	**	**	ns	ns
Macro-minerals											
Ca	8.8^a	4.7^b	4.4^b	4.7	7.2	0.32	**	**	**	ns	ns
P	3.6^a	3.0^b	2.7^c	3.0	3.2	0.12	**	**	**	*	ns
K	29.2^a	22.7^b	21.4^b	22.2	26.7	1.28	**	**	*	ns	ns
Na	2.2^a	0.4^b	0.2^b	0.8	1.1	0.18	**	**	ns	*	ns
Micro-minerals (mg kg^{-1} DM)											
Fe	236^a	115^b	126^b	116	202	26.3	**	**	*	ns	ns
Zn	42.8^a	24.5^b	23.6^b	27.9	32.7	2.94	**	*	ns	ns	ns
Cu	6.64^a	4.61^b	4.36^b	4.17	6.24	0.221	**	**	**	ns	ns
Mn	89.0^a	62.7^b	56.2^b	58.8	79.9	5.55	**	**	*	ns	ns
Mo	1.93	1.48	1.49	1.87	1.40	0.385	ns	ns	ns	ns	ns
Total digestible nutrient (TDN, %), net energy for maintenance and for gain (MJ kg^{-1} DM)											
TDN	61.9^a	59.1^b	59.1^b	59.9	60.1	0.37	**	ns	**	ns	ns
NE_m	5.5^a	5.0^b	5.0^b	5.2	5.2	0.07	**	ns	**	ns	ns
NE_g	3.1^a	2.7^b	2.7^b	2.8	2.8	0.06	**	ns	**	ns	ns

[1] Standard error of forage type by experiment interaction term; CHA = chicory alone stand; GCC = grass-clover-chicory mixed stand; GCM = grass-clover mixed stand; *ft* = forage type; *exp* = experiment; *cd* = cutting date; DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; NFC = non-fibre carbohydrate; NE = net energy; SE = standard error; ** $P<0.01$; * $P<0.05$ and ns = not statistically significant. Means in a row with different superscripts are different at α=0.05.

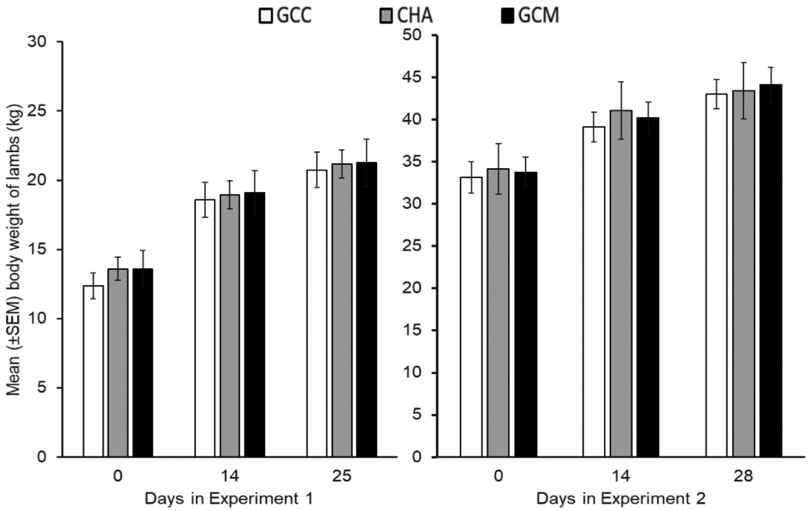

Figure 1. Body weight development of lambs during spring (Exp. 1) and summer (Exp. 2) grazing period. GCC= grass-clover-chicory mixed stand; CHA= chicory alone stand; GCM= grass-clover mixed stand; SEM = standard error of the mean.

intake achieved by lambs may not have been as contrasting as what is reported here due to the effects of selective grazing behaviour (Hodgson *et al.,* 1991) either averting or preferring a plant species (Nielsen *et al.,* 2009).

Conclusions

Inclusion of chicory in sown pasture swards improved nutrient composition and energy values but did not influence growth performance of lambs. This could be due to the short duration of our experiments in contrast to that of other reported results (Kidane *et al.,* 2009; Komolong, 1994).

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References

Golding K.P., Wilson E.D., Kemp P.D., Pain S.J., Kenyon P.R., Morris S.T. and Hutton P.G. (2011) Mixed herb and legume pasture improves the growth of lambs post-weaning. *Journal of Animal Production Science* 51, 717-723.

Hodgson J., Forbes T.D.A., Armstrong R.H., Beattie M.M. and Hunter E.A. (1991) Comparative studies of the ingestive behaviour and herbage intake of sheep and cattle grazing indigenous hill plant communities. *Journal of Applied Ecology* 28, 205-227.

Kidane A., Houdijk J.G.M., Athanasiadou S., Tolkamp B.J. and Kyriazakis I. (2010) Effects of maternal protein nutrition and subsequent grazing on chicory (*Cichorium intybus*) on parasitism and performance of lambs. *Journal of Animal Science* 88, 1513-1521.

Kidane A., Parissi Z.M., Houdijk J.G.M., Athanasiadou S., Tolkamp B.J. and Kyriazakis I. (2009) Chicory as promising bioactive forage for sheep production. *Grassland Science in Europe* 14, 394-397.

Kidane A., Sorheim K., Eik L.O. and Steinshamn H. (2014) Growth and chemical composition of chicory and performance of lambs grazing chicory relative to grass-clover mixtures. *Acta Agriculturae Scandinavica Section A-Animal Science* 64, 233-242.

Komolong M.K. (1994) Nutrient supply for lamb growth from Grasslands Puna chicory (*Cichorium intybus*) and Wana cocksfoot (*Dactylis glomerata*), Lincoln University, New Zealand, pp. 139.

Nielsen B.K., Thamsborg S.M., Hansen H., Ranvig H. and Hogh-Jensen H. (2009) Effects of including chicory in perennial ryegrass-white clover leys on production and health in organic lambs. *Livestock Science* 125, 66-73.

The effects of close-up concentrate feeding in grass silage-based diet of dairy cows

Kokkonen T., Halmemies-Beauchet-Filleau A., Jaakkola S. and Vanhatalo A.
Department of Agricultural Sciences, University of Helsinki, Finland

Abstract

We studied whether the inclusion of concentrate in a grass silage and straw-based close-up diet during the dry period is needed to prime the rumen for high-concentrate feeding after calving, potentially improving feed intake and production performance. During the 3-week close-up period, 16 multiparous cows received either *ad libitum* feeding of a mixture of grass silage (64.4% of dry matter), straw (27.6%) and rapeseed meal (8%), or a mixture of grass silage (49%), straw (21%) and concentrate (30%). After parturition, both groups were offered *ad libitum* partial mixed ration of grass silage (45.5%), whole-crop barley silage (19.5%) and concentrate mixture (35%). In addition, all cows received the same amount of concentrate (max. 8 kg d^{-1}) during milking. Close-up concentrate feeding did not affect total dry matter intake, body condition score and milk yield. In conclusion, we observed only transient benefits of concentrate supplementation in a grass silage and straw-based close-up diet.

Keywords: grass silage, straw, transition period

Introduction

During the transition from pregnancy to lactation dairy cows must adapt to a two- to three-fold increase in energy requirement due to onset of milk production (Bell, 1995). Although cows may utilise their lipid reserves to fill the gap between energy intake and demand during early lactation, a deep negative energy balance predisposes the cows to metabolic diseases and may disturb fertility. Therefore, an increase in feed intake after calving is an essential factor for successful transition. Inclusion of cereal concentrates in the dairy cow diet during the last few weeks before calving is used to prime the rumen for the early lactation diet with high concentrate proportion (Grummer *et al.*, 2004). The rationale for concentrate feeding in the close-up period is to increase volatile fatty acid absorption capacity by enhancing the surface area of the rumen papillae (Dirksen *et al.*, 1985). Further, rumen microorganisms may need time to adapt to the lactation diet with increased proportion of non-structural carbohydrates. Recent studies have shown major changes in relative abundancies of ruminal fibrolytic, proteolytic, amylolytic, and lactate-producing bacteria during close-up feeding and after a shift to a lactation diet (Derakshani *et al.*, 2017). The adaptation of rumen microbiota and the increase of volatile fatty acid absorption capacity may prevent the development of rumen acidosis during early lactation. We hypothesised that close-up concentrate feeding increases feed intake and milk production during early lactation.

Materials and methods

Sixteen Finnish Ayrshire cows were used in a randomized complete block design. The cows were paired based on parity (second or later calving), expected calving date and body weight. Within pairs, the cows were then allocated to one of the two treatment groups three weeks before the expected calving date. Treatments consisted of *ad libitum* feeding of forage-rich mixture of grass silage (64.4% of dry matter (DM)), straw (27.6%) and rapeseed meal (8%) (FOR), or mixture of grass silage (49%), straw (21%) and concentrate (30%) (CONC). After parturition, both groups were offered *ad libitum* partial mixed ration of grass silage (45.5%), whole-crop barley silage (19.5%) and concentrate mixture (35%). In addition, all cows received the same amount of concentrate (max. 8 kg d^{-1}) during milking. Feed intake was recorded daily with roughage intake control system (Insentec BV, Marknesse, the Netherlands). Cows were housed in tie stalls throughout the dry period and first ten days of lactation. In tie stalls, the cows were milked twice daily. After d 10 of lactation, the cows were moved to free stall and milked with an automated milking system (Lely Astronaut A3, Lely Industries N.V., Maassluis, the Netherlands). Milk yield was recorded for every milking. Samples were taken on four consecutive milkings, at 1, 2, 4, 6 and 8 weeks after parturition. Body condition score (BCS) was assessed on a 5-point scale (Edmonson *et al.,* 1989) 1 wk prior to calving, and 2, 10, 28, 42 and 56 d after calving. Data for feed intake, milk production and postpartal BCS were analysed as repeated measures ANOVA using the Mixed procedure of SAS version 9.4 (SAS Institute, Cary, NC, USA) with a model that included fixed effects of treatment and time and their interaction, and random effects of block and interaction between block and time. Prepartal BCS was analysed with the PROC MIXED procedure with a model that included the fixed effect of treatment and the random effect of block.

Results and discussion

Close-up concentrate feeding did not affect feed intake prepartum or postpartum (Table 1). The absence of effects on feed intake postpartum is in agreement with Keady *et al.* (2001). In contrast, McNamara *et al.* (2003) observed that increasing energy density of the grass-silage based diet in the last 4 wk of the dry period increased feed intake after calving. Based on body condition score, body energy reserves before calving and their mobilization after calving was similar in the treatment groups. Milk yield was unaffected by the close-up concentrate feeding. This is in line with the results by Keady *et al.* (2001), whereas McNamara *et al.* (2003) reported higher milk yield with close-up concentrate feeding. In the current study, the cows in close-up concentrate feeding group tended to have higher energy corrected milk yield (Figure 1) and milk lactose concentration during lactation weeks 1 and 2. Milk fat content was lower in CONC at lactation week 1 but higher at weeks 2 and 4 than in FOR.

Table 1. The effect of close-up concentrate feeding on dairy cow performance on grass-silage-based diet.[1]

	Diet[2]		SEM	Significance		
	CONC	FOR		Treatment	Time	Treatment × time
Feed intake, kg DM d^{-1}						
Prepartum, d -21 to -1	12.2	12.2	0.48	0.97	0.14	0.84
Postpartum, d +1 to 56	24.0	23.9	0.76	0.93	<0.001	0.44
Body condition score						
Prepartum, d -7	3.43	3.33	0.09	0.42	na	na
Postpartum, d +1 to 56	3.05	2.95	0.09	0.44	<0.001	0.93
Yield						
Milk, kg d^{-1}	45.4	44.0	1.62	0.56	<0.001	0.56
Energy corrected milk, kg d^{-1}	43.1	41.9	1.43	0.51	<0.001	0.07
Content						
Fat, g kg^{-1}	42.1	42.4	1.10	0.85	<0.001	<0.01
Protein, g kg^{-1}	33.0	34.0	0.71	0.14	<0.001	0.88
Lactose, g kg^{-1}	45.6	45.3	0.41	0.61	<0.001	<0.01
Urea, mg dl^{-1}	23.4	23.5	1.08	0.93	<0.001	0.99

[1] DM = dry matter; na = not applicable; SEM = standard error of the mean.

[2] CONC = mixture of grass silage (49%), straw (21%) and concentrate (30%); FOR = forage-rich mixture of grass silage (64.4% of DM), straw (27.6%) and rapeseed meal (8%).

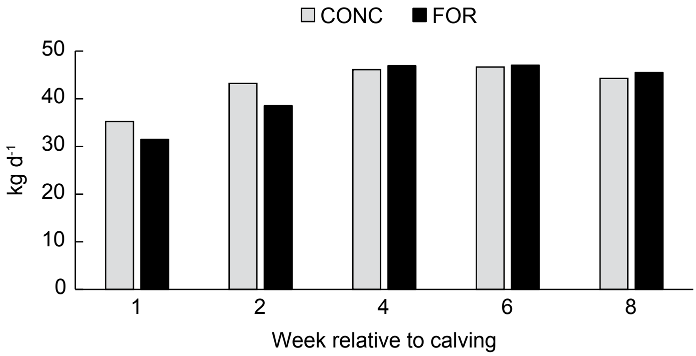

Figure 1. Energy corrected milk yield (CONC = mixture of grass silage (49%), straw (21%) and concentrate (30%); FOR = forage-rich mixture of grass silage (64.4% of dry matter), straw (27.6%) and rapeseed meal (8%)).

Conclusions

Close-up feeding did not affect total dry matter intake and body condition score pre- or postpartum but tended to induce a more rapid increase of energy corrected milk yield. In conclusion, we observed only transient benefits of concentrate supplementation in grass silage and straw-based close-up diet.

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References

Bell A.W. (1995) Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *Journal of Animal Science* 73, 2804-2819.

Derakhshani H., Tun H.M., Cardoso F.C., Plaizier J.C., Khafipour E. and Loor J.J. (2017) Linking peripartal dynamics of ruminal microbiota to dietary changes and production parameters. *Frontiers in Microbiology* 7, 2143.

Dirksen G.U., Liebich H.G. and Mayer E. (1985) Adaptive changes of the ruminal mucosa and their functional and clinical significance. *Bovine Practitioner* 20, 116-120.

Edmonson A.J., Lean I.J., Weaver L.D., Farver T. and Webster G. (1989) A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science* 72, 68-78.

Grummer R.R., Mashek D.G. and Hayirli A. (2004) Dry matter intake and energy balance in the transition period. *Veterinary Clinics of North America: Food Animal Practice* 20, 447-470.

Keady T.W.J., Mayne C.S., Fitzpatrick D.A. and McCoy M.A. (2001) Effect of concentrate feed level in late gestation on subsequent milk yield, milk composition, and fertility of dairy cows. *Journal of Dairy Science* 84, 1468-1479.

McNamara S., O'Mara F.P., Rath M. and Murphy J.J. (2003) Effects of different transition diets on dry matter intake, milk production, and milk composition in dairy cows. *Journal of Dairy Science* 86, 2397-2408.

Nutritive value of *Dactylis glomerata* L. is affected by temperature increase and CO_2-enhancement

Küsters J.[1], Pötsch E.M.[2], Resch R.[2] and Gierus M.[1]

[1]*Institute of Animal Nutrition, Livestock Products, and Nutrition Physiology, University of Natural Resources and Life Sciences, Vienna, Austria;* [2]*Department of Grassland Management and Cultural Landscape, Agricultural Research and Education Centre Raumberg, Gumpenstein, Irdning, Austria*

Abstract

High yielding ruminants require high nutritive value in forage for maintenance and production of animal products. However, climate change will impact on grassland, altering the nutritive value of forage. The aim of this study was to investigate the impact of elevated temperature and CO_2-enhancement on the nutritive value of orchard grass (*Dactylis glomerata* L.) growing in mountainous permanent grassland. The experiment took place within the ClimGrass-project of the AREC Raumberg-Gumpenstein in Austria. Next to ambient climate conditions, treatments with increased temperature (+3 °C) and higher CO_2 levels (+0.3 g kg^{-1}) were carried out, simulating future climate conditions (SCC). From 2016-18, plots were sampled in a 3-cut system. Measurements on development stage, leaf weight ratio, tiller height and weight were performed, and nutritive value determined for orchard grass. SCC influenced tiller height and weight. Crude protein and carbohydrate fractionation, as well as energy content and digestibility were positively affected in SCC. In conclusion, ruminant production systems will face climate change advantageously regarding energy content and digestibility, but decreased biomass production will worsen forage provision.

Keywords: climate change, grassland, temperature increase, CO_2-enhancement, ruminants

Introduction

Agriculture, as a sensitive ecosystem, is severely threatened by climate change and variability (Wheeler and Reynolds, 2013). This will result in a range of management problems, including heat stress and thus decreased animal welfare, manure storage and application and forage production, among others. Livestock systems may be influenced by climate change indirectly through impacts on productivity and changes in nutritive value of pastures and forage crops. Short-term fluctuations in weather conditions impact nutritive value. This fluctuation can mainly be monitored with the determination of water-soluble carbohydrates (WSC) contents, supported by plant-related measurements like development stage determination, and leaf weight ratio (LWR). However, short-term fluctuations in weather on variation in the non-protein nitrogen (NPN) of plants have not been considered up to now. In ruminants, microbial activity in the rumen converts forage protein into NPN, which serves as amino acids source to fulfil the requirements of the host. Therefore, detecting the content of crude protein in forage for ruminants only is not enough to evaluate the quality of protein in forage. Predicting the response of forage crops to stress conditions will help to adjust management practices, determine the optimum harvest date at the proper stage of maturity to achieve highest nutritive value, which will be positively reflected on the animal performance. The aim of this project was to evaluate the influence of climate change on the nutritive value of permanent grassland as important feed basis in livestock production. It is hypothesized that WSC and NPN are equally dependent on variable climate conditions in orchard grass, and that development stage is altered by increased temperature and elevated CO_2-concentration. Orchard grass will serve as reference species being the dominant grass in the experimental site.

Materials and methods

The sampling took place in the ClimGrass-project at AREC Raumberg – Gumpenstein in Styria, Austria (Pötsch *et al.*, 2017). Experimental plots were equipped with infrared heaters for increasing air temperature, and with a central fumigation ring for enhancing the concentration of CO_2. The sampling of orchard grass was carried out in three successive years (2016-2018) at each growth in a 3-cut system. One part of the samples was taken right before harvesting to identify the development stage of orchard grass and to determine height, weight and LWR of the tillers. Based on these measurements mean stage by count (MSC) and mean stage by weight (MSW) were estimated (Salama *et al.*, 2017). The samples were stored and analysed in Vienna, according to the proximal analysis methods. Additionally, the protein fractionation (Licitra *et al.*, 1996), the carbohydrate fractionation, as well as the *in vitro* digestibility (DOM) and the metabolizable energy (ME) content were analysed. The results were statistically evaluated with a mixed linear model. Treatment and cut are fix effects and year functions as repetition, considering possible interactions between treatment and cut. MSC and MSW are non-parametric variables and were evaluated with a Wilcoxon-test.

Results and discussion

Both MSC and MSW were similar between treatments. Regarding agronomic measurements, treatments differed in height and weight of the tillers, simulating future climate conditions (SCC) being inferior to ambient climate conditions (ACC). LWR, dry matter content (DM) and crude protein (CP) did not show any differences between the treatments. The CP fractionation, however, revealed significant differences in NPN and neutral detergent – insoluble protein (NDIP). The NPN content under SCC was significantly lower than in ACC. On the other hand, SCC was higher in NDIP than under ACC. The content of WSC was higher whereas NDF and ADF were lower under future climate conditions. The results show that the ME content and the DOM were higher in SCC than in ACC.

Higher temperature and enhanced CO_2 were assumed to be advantageous for orchard grass development, compared with ACC. This could not be proven in this experiment. In contrast, under SCC tiller height and weight were lower than under ACC. Since harvesting takes place during the summer months, the rising temperature can become disadvantageous for plants when water supply is limited at the same time, due to infrequent precipitation, thus reducing biomass production. Dumont *et al.* (2015) suggested that higher temperature leads to lower LWR, but this was not the case in our study. Elevated temperature did not influence DM content. Kyriazopoulos *et al.* (2012) found that shading does not affect the DM content of orchard grass, so this could be a similar ineffectiveness to that of different temperatures.

It was hypothesised that NPN and WSC are most likely to be sensitive parameters regarding short-term changes of climate. With SCC being lower, there is indeed a climate-related influence on NPN. In contrast, WSC increased with SCC. Higher temperatures lead to an increased and accelerated conversion of N into complex N compounds, reducing NPN content in SCC. Corresponding to this is the higher content of NDIP in SCC, which reflects the conversion into cell wall bonded proteins. However, the elevated temperature and enhanced CO_2 led to an increased carbohydrate synthesis in the plant as indicated by higher WSC content. Dumont *et al.* (2015) revealed that elevated CO_2 has no effect on structural carbohydrates. So, in this case the lower content of NDF and ADF in SCC must be attributed to the difference in the WSC content as well as in the height of the tillers. As increased CO_2 allows more storage of carbohydrates, the WSC content accounted mainly for the higher ME content in SCC, when there is not simultaneously advantage in growth. In addition, the higher DOM can partly be related to the lower content of NDF and ADF, as cell wall constituents contain high rates of indigestible fibrous substances like lignin and insoluble parts of hemicellulose and cellulose lowering total digestibility.

Table 1. Measurements and nutritive value in orchard grass.[1]

	ACC	SCC	*P*-value	SEM
Mean stage by count	6	7	0.903	0.49
Mean stage by weight	7	7	0.815	0.55
Leaf weight ratio (%)	42.3	44.7	0.088	2.56
Height of tillers (cm)	70.6	54.4	<0.001	2.47
Weight of tillers (g of fresh sample)	3.22	2.42	<0.001	0.17
Dry matter (g kg fresh sample)	226	216	0.065	6.91
Crude protein (g kg^{-1} DM)	119	121	0.343	2.79
Non-protein N (g kg^{-1} CP)	339	312	0.003	5.43
Neutral detergent insoluble protein (g kg^{-1} CP)	371	397	0.013	7.17
Acid detergent insoluble protein (g kg^{-1} CP)	81	81	0.981	2.90
Water soluble carbohydrates (g kg^{-1} DM)	83	90	<0.001	2.45
Neutral detergent fibre (g kg^{-1} DM)	648	619	<0.001	6.37
Acid detergent fibre (g kg^{-1} DM)	387	368	<0.001	4.95
Metabolizable energy (MJ kg^{-1} DM)	8.86	9.18	<0.001	0.10
In vitro digestibility (%)	65.4	68.2	<0.001	0.77

[1] ACC = ambient climate condition; SCC = simulated climate condition; DM = dry matter; CP = crude protein; SEM = standard error of the mean.

Conclusions

It was hypothesised that WSC and NPN are equally dependent on variable climate conditions in orchard grass. This was proven by lower NPN and higher WSC contents under future climate conditions with elevated temperature and enhanced CO_2. On the contrary, there was no alteration of the development stage due to climate variation. However, height and weight decreased with elevated temperature and enhanced CO_2, which means that a decline in biomass production is therefore likely to occur under future scenarios. The outcome of this project reveals circumstances of climate in the near future, in terms of plant productivity, nutritive value and consequently utilisation by livestock.

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References

Dumont B., Andueza D., Niderkorn V., Lüscher A., Porqueddu C. and Picon-Cochard C. (2015) A meta-analysis of climate change effects on forage quality in grasslands: specificities of mountain and Mediterranean areas. *Grass and Forage Science* 70, 239-254.

Kyriazopoulos A.P., Abraham E.M., Parissi Z.M., Koukoura Z. and Nastis A.S. (2012) Forage production and nutritive value of *Dactylis glomerata* and *Trifolium subterraneum* mixtures under different shading treatments. *Grass and Forage Science* 68, 72-82.

Licitra G., Hernandez T.M. and Van Soest P.J. (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology* 57, 347-358.

Pötsch E.M., Herndl M., Bahn M., Schaumberger A., Schweiger M., Kandolf M., Reinthaler D., Schink M. and Adelwöhrer M. (2017) ClimGrass – ein innovatives Freilandexperiment zur Erforschung der Folgen des Klimawandels im Grünland. In: Bericht zum 21. Alpenländischen Expertenforum *'Klimawandel im Alpenraum – Auswirkungen auf das Ökosystem Grünland und dessen Bewirtschaftung'*, HBLFA Raumberg-Gumpenstein, pp. 3-9.

Salama H., Loesche M., Herrmann A., Taube F. and Gierus M. (2017) A simplified maturity index to quantify the development stage of perennial ryegrass (*Lolium perenne* L.) and its relationship with yield and nutritive value. *Bodenkultur* 68, 89-101.

Wheeler T. and Reynolds C. (2013) Predicting the risks from climate change to forage and crop production for animal feed. *Animal Frontiers* 3, 36-41.

The effect of forage to concentrate ratio and forage type on fat globule size of cow milk

Leskinen H., Jaakamo M., Luukkonen T., Kairenius P., Bayat A.R., Ahvenjärvi S., Tupasela T., Vilkki J. and Shingfield K.

Production Systems, Natural Resources Institute Finland (Luke), 31600 Jokioinen, Finland

Abstract

The effects of two grass silage-based diets with different forage:concentrate (FC) ratio (70:30, HG; 30:70, LG) and a red clover silage-based diet (FC 50:50, RC) on intake, milk production, ruminal fatty acid (FA) biohydrogenation, milk FA composition and milk fat globule (MFG) size distribution were examined in ten multiparous Nordic Red cows. LG led to 13% lower milk fat concentration compared with HG. The effect of FC ratio on MFG size was moderate and LG decreased the volume-surface diameter of MFG in the size class >1 μm compared with HG. LG increased the level of polyunsaturated FA (PUFA) in milk fat compared with HG. RC lowered milk fat yield compared with grass silage-based diets and decreased the volume-weighted diameter of MFG in the size class >1 μm. RC increased the flow of 18:3n-3 FA at the omasum by 2.4-fold due to decreased biohydrogenation of 18:3n-3. RC milk had the lowest amount of saturated FA and the highest amounts of *cis*-9 18:1, 18:3n-3 and PUFA in milk fat. In conclusion, LG induced only minor changes in MFG size distribution compared with HG, whereas RC led to smaller MFG compared with grass silage-based diets.

Keywords: milk fat globule, grass silage, red clover silage, fatty acid composition, dairy, cow

Introduction

Milk fat is secreted from the mammary gland as milk fat globule (MFG) surrounded by a trilayer membrane, the MFG membrane, which consists of polar lipids, proteins, cholesterol, and various minor components (Lopez *et al.*, 2008). The emerging evidence on the potential functional properties of MFG and health benefits of MFG membrane components has increased interest in examining the effect of dairy cow feeding on MFG size and membrane composition. Previous reports suggest that diet of dairy cows affects MFG size (Argov-Argaman *et al.*, 2014; Lopez *et al.*, 2008; Wiking *et al.*, 2010), but the results have been inconclusive. Red clover is known to increase PUFA, especially 18:3n-3, and to decrease saturated FA concentrations in milk fat (Halmemies-Beauchet-Filleau *et al.*, 2014). However, published data regarding the effect of grass silage-based diets, the predominant feeding system in Nordic countries, and red clover-based diets on MFG are scarce. Our aim was to investigate how FC ratio in grass silage-based diets and a diet based on red clover silage affects MFG size distribution and FA metabolism in dairy cow. We hypothesised that the changes in the biohydrogenation of FA in the rumen would affect the FA composition in milk fat and thus affect MFG sizes.

Materials and methods

Ten multiparous Nordic Red cows (74.4±21.0 postpartum) fitted with rumen cannulas were randomly allocated to experimental diets in a 3 times replicated 3×3 Latin square with an extra replicate that was randomly allocated to one of the diets in each 35-d experimental period (n=10 for each diet throughout the study). The trial was split into 2 blocks of 4 or 6 animals over time (7-wk interval) to have all cows in the same lactation stage. Cows were offered cereal-based concentrate and grass silage (a mixture of timothy, *Phleum pratense*, and meadow fescue, *Festuca pratensis*) as total mixed ration with either 70:30 (HG) or 30:70 (LG) FC ratio (on a dry matter basis) and a red clover silage (*Trifolium pratense*) with 50:50 FC ratio (RC). Feed intake and milk yield were recorded daily, and representative samples were analysed as reported elsewhere (Halmemies-Beauchet-Filleau *et al.*, 2011; Shingfield *et al.*, 2002, 2003). Samples of omasal canal digesta were collected over four days to obtain samples representative of the entire feeding cycle, and the omasal canal digesta flow was measured using a triple marker system (Ahvenjärvi *et al.*, 2000). Size distributions of MFG (n=6 per diet, 2nd block) were determined using a Mastersizer 2000 (Malvern Panalytical Ltd., Malvern, UK) particle size analyser (Lopez *et al.*, 2008). Calculations were conducted only for the >1 μm size class as casein micelles contributing to the <1 μm class were present in the fresh milk samples. All statistical analyses of data were performed by ANOVA, using SAS (version 9.4; SAS Institute Inc., NC, USA) with PROC GLIMMIX model, including period, diet and diet by period interaction as fixed effects and cow, block and period by block interaction as random effects. Data on MFG size distribution were analysed with the same model, but block and period × block interaction were excluded. Orthogonal contrasts were (1) LG vs HG (FC effect in grass silage-based diets) and (2) RC vs grass silage diets (forage type effect).

Results and discussion

High grass diet increased volume-surface diameter of MFG from 3.72 to 3.87 μm and decreased specific surface area from 1,618 to 1,549 $m^2 kg^{-1}$ compared with LG (P<0.05). In a previous study with corn silage and oat and clover hay, MFG diameter tended also to increase with lower concentrate content (Argov-Argaman *et al.*, 2014). The red clover-based diet resulted in 0.18 μm smaller MFG (4.15 vs 4.33 μm) and elevated specific surface area (1,647 vs 1,584 $m^2 kg^{-1}$) compared with grass silage-based diets (P<0.05). However, in the study of Wiking *et al.* (2010), the MFG diameter did not change in a comparison of a diet consisting of red clover and ryegrass and a diet based on corn and grass silage. In addition to the diet effect, some studies have reported that when the mammary gland secretes higher level of fat the MFG size increases, possibly because the membrane material becomes a limiting factor (Wiking *et al.*, 2004). In this study, both MFG size and milk fat yield were reduced (P<0.05) in RC diet, and LG led to 13% lower milk fat concentration (P<0.001) compared with HG, but no correlation between MFG size and milk fat yield was observed, probably due to a high variation between individual cows in general or variation in their individual responses to the diets.

Low grass diet increased PUFA content in milk fat compared with HG (P<0.001; 4.30 and 3.58 g per 100 g FA, respectively) due to higher (P<0.001) intake and omasal flow of 18:2n-6 in LG. Although 18:3n-3 intake in RC was rather moderate, higher ruminal escape of 18:3n-3 (P<0.001; 21.1 and 8.8 g d^{-1} for RC and grass diets, respectively) led to 1.7-fold increase in the proportion of 18:3n-3 in milk (P<0.001; 0.96 and 0.56 g per 100 g FA for RC and grass diets, respectively) compared with grass silage-based diets, which was consistent with previous studies (Halmemies-Beauchet-Filleau *et al.*, 2013, 2014; Moorby *et al.*, 2009). Consistent with earlier studies (Moorby *et al.*, 2009), RC decreased (P<0.001) saturated FA concentration in milk fat and led to higher levels of monounsaturated FA (MUFA), mainly *cis*-9 18:1, and PUFA in milk fat compared with grass silage-based diets (P<0.001; 25.8 and 22.2 g MUFA per 100 g FA and 4.80 and 3.9 g PUFA per 100 g FA, respectively). Smaller MFG contain relatively more polar lipids that are rich in PUFA (Heid and Keenan, 2005; Mesilati-Stahy *et al.*, 2011). Whether high omasal canal flow of unsaturated FA available for absorption and high *cis*-9 18:1 and total PUFA in milk fat in RC are reflected in MFGM structure and contributes to the smaller MFG sizes remains a question.

Conclusions

The results show that dairy cow diets affect MFG size of raw milk in >1 μm size class. Low grass diet induced only minor changes in MFG size distribution compared with HG, whereas RC led to smaller MFG compared with grass silage-based diets. Red clover-based diet led to higher MUFA and PUFA levels at the omasal canal and in milk fat. These changes are beneficial for human health and could reflect the smaller MFG in RC compared with grass silage-based diets.

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References

Ahvenjärvi S., Vanhatalo A., Huhtanen P. and Varvikko T. (2000) Determination of reticulo-rumen and whole-stomach digestion in lactating cows by omasal canal or duodenal sampling. *British Journal of Nutrition* 83, 67-77.

Argov-Argaman N., Mesilati-Stahy R., Magen Y. and Moallem U. (2014) Elevated concentrate-to-forage ratio in dairy cow rations is associated with a shift in the diameter of milk fat globules and remodeling of their membranes. *Journal of Dairy Science* 97, 6286-6295.

Halmemies-Beauchet-Filleau A., Kokkonen T., Lampi A.-M., Toivonen V., Shingfield K. J. and Vanhatalo A. (2011) Effect of plant oils and camelina expeller on milk fatty acid composition in lactating cows fed diets based on red clover silage. *Journal of Dairy Science* 94, 4413-4430.

Halmemies-Beauchet-Filleau A., Vanhatalo A., Toivonen V., Heikkilä T., Lee M.R.F. and Shingfield K.J. (2013) Effect of replacing grass silage with red clover silage on ruminal lipid metabolism in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. *Journal of Dairy Science* 96, 5882-5900.

Halmemies-Beauchet-Filleau A., Vanhatalo A., Toivonen V., Heikkilä T., Lee M. and Shingfield K.J. (2014) Effect of replacing grass silage with red clover silage on nutrient digestion, nitrogen metabolism, and milk fat composition in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. *Journal of Dairy Science* 97, 3761-3776.

Heid H.W. and Keenan T.W. (2005) Intracellular origin and secretion of milk fat globules. *European Journal of Cell Biology* 84, 245-258.

Lopez C., Briard-Bion V., Menard O., Rousseau F., Pradel P. and Besle J.M. (2008) Phospholipid, sphingolipid, and fatty acid compositions of the milk fat globule membrane are modified by diet. *Journal of Agricultural and Food Chemistry* 56, 5226-5236.

Mesilati-Stahy R., Mida K. and Argov-Argaman N. (2011) Size-dependent lipid content of bovine milk fat globule and membrane phospholipids. *Journal of Agricultural and Food Chemistry* 59, 7427-7435.

Moorby J.M., Lee M.R.F., Davies D.R., Kim E.J., Nute G.R., Ellis N.M. and Scollan N.D. (2009) Assessment of dietary ratios of red clover and grass silages on milk production and milk quality in dairy cows. *Journal of Dairy Science* 92, 1148-1160.

Shingfield K.J., Ahvenjärvi S., Toivonen V., Ärölä A., Nurmela K.V.V., Huhtanen P. and Griinari J.M. (2003) Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. *Animal Science* 77, 165-179.

Shingfield K.J., Jaakkola S. and Huhtanen P. (2002) Effect of forage conservation method, concentrate level and propylene glycol on diet digestibility, rumen fermentation, blood metabolite concentrations and nutrient utilisation of dairy cows. *Animal Feed Science and Technology* 97, 1-21.

Wiking L., Stagsted J., Björck L. and Nielsen J.H. (2004) Milk fat globule size is affected by fat production in dairy cows. *International Dairy Journal* 14, 909-913.

Wiking L., Theil P.K., Nielsen J.H. and Sørensen M.T. (2010) Effect of grazing fresh legumes or feeding silage on fatty acids and enzymes involved in the synthesis of milk fat in dairy cows. *Journal of Dairy Research* 77, 337-342.

Enteric methane emissions from sheep fed diets including biochar

Lind L.[1], Sizmaz Ö.[2], Weldon S.[1], Dragan Miladinovic D.[3] and Jørgensen G.M.[1]
[1]*Norwegian Institute of Bioeconomy Research (NIBIO), PB 118, 1432 Aas, Norway;* [2]*University of Ankara, Dep. Animal Nutrition and Nutritional Diseases, 06110 Diskapi, Ankara, Turkey;* [3]*Center for Feed Technology-FôrTek, Norwegian University of Life Sciences, Arboretveien 10, 1430 Aas, Norway*

Abstract

Several scientific groups have concluded that the use of biochar as an on-farm management tool for carbon sequestration should be further investigated. Review articles also pinpoint the use of biochar to reduce greenhouse gas emissions from the entire agricultural production, and this should be studied using whole-chain models. Biochar is added to animal diets with the main purpose of enhancing animal health. There are indications that biochar fed to ruminants may reduce enteric methane emission. Twenty-four ewe lambs were fed one of two diets, a control diet (no biochar) and a biochar diet (1.4% biochar). There were no differences in dry matter intake and average daily growth rate between animals. An expected reduction in enteric methane emissions from animals fed the biochar diet was not detected. We conclude that the effect on enteric methane emissions may depend on structure and properties of the biochar offered. We suggest further research on biomass and pyrolysis of biochar to accommodate several properties as a feed additive for farm animals.

Keywords: lamb, growth rate, feed intake, CH_4

Introduction

Biochar has been used for centuries as a food additive for humans and animals. Biochar properties vary with feedstock, pyrolysis temperature and method making it difficult to generalize biochar effects on animal metabolism. A literature analysis (Gerlach and Schmidt, 2012) showed mostly positive effects on toxin adsorption, digestion, blood values, feed efficiency, meat quality, greenhouse gas (GHG) emissions and soil organic matter when biochar was added to animal feed. Rare negative effects were identified regarding the immobilization of liposoluble feed ingredients (e.g. vitamin E or carotenoids) which may limit long-term biochar feeding. The studies did not systematically investigate biochar properties, initial biomass and dosage. Research in Norway using biochar *in vivo* for ruminant feed is new and undeveloped. A pre-trial including biochar at levels between 0 and 1% of the total diet for sheep showed no changes in total feed intake, and intake was not affected by palatability with biochar inclusions (Nilsen and Lidtvedt, 2019). Leng *et al.* (2013) showed in an *in vivo* experiment that methane (CH_4) production in cattle could be reduced by 20% when 0.6% of biochar was added to the diet. A Norwegian pre-trial with castrated rams showed differences in enteric CH_4 production when 1% biochar was included in the experimental diet. The pre-trial was followed up by a full-scale trial using the same diets with the hypothesis that biochar can reduce enteric CH_4 emissions from sheep.

Materials and methods

The two diets tested were based on first-cut grass silage (*Bromus inermis, Poa pratensis, Festuca pratensis*). Two batches of concentrate were composed by FôrTek, Norway based on rapeseed meal, barley and wheat (Table 1). For the experimental concentrate, 4.6% biochar on dry matter (DM) basis was added in the mixture and two diets were created: Control (CON) and Biochar (CHAR). Twenty-four Norwegian White sheep lambs of age five months and average body weight ± standard deviation of 44.4±6.67 kg were randomly distributed into one of two groups of 12 animals and offered one of the two diets (CON or CHAR). The lambs were stalled individually in an uninsulated barn at NIBIO Tjøtta (65°49'22 N,

Table 1. Composition of ingredients (g kg^{-1}) in concentrate feeds.

Ingredient	Control diet	Biochar diet
Corn flour	48.5	46.4
Broad bean	90.0	86.1
Rapeseed cake	68.1	66.1
Barley Roller mill (0.75 mm)	503	491
Wheat	100	82.7
Wheat bran	66.2	63.3
Rapeseed oil	20.0	19.1
Molasses	50.0	47.8
Vitamins and minerals	54.2	51.5
Biochar		46.0

12°25'37 E) with free access to water. Animals were fed their daily allowances in two equal portions at 08:30 and 14:30 h. Animals were fed grass silage *ad libitum* allowing a minimum of 10% leftovers and 400 g concentrate per animal and day. After a 20-d adaptation period to the corresponding diet, individually lambs were put in respiration chambers for measuring enteric (CH_4) emission. Methane concentrations in ambient air and in exhaust gas leaving each chamber (in total seven inlets) were measured on a rotational basis, taking one gas reading after 3 min per inlet, measuring ambient air, and each chamber every 21 min. The animals were kept in the chambers for 72 h during which they were fed and managed in a similar way to the barn. Enteric CH_4 was sampled via plastic tubes into a seven port Servopro Multiexact 4100 Analyzer (Servomex Group Inc, Woburn, MA, USA). To determine lambs' daily growth rate and enteric CH_4 production between diets, data were analysed using one-way ANOVA procedure considering diets as fixed effects and lambs as random effects. Differences were considered significant at P<0.05.

Results and discussion

The chemical composition of the concentrate diets is shown in Table 2. Average DM content of grass silage during the experimental period was 366 g kg^{-1} DM. Biochar consists mainly of carbon, which explains the differences in the NDF and ash content between the two concentrate diets. The protein content did not differ between the diets. Animals' average daily DM intake was the same (P=0.288) between the two groups of sheep (1.18 and 1.26 kg DM for the CON and CHAR, respectively). Nilsen and Lidtvedt (2019) examined the feed intake of 20 sheep fed grass silage and concentrate diets with increasing inclusion of biochar (0, 1, 2.3 and 4.6% of DM). They too found that inclusion of biochar in the diet had no effect on total DM intake. In Nilsen and Lidtvedt (2019), the biochar content in the total diets ranged from 0 to 1.0%, whereas in the present trial the content of biochar in the total diet was 1.4% of DM. This is higher than recommended than in previously reported inclusion in *in vivo* experiments (e.g. Leng *et al.*, 2013). The higher inclusion of biochar in our trial did not affect average weight gain of lambs (P=0.983; 338 and 344 g day^{-1} for CON and CHAR, respectively).

Table 2. Chemical composition (g kg^{-1}) in concentrate feeds.

	Control diet	Biochar diet
Dry matter	134	128
Crude protein	146	148
Neutral detergent fibre	130	144
Ash	53.5	61.7

We found no significant reduction (*P*=0.304) in enteric CH_4 production from the lambs fed the CHAR diet compared with those fed the CON diet (23.6 and 22.8 l CH_4 kg^{-1} DM intake). A pre-trial using only 6 castrated rams fed the same diets, however, showed a 30% reduction of CH_4 from the animals fed the biochar diet. However, the level of CH_4 production from the animals fed the control diet in that pre-trial was high (33.7 l CH_4 kg^{-1} DM intake) compared with studies performed with goats (Romera-Huelva and Molina-Alcaide, 2013; Romera-Huelva *et al.*, 2017) and sheep (Martinez-Fernandez *et al.*, 2014). The average emissions from those experiments are more in accordance with the emissions found in the present experiment and with the emissions from the biochar-fed castrated rams in the pre-trial, closer to 22 l CH_4 kg^{-1} DM intake. Structure and properties of biochar depend on feedstock and pyrolysis method and temperature. Therefore, results of reducing enteric CH_4 from ruminants fed biochar might differ.

Conclusions

From the present experiment we conclude that inclusion of biochar has no negative effect on DM intake or growth rate of lambs. However, an expected reduction in enteric CH_4 emission from sheep fed biochar was not detected. Structure and properties of biochar depend on biomass and pyrolysis and thus more research is needed to determine the effect of biochar as a compound for reducing enteric CH_4 emissions from ruminants.

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References

Gerlach A. and Schmidt H-P. (2012) The use of biochar in cattle farming. *Journal of terroir – Wine and biodiversity.* ISSN 1663-0521 http://www.ithaka-journal.net/pflanzenkohle-in-der-rinderhaltung?lang=en.

Leng R.A., Preston T.R. and Inthapanya S. (2013) Biochar reduces enteric methane and improves growth and feed conversion in local 'Yellow' cattle fed cassava root chips and fresh cassava foliage. *Livestock Research for Rural Development* 24(11).

Martínez-Fernández G., Abecia L., Arco L., Cantalapiedra-Hijar G., Martín-García A.I., Molina-Alcaide E., Kindermann M., Duval S. and Yáñez-Ruiz D.R. (2014) Effects of ethyl-3-nitrooxy propionate and 3-nitrooxypropanol on ruminal fermentation, microbial abundance, and methane emissions in sheep. *Journal of Dairy Science.* 97, 3790-3799.

Nilsen T.B. and Lidtvedt G. (2019) Biokullets påvirkning på sauen sitt grovfôropptak [*in Norwegian*]. Bacheloroppgave Nord Universitet. 29 pp.

Romero-Huelva M. and Molina-Alcaide E. (2013) Nutrient utilization, ruminal fermentation, microbial nitrogen flow, microbial abundances, and methane emissions in goats fed diets including tomato and cucumber waste fruits. *Journal of Animal Science* 91, 914-923.

Romero-Huelva M., Ramírez-Fenosa M.A., Planelles-González R., García-Casado P. and Molina-Alcaide E. (2017) Can by-products replace conventional ingredients in concentrate of dairy goat diet? *Journal of Dairy Science* 100, 4500-4512.

Red macroalgae *Porphyra* as a protein source in lamb diets

Lind V.[1], Weisbjerg M.R.[2], Jørgensen G.M.[1] and Molina-Alcaide E.[3]

[1]*Norwegian Institute of Bioeconomy Research (NIBIO), PB 118, 1432 Ås, Norway;* [2]*Aarhus University, AU Foulum, Blichers Allé 20, 8830 Tjele, Denmark;* [3]*Estación Experimental del Zaidin (Consejo Superior de Investigaciones Cientificas), Profesor Albareda, 1, 18008 Granada, Spain*

Abstract

Edible seaweed biomass is a valuable alternative feed ingredient for livestock. Seaweeds contain a variable amount of proteins, minerals, lipids and carbohydrates. Species, season, harvesting year, habitat and environmental conditions account for this variation. Using seaweeds as an alternative protein source of animal feed is of great interest. To investigate the red seaweed *Porphyra* sp. as a protein source for sheep, an *in vivo* study was carried out with 24 Norwegian White lambs. The lambs were allocated into four groups receiving a control diet or one of three supplemented diets. All groups were fed grass silage *ad libitum*, crushed oats and mineral pellets. Growth rate when lambs were given dried and powdered *Porphyra* sp. was compared with white clover silage or pelleted soybean meal. Weight changes were analysed using the GLM procedure with diet as fixed effect. Crude protein intake per lamb was between 202 g day^{-1} (control diet) and 322 g day^{-1} (clover diet). Lambs fed the diets including soybean meal or *Porphyra* had a significantly (P<0.001) higher growth rate compared with control and clover diets. The results show that *Porphyra* sp. can replace soybean meal as a protein source.

Keywords: growth rate, sheep, feed intake

Introduction

In Europe, prohibition of the use of animal and fish protein subsequent to the Bovine spongiform encephalopathy (BSE) crisis in the 1990s has led to a gap in the supply of protein for ruminants. Additional protein was needed and imported soybean meal seemed to be the most suitable. In Norway, more than 80% of the protein and 40% of all the ingredients in commercial concentrates for animal feeding are imported. Thus, future development of feeds should find and include locally produced protein sources. Traditionally, seaweed has been used in Norway in the coastal communities as a supplement in the animal diet especially when there was shortage of forage (Bay-Larsen *et al.*, 2018). Due to geography, environment and the characteristics of aquaculture production, conditions along the Norwegian coast are well suited for production of seaweed. The protein concentration is high in *Rhodophyta* (red) and *Chlorophyta* (green) seaweed species (100-470 g kg^{-1} of dry matter (DM)) compared with that of *Phaeophyta* (brown) seaweed species (30-150 g kg^{-1} of DM) (Gaillard *et al.*, 2018; Molina-Alcaide *et al.*, 2017; Tayyab *et al.*, 2016). For the red specie *Porphyra* sp. the protein concentration may be as high as 470 g kg^{-1} of DM in the spring and it could be relevant as a substitute for soybean meal (Tayyab *et al.*, 2016). In general, amino acid composition of *Porphyra* sp. is similar to proteins from soybean and eggs (Gaillard *et al.*, 2018).

In vitro and *in situ* techniques are used in animal nutrition research as they are less expensive, less time-consuming and less laborious compared to *in vivo*. There are some *in vitro* – *in situ* studies that investigated the nutritive value of seaweeds (Gaillard *et al.*, 2018; Molina-Alcaide *et al.*, 2017; Tayyab *et al.*, 2016) but to our knowledge, very few *in vivo* experiments have been performed using seaweed as a protein source for ruminants (Makkar *et al.*, 2016). Literature is especially scarce concerning sheep. Therefore, the most promising seaweed species, *Porphyra* sp. found in the experiments by Gaillard *et al.* (2018), Molina-Alcaide *et al.* (2017) and Tayyab *et al.* (2016) was used in the present work. The effect of including *Porphyra* sp. as a source of protein in diets for sheep was compared with the protein sources clover silage and soybean meal on animal intake and performance.

Materials and methods

A control diet (CON) based on grass silage, crushed oats (100 g d^{-1}) and a vitamin-mineral mixture (VMM; 25 g d^{-1}) was compared with 3 experimental diets with an additional protein source of either clover silage (CLO), soybean meal (200 g d^{-1}; SOY) or *Porphyra* sp. (200 g d^{-1}; POR). Oats, VMM and soybean meal or *Porphyra* sp. was offered in separate buckets from the silages. Twenty-four Norwegian White sheep lambs at the age of five months and average body weight ± standard deviation of 32.9±3.69 kg were used in the experiment. The animals were blocked according to initial live weight. The lambs were stalled individually in an uninsulated barn at NIBIO Tjøtta (65°49'22 N 12°25'37 E) with free access to water.

Animals were fed their daily allowances in two equal portions at 08:30 and 14:30 h. Grass silage and white clover silage was supplied *ad libitum* allowing a minimum of 10% leftovers. Leftovers of white clover silage were measured daily, and daily intake calculated. Powdered *Porphyra* sp. was mixed with 5 dl of cold water, the crushed oats and the mineral and vitamin mixture and offered as a 'porridge'. Samples of grass and clover silages were sampled weekly during the trial for analysis. Samples of the other ingredients (oats, soybean pellets and *Porphyra* sp.) were collected every second week and pooled for analysis (Table 1). The lambs were adapted to their diet during a 20-d period followed by a recording period of six weeks. Weight gain was calculated based on start weight and weight after the six weeks recording period. Feed intake was measured four days every week during the six weeks of recording. The trial was conducted in accordance with the regulation for use of animals in experiments, adopted by the Norwegian Ministry of Agriculture and Food, and approved by the Ethics Commission on Animal Use by the Norwegian Food and Safety Authority, application number FOTS ID 8836.

Results and discussion

Lambs fed the CLO diet had higher (P<0.001) DM, crude protein, neutral detergent fibre, digestible organic matter and energy (FU) intake (Table 2) compared with lambs fed the CON diet. The lambs fed the SOY and POR diets had intermediate intakes, and were different from the animals receiving CON and CLO diets except for FU which was also higher for the POR diet. However, the growth rate was higher (P<0.001) for SOY and POR diets compared with CON and CLO diets. The protein content differed between the protein sources with soybean meal having higher content than *Porphyra* sp. and clover silage. Diets with protein enrichment (CLO, SOY and POR) were planned to be isoenergetic and isoproteinic but chemical analysis showed that SOY diet had higher crude protein content compared to CLO and POR diets (data not shown). Tayyab *et al.* (2016) demonstrated using *in situ* methods that *Porphyra* sp. can supply the rumen with high amounts of degradable protein and supply a high amount of rumen escapable protein, digestible in the small intestine. The crude protein concentration in *Porphyra*

Table 1. Average chemical composition (g kg^{-1} DM) of diet ingredients.[1]

Item	Grass silage	White clover silage	Crushed oats	Soybean meal pellets	*Porphyra* sp.
DM g kg^{-1} fresh	383	352	943	932	947
Ash	60.9	86.8	28.0	60.0	21.6
Crude protein	137	137	325	545	371
NDF	521	433	115	120	58
DOM	707	721	972	929	898
FU kg^{-1} DM	0.83	0.83	1.09	1.10	0.80

[1] DM = dry matter; NDF = neutral detergent fibre; DOM = *in vitro* digestible organic matter; FU = Scandinavian feed unit: 1 FU = 7.89 MJ net energy.

Table 2. Average dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), digestible organic matter (DOM) (g d^{-1}) and feed unit (FU) intake, growth rate (g d^{-1}) of lambs.[1,2]

Diet	DM	CP	NDF	DOM	Feed units[3]	Growth rate
CON	1,395^{a}	202^{a}	672^{a}	1,046^{a}	1.17^{a}	163.3^{a}
CLO	1,967^{c}	322^{c}	787^{c}	1,222^{b}	1.38^{bc}	160.5^{a}
SOY	1,625^{b}	309^{b}	719^{b}	1,251^{b}	1.42^{c}	253.8^{b}
POR	1,600^{b}	305^{b}	694^{ab}	1,227^{b}	1.34^{b}	249.0^{b}
SEM[2]	11.6	2.32	4.10	127.8	0.14	12.3
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1] SEM = standard error of the mean; FU = Scandinavian Feed Unit – 1 FU = 7.89 MJ net energy; CON = control diet; CLO = diet including clover silage; SOY = diet including soybean meal; POR = diet including *Porphyra* sp.; SEM = standard error of the mean.

[2] Means that do not share a letter within a row are significantly different.

sp. was comparable to that of oilseed by-products such as sunflower meal and rapeseed meal. The growth rate for SOY and POR animals were 254 and 249 g day^{-1} respectively and 163 and 160 g day^{-1} for the CON and CLO lambs, respectively (Table 2; $P<0.001$). The result confirms the findings of Tayyab *et al.* (2016) that the protein value of *Porphyra* sp. is comparable to high valuable protein sources such as soybean meal.

Conclusions

This study confirms that *Porphyra* sp. seems to be as good a protein source for ruminants as soybean meal. The use of the seaweed species, however, faces some challenges due to commercial production and costs before it can substitute for soybean meal and be implemented in the daily diet to ruminants.

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References

Bay-Larsen I., Risvoll C., Vestrum I. and Bjørkhaug H. (2018) Local protein sources in animal feed – perceptions among arctic sheep farmers. *Journal of Rural Studies* 59, 98-110.

Gaillard C., Bhatti H.S., Novoa-Garrido M., Lind V., Roleda M.Y. and Weisbjerg M.R. (2018) Amino acid profiles of nine seaweed species and their *in situ* degradability in dairy cows. *Animal Feed Science and Technology* 241, 210-222.

Makkar H.P.S., Tran G., Heuzé V., Giger-Reverdin S., Lessire M., Lebas F. and Ankers P. (2016) Seaweeds for livestock diets: a review. *Animal Feed Science and Technology* 212, 1-17.

Molina-Alcaide E., Carro M.D., Roleda M.Y., Weisbjerg M.R., Lind V. and Novoa-Garrido M. (2017) *In vitro* ruminal fermentation and methane production of different seaweed species. *Animal Feed Science and Technology* 228, 1-12.

Tayyab U., Novoa-Garrido M., Roleda M.Y., Lind V. and Weisbjerg M.R. (2016) Ruminal and intestinal protein degradability of various seaweed species measured *in situ* in dairy cows. *Animal Feed Science and Technology* 213, 44-54.

The potential of multispecies swards for eco-efficient dairy production in Northern Germany

Loges R., Loza C., Voss P., Kluß C., Malisch C. and Taube F.
Group of Grass and Forage Science/Organic Agriculture, Kiel University, Kiel, Germany

Abstract

Increased plant diversity in cultivated grassland possesses the potential to combine both environmental and agronomic benefits. The use of legumes reduces the need for N fertiliser whilst including herbs can have a positive impact on nutritive value of forages and benefit animal health. Mixed swards can increase herbage production if plants with diverse functional traits are combined. To prove whether this also holds true for intensive forage production with at least five defoliations per year, we conducted a two-year experiment, hypothesizing that the introduction of deep-rooting legumes and herbs into a binary mixture of perennial ryegrass and white clover will outperform the latter. Yield, forage quality and sward botanical composition were examined in an orthogonal experimental approach also considering defoliation (intensive rotational grazing versus mechanical harvesting). While integration of only red clover led to yield increases in all systems, a multispecies mixture also containing herbs, birdsfoot trefoil and red clover had only small additional effects on yield performance. When grazed in early growth stages the dairy cows preferred the complex swards, resulting in higher forage use efficiency. Under a high frequency of defoliation per year all seed mixtures tested were suited to provide high quality forage for lactating dairy cows.

Keywords: grazing, grass clover, zero-grazing, multi-species ley, defoliation frequency

Introduction

Several publications indicate that the inclusion of legumes and forbs into grass swards can reduce the environmental impact of milk production (Carlton *et al.*, 2019; Simon *et al.,* 2019) and simultaneously affect herbage yield, feeding quality (Roca-Fernández *et al.,* 2016), or agronomic traits (Hoekstra *et al.,* 2015) positively. The inclusion of chicory into grass-clover swards was found to increase dry matter (DM) intake and organic matter digestibility, leading to higher milk yields of the grazing dairy cows (Roca-Fernández *et al.,* 2016). However, if managed very intensively, only few species end up contributing to the harvested yields due to increased asynchrony in plant development with each new species. To validate the potential of diverse pastures under intensive grazing, zero grazing or cutting management systems, we conducted a two-year experiment with a minimum of 5 defoliations per year. We hypothesized that the introduction of deep-rooting legumes like red clover and birdsfoot trefoil, as well as of herbs, into a binary mixture of perennial ryegrass plus white clover will outperform the latter independent of the harvest regime.

Materials and methods

The field experiment was conducted at Kiel University's experimental farm for organic agriculture 'Lindhof' in Northern Germany (N 54°27; E 9°57; mean air temperature 8.7 °C; mean annual precipitation 785 mm) during two years (2017 and 2018). The experimental layout was an orthogonal split plot design with two experimental factors in four replicates: (1) seed mixture and (2) defoliation system. The seed mixtures used were (1) a binary mixture of 24 kg ha^{-1} perennial ryegrass (*Lolium perenne*, PR) + 4 kg ha^{-1} white clover (*Trifolium repens*, WC) (PR+WC), (2) a tertiary mixture of 24 kg ha^{-1} PR+ 2 kg ha^{-1} WC + 6 kg ha^{-1} red clover (*Trifolium pratense*, RC) (PR+WC+RC) and (3) a multi-species mixture of 16 kg ha^{-1} PR+ 1.5 kg ha^{-1} WC + 3 kg ha^{-1} RC that additionally contained herbs (1 kg ha^{-1} ribwort plantain (*Plantago lanceolata*) + 2 kg ha^{-1} of each chicory (*Cichorium intybus*), sheep's burnet (*Sanguisorba minor*), caraway (*Carum carvi*) and 5 kg ha^{-1} birdsfoot trefoil (*Lotus corniculatus*) (PR+WC+RC+Herbs). The 3 different defoliation systems were rotational grazing (8 times per year), zero grazing (harvested 8 times per year) and a silage cut system with 5 cuts per year. Grazing was performed with a pasture-based Jersey dairy herd. Mechanical harvest was carried out by a plot harvester. In each experimental year grass-clover swards in their first production year were used. Before machine harvesting at 5 cm and grazing at 4 cm residual height, forage yield had been determined by hand clipping 2 squares of 0.5 m^2 in each of the 4 plot replicates. Post-grazing biomass was sampled in the morning after day and night grazing. Forage quality was determined using NIRS spectroscopy. A linear mixed model with seed mixture and defoliation system as fixed and experimental year as random factor was used for statistical analysis with the statistical software R.

Results and discussion

Table 1 shows clear effects of both seed mixture and defoliation system on potential harvestable DM yield, forage quality and botanical composition of grass-clover leys at Lindhof. In all defoliation systems the integration of red clover led to yield increases compared to the binary mixture PR+WC. In none of the defoliation systems could yield differences be detected between the binary mixture and the multispecies mixture. Independent of seed mixture, grazing led to higher yields of potential harvestable biomass compared to the mechanical defoliated systems. This was accompanied by higher sward grass contents. No effects of seed mixture could be detected on the content of metabolizable energy (ME), while there were strong effects of the defoliation system on this parameter.

Table 1. Effect of seed mixture and defoliation system on potential harvestable DM yield, forage quality and sward grass-, legume- and herb-content of grass clover leys at Lindhof as average over 2 experimental years (2017 + 2018).[1,2]

Parameter (unit)	Defoliation system	Seed mixture					
		PR+WC		PR+WC+RC		PR+WC+RC+Herbs	
Potential harvestable DM (Mg DM ha^{-1})	Grazing	11.42	bA	13.26	aA	11.93	aA
	Zero-grazing	9.48	bB	10.87	aB	10.54	abB
	5-cut	9.41	bB	10.41	aB	9.19	bB
Forage ME content (MJ kg^{-1} DM)	Grazing	11.1	A	11.0	A	11.0	A
	Zero-grazing	11.0	A	10.9	A	10.9	A
	5-cut	10.6	B	10.7	B	10.7	B
Forage CP content (% of DM)	Grazing	22.5	aA	21.9	abA	21.3	bA
	Zero-grazing	21.9	aA	21.3	abB	20.0	bA
	5-cut	19.4	aB	19.4	aB	18.1	bB
Sward grass content (% of DM)	Grazing	40.0	A	38.8	A	33.4	A
	Zero-grazing	30.5	B	25.5	B	23.5	B
	5-cut	28.8	B	17.8	C	19.0	C
Sward legume content (% of DM)	Grazing	59.9	B	61.1	B	52.9	B
	Zero-grazing	68.3	A	73.8	AB	65.2	A
	5-cut	70.6	aA	81.8	aA	67.6	bA
Sward herb-content (% of DM))	Grazing	0.2	b	0.2	b	13.7	a
	Zero-grazing	1.2	b	0.7	b	11.4	a
	5-cut	0.6	b	0.4	b	13.4	a

[1] Different lower-case letters indicate significant differences between seed mixtures in the same defoliation system, different upper-case letters indicate significant differences between defoliation systems in the same seed mixture at P<0.05.

[2] DM = dry matter; ME = metabolizable energy; CP = crude protein; PR = perennial ryegrass; WC = white clover; RC = red clover.

An 8-time defoliation (grazing and zero grazing) led to higher ME concentrations compared to forage harvested in the 5-cut system. In all defoliation systems the multispecies mixture showed lower concentrations of crude protein (CP) compared to the binary mixture PR+WC. Despite lower legume contents, CP concentrations of the grazed swards were higher compared to material harvested in the 5-cut system. Table 2 shows potential effects of the factor seed mixture on amount and forage quality of different sward fractions of the grazed grass-clover leys. Despite statistical differences in pre-grazing biomass, all mixtures resulted in the same DM intake. Having free choice, cows seem to prefer the multispecies mixture to the tertiary mixture PR+WC+RC and left less post-grazing biomass. The results clearly indicate selective grazing in all mixtures. CP and ME contents in the rejected post-grazing biomass were lower than in the offered pre-grazing biomass. Thus, forage quality of the biomass chosen by the cows was higher than the quality of the offered pre-grazing biomass.

Table 2. Effect of seed mixture on pre-grazing, rejected post-grazing and eaten biomass, as well as on contents of metabolizable energy (ME) and crude protein (CP) of these biomass fractions as average over 2 experimental years at Lindhof (2017 + 2018).[1,2]

Parameter (unit)	Sward fraction	Seed mixture					
		PR+WC		PR+WC+RC		PR+WC+RC+Herbs	
Biomass (Mg DM ha^{-1})	Pre grazing	11.42	b	13.26	a	11.93	b
	Post grazing	3.01	ab	4.52	a	2.90	b
	Intake via grazing	8.42		8.74		9.04	
ME content (MJ kg^{-1} DM)	Pre grazing	11.1	A	11.0	B	11.0	B
	Post grazing	10.6	B	10.5	C	10.4	C
	Intake via grazing	11.2	A	11.3	A	11.2	A
CP content (% of DM)	Pre grazing	22.5	aB	21.9	abB	21.3	bA
	Post grazing	18.8	aC	18.1	a C	16.3	bB
	Intake via grazing	23.8	aA	24.4	a A	22.5	bA

[1] Different lower-case letters indicate significant differences between seed mixtures in the same biomass fraction, different upper-case letters indicate significant differences in forage quality between the biomass fractions of the same seed mixture at $P<0.05$.

[2] DM = dry matter; ME = metabolizable energy; CP = crude protein; PR = perennial ryegrass; WC = white clover; RC = red clover.

Conclusions

The addition of deep-rooted red clover to shallow-rooted grass-white clover leys can increase herbage production because of combination of diverse functional traits. Further addition of species had no positive yield effects. When grazed in early growth stages the dairy cows preferred the complex multispecies swards resulting in higher forage use efficiency. Under a high frequency of defoliation all seed mixtures tested are suited to provide high quality forage for lactating dairy cows. Under the conditions of Northern Germany, multispecies leys are a good measure to increase biodiversity without decreases in yield and forage quality.

References

Carlton A.J., Camero K.C., Di H.J., Edwards G.R. and Clough T.J. (2019) Nitrate leaching losses are lower from ryegrass/white clover forages containing plantain than from ryegrass/white clover forages under different irrigation. *New Zealand Journal of Agricultural Research* 62 (2), 150-172.

Hoekstra N.J., Suter M., Finn J.A., Husse S. and Lüscher A. (2015) Do belowground vertical niche differences between deep- and shallow-rooted species enhance resource uptake and drought resistance in grassland mixtures? *Plant and Soil* 394, 21-34.

Roca-Fernández AI., Peyraud J.L, Delaby L. and Delagarde R. (2016) Pasture intake and milk production of dairy cows rotationally grazing on multi-species swards. *International Journal of Animal Bioscience* 10 (9), 1448-1456.

Simon P.L., De Klein C.A., Worth W., Rutherford A.J. and Dieckow J. (2019) The efficacy of *Plantago lanceolata* for mitigating nitrous oxide emissions from cattle urine patches. *Science of the Total Environment* 691, 430-441.

The effect of dairy cow feeding system on rumen function and milk production

McAuliffe S.[1,2], Gilliland T.J.[2], Lewis E.[3] and Hennessy D.[1]

[1] *Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland;* [2] *Institute of Global Food Security, Queen's University Belfast, Northern Ireland; United Kingdom;* [3] *Devenish, Lagan House, 19 Clarendon Road, Belfast BT1 3BG, Northern Ireland, United Kingdom*

Abstract

Dairy cow feeding system is one of the main determinants of milk production. Good rumen function is essential to optimise animal performance and is influenced by what the cow consumes. The rumen function and milk production of cows offered one of three dietary treatments over a full lactation was examined. The three treatments were: (1) cows grazing grass-only swards receiving 250 kg N ha^{-1} $year^{-1}$ (Grass); (2) cows grazing grass-white clover swards receiving 250 kg N ha^{-1} $year^{-1}$ (Grass-Clover); and (3) cows housed indoors and offered a total mixed ration diet (TMR). Grass cows had the lowest MS production in all seasons. While treatment had no effect on rumen pH, it significantly altered the rumen volatile fatty acid (VFA), ammonia and lactic acid profiles. The Grass-Clover treatment had significantly greater ($P<0.05$) total VFA concentration as well as increased ammonia concentration compared with the Grass and TMR treatments. The increased ammonia concentration from the Grass-Clover treatment corresponded with the significantly greater milk urea content for the cows fed on Grass-Clover, indicating a greater excess of dietary protein in that treatment.

Keywords: rumen, grass white clover, total mixed ration, ammonia, volatile fatty acid

Introduction

Feed system is one of the main determinants of milk production (Bargo *et al.*, 2002). The ability to alter the diet to suit the cows' requirements (age, stage of lactation, etc.) gives total mixed ration (TMR) diets an advantage over pasture-based systems in terms of attaining maximum output per cow (Kolver and Muller, 1998). In temperate regions with a long grass growing season, low cost grass based systems are the most advantageous milk production systems from an economic perspective (Dillon *et al.*, 2016). White clover (*Trifolium repens* L.; clover) inclusion in the sward has been shown to improve pasture quality and animal performance compared to a grass-only sward (Egan *et al.*, 2018). The effect on rumen function and possible subsequent environmental effects of clover inclusion in the diet is poorly understood, particularly in comparison with TMR feeding systems. Differences in rumen characteristics, such as the quantity and proportions of volatile fatty acids (VFA), have been reported between animals fed clover (e.g. Dewhurst *et al.*, 2003) or mixed swards (Ribeiro Filho *et al.*, 2003) compared with perennial ryegrass only. While previous studies have compared grass-only with grass-clover diets (Egan *et al.*, 2018) or grass-only with TMR diets (Kolver and Muller, 1998), little work has been carried out simultaneously comparing both sward types with a TMR system. The objective of this study was to determine the effects, if any, of three different dietary treatments (Grass, Grass-Clover, TMR) on dairy cow milk production and rumen function.

Materials and methods

The experiment was undertaken at Teagasc, AGRIC, Moorepark, Fermoy, Co. Cork, Ireland (520 9' N; 80 16' W) in 2016. The experiment had three treatments: (1) cows grazing grass-only swards receiving 250 kg N ha^{-1} $year^{-1}$ (Grass); (2) cows grazing grass-white clover swards receiving 250 kg N ha^{-1} $year^{-1}$ (Grass-Clover); and (3) cows offered a total mixed ration diet and housed indoors (TMR). Swards in the Grass and Grass-Clover treatments were rotationally grazed and daily herbage allowance was 18 kg DM cow^{-1} per day. No concentrate was fed. The TMR consisted of 7.15 kg DM grass silage, 7.15 kg DM maize silage and 8.3 kg DM concentrates. There were three seasons (spring, summer and autumn). Within each season a 3 (treatments) × 3 (periods) Latin square was carried out. Each period in the Latin square lasted two weeks, allowing 10 days for acclimatisation, with measurements taken in the final four days. Nine primiparous rumen-cannulated spring calving dairy cows (mean calving date 07 February 2016 ± 6.7 days) were used, three per treatment per period. The cannulated cows were blocked according to calving date, body weight, body condition score and milk production for the 3-week period prior to each measurement season and added to the three herds for each season and removed from those herds between seasons. Between seasons, cows grazed predominantly grass swards. There were 17 'intact' cows in each of the three treatment herds. Rumen samples were taken from each of the cows after morning (AM) and evening (PM) milking on days 11 and 12 of each period. Representative samples of both solid and liquid rumen contents were taken and strained through three layers of synthetic cheese cloth to separate solids from liquids. The liquid fraction was used for VFA and ammonia analyses. Milk yield was recorded daily and milk solids (MS; fat + protein) and milk urea weekly. Rumen VFA, ammonia, lactic acid and pH data are from the rumen-cannulated cows. Milk production results are from the herd fed each diet during the three seasons: spring (April/May), summer (June/July) and autumn (August/September). Data were analysed using SAS. Data were checked for normality using PROC UNIVARIATE. Data were analysed using linear mixed models that allowed for repeated measurements using the PROC MIXED procedure in SAS (2003).

Results and discussion

The TMR treatment had the greatest MS yield in summer and autumn, being significantly greater than Grass (Table 1). Grass-Clover and TMR had similar MS production in summer but TMR had significantly greater MS yield in autumn. The milk urea (MU) concentrations were similar on all treatments in spring (Table 1). They were significantly (P<0.05) greater on Grass-Clover in summer and autumn than Grass and TMR, while in autumn Grass had significantly greater MU concentration than TMR, indicating that there is surplus protein in the diet and inefficient N use (Roseler *et al.*, 1993). There was a significant (P<0.05) treatment × season interaction on rumen ammonia concentrations (Table 1). All treatments had similar rumen ammonia in the spring AM samples. Grass-Clover had significantly greater rumen ammonia than Grass and TMR in summer AM samples, and also than TMR in autumn AM samples. For the PM samples (Table 1), Grass-Clover had significantly greater rumen ammonia than TMR across all three seasons and greater than Grass in summer PM samples. Grass had greater rumen ammonia than TMR in the autumn PM. There was a significant (P<0.05) treatment × season interaction effect on total VFA concentration (Table 1). For the AM samples, in autumn Grass-Clover had a significantly greater

Table 1. Effect of treatment on milk production, milk urea concentration, and AM and PM total rumen VFA and ammonia concentrations in Spring, Summer and Autumn (n=9).[1]

	Spring			Summer			Autumn			SE	Treat	Season	Treat × Season
	G	GC	TMR	G	GC	TMR	G	GC	TMR				
Daily milk yield (kg cow^{-1})	26.7^{abc}	28.6^{a}	26.8^{ab}	20.9^{def}	23.9^{bcde}	24.4^{cd}	15.9^{g}	18.5^{fg}	21.6^{ef}	0.99	<0.05	<0.001	<0.001
Daily MS yield (kg cow^{-1})	1.95^{ab}	2.14^{a}	1.85^{bc}	1.61^{cd}	1.81^{bc}	1.90^{ab}	1.28^{e}	1.50^{de}	1.76^{bc}	0.072	<0.05	<0.001	<0.001
Milk urea (mg dl^{-1})	20.5^{a}	25.5^{ac}	22.7^{ad}	27.6^{dc}	34.3^{bg}	26.1^{dc}	41.1^{e}	46.7^{f}	29.5^{cg}	1.16	<0.001	<0.001	<0.001
Rumen measurements													
AM Total VFA (mmol l^{-1})	123^{abc}	122^{ab}	121^{ab}	126^{abc}	137^{c}	122^{abc}	124^{abc}	135^{ac}	111^{b}	4.0	<0.001	<0.05	<0.05
Ammonia (mmol l^{-1})	3.9^{a}	4.1^{a}	8.4^{abc}	9.1^{bc}	15.8^{d}	5.5^{ab}	11.6^{cd}	16.1^{d}	8.1^{abc}	1.01	<0.001	<0.001	<0.001
PM Total VFA (mmol l^{-1})	145^{abcd}	146^{abc}	123de	128^{cde}	153^{ab}	132^{bcde}	152^{ab}	164^{a}	121^{e}	5.3	<0.001	<0.05	<0.05
Ammonia (mmol l^{-1})	4.5^{ab}	11.2^{bc}	3.2^{a}	11.9^{c}	21.9^{d}	9.5^{abc}	25.6^{de}	30.4^{e}	9.0^{abc}	1.63	<0.001	<0.001	<0.001

[1] G = grass only; GC = grass clover; TMR = total mixed ration, indoors; VFA = volatile fatty acids; MS = milk solids; AM <12:00 h; PM >12:00 h.

total VFA than TMR. For the PM samples, in spring Grass-Clover had a higher total VFA than TMR, in summer, Grass-Clover were higher than Grass and in autumn Grass-Clover and Grass had greater total VFA than TMR. On average, Grass-Clover had a significantly (P<0.05) greater total VFA than Grass and TMR (Table 1). The higher total VFA of Grass-Clover compared to Grass is similar to Dewhurst *et al.* (2003) and Ribeiro Filho *et al.* (2003). Grass resulted in a significantly greater total VFA than TMR.

Conclusions

The beneficial aspects of white clover inclusion in grazed grass swards were evident in this study through increased animal production compared with that of Grass; similar milk solids production to the TMR treatment; and the greater total rumen VFA concentration compared with the Grass and TMR treatments. However, the increased rumen ammonia and MU concentrations on the Grass-Clover treatment may be a cause of concern from an environmental perspective.

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References

Bargo F., Muller L.D., DelahoY.J.E. and Cassidy T.W. (2002) Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations. *Journal of Dairy Science* 85, 2948-2963.

Dewhurst R.J., Evans R., Scollan N., Moorby J., Merry R. and Wilkins R. (2003) Comparison of grass and legume silages for milk production. 2. *In vivo* and in sacco evaluations of rumen function. *Journal of Dairy Science* 86, 2612-2621.

Dillon P., Hanrahan L., Thorne F., Kelly P., Shalloo L. and Moran B. (2016) Irish dairying: Well positioned for the upturn. In: *Technologies for Success,* Teagasc National Dairy Conference, 2016. pp 8-27.

Egan M., Galvin N. and Hennessy D. (2018) Incorporating white clover (*Trifolium repens* L.) into perennial ryegrass (*Lolium perenne* L.) swards receiving varying levels of nitrogen fertilizer: effects on milk and herbage production. *Journal of Dairy Science* 101, 3412-3427.

Kolver E.S. and Muller L.D. (1998) Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. *Journal of Dairy Science* 81, 1403-1411.

Ribeiro Filho H., Delagarde R. and Peyraud J. (2003) Inclusion of white clover in strip-grazed perennial ryegrass swards: herbage intake and milk yield of dairy cows at different ages of sward regrowth. *Animal Science* 77, 499-510.

Roseler D.K., Ferguson J.D., Sniffen C.J. and Herrema, J. (1993) Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. *Journal of Dairy Science* 76, 525-534.

Validating the n-alkane technique for determining intake in grazing sheep

McGovern F.[1], Beecher M.[2], Creighton P.[1], Galvin N.[2], Hennessy D.[2], McHugh N.[2], O'Donovan M.[2] and Garry B.[2]
[1]Teagasc, Animal and Grassland Research and Innovation Centre, Athenry, Co. Galway, Ireland;
[2]Teagasc, Animal and Grassland Research Centre, Moorepark, Fermoy, Co. Cork, Ireland

Abstract

Optimizing nutrition for animals requires accurate estimates of feed intake and the nutritive value of the feed. Feed intake in grazing animal systems is routinely measured using the n-alkane technique, whereby long chain alkanes are used as internal markers for predicting dry matter intake (DMI). The aim of this study was to validate the suitability of the n-alkane technique for determining grass DMI in Irish sheep systems. Detailed total DMI measurements were obtained from over 200 Texel wether animals from 2012 to 2014. *In vivo* DMI was determined using the total collection method with animals offered perennial ryegrass *ad libitum*. All animals were dosed once daily with an n-alkane bolus for 12 days with faeces samples collected for the final six days of each intake run. The r-squared value between *in vivo* DMI and n-alkane estimated DMI was 0.75. This is dependent on multiple factors, most importantly grass quality. This data highlights the suitability of the n-alkanes technique for measuring grass DMI in sheep.

Keywords: grass, sheep, DMI, n-alkane

Introduction

Livestock production systems are being put under increasing pressure to maintain an optimum level of production efficiency. Opportunities to optimise whole farm efficiency and profitability are continually being investigated and include improving the efficient conversion of forage to meat and/or milk (Boland *et al.*, 2013). In pastoral grazing systems animal performance is highly influenced by dry matter intake (DMI). Determining accurate DMI of perennial ryegrass in a grazing scenario can be difficult. The 'n-alkane technique' as described by Mayes *et al.* (1986) and modified by Dillon and Stakelum (1989) is routinely used in animal research for estimating DMI in grazing dairy cows. This technique involves determining the faecal concentration of an inert marker, dosed intra-ruminally in known amounts, relative to the concentration of a homologous internal marker present in a known concentration in the diet (Mayes *et al.*, 1986). The objective of this study was to investigate the n-alkane technique for estimating DMI in sheep offered a perennial ryegrass diet and dosed with an external marker, ^{33}C, once daily.

Materials and methods

A series of experiments was undertaken at the Teagasc Animal and Grassland Research Centre, Moorepark, Fermoy, Co. Cork, Ireland (50°09'N; 8°16'W). The procedures formed part of three larger scale studies determining the effect of perennial ryegrass herbage mass (HM) and cultivar on *in vivo* DMI and digestibility in sheep (Beecher *et al.* 2018; Garry, 2016). In each of the HM experiments a 3×3 Latin square experimental design was used while a 4×2 incomplete Latin square design was used in the cultivar study.

In vivo DMI was measured as described by Demarquilly *et al.* (1995) and Baumont *et al.* (2004) and were conducted across seasons (spring, summer, autumn) during periods of 12 days using one-year-old Texel wether sheep. Each animal was individually housed in stalls allowing for the total collection of urine and faeces. Animals had *ad libitum* access to water. On days 1 to 6 the animals were allowed to adapt to the diet and stalls (adaption phase), and on days 7 to 12 DMI was recorded and total collection of faeces was made (measurement phase). Following weighing, the faeces were homogenised by mixing and a 20% representative sample per sheep was retained daily. Measurements of DMI were also estimated using the n-alkane technique (Mayes *et al.*, 1986). All animals were dosed once daily, prior to morning feeding, with paper boluses containing 132 mg of ^{32}C alkane (n-dotriacontane) for 12 days with herbage and faeces samples collected during the measurement phase. The ratio of herbage ^{33}C alkane (tritriacontane) to dosed ^{32}C alkane was used to calculate estimated DMI. Liveweight was measured before each period.

During each period grass was harvested daily at 8:30 h with an Etesia mower (Etesia UK Ltd., Warwick, United Kingdom). The particle size of grass harvested with the Etesia was 52±25 mm. The grass was offered to the sheep *ad libitum* to allow a 10% refusal rate. Animals were offered grass twice daily at 8:30 and 16:00 h. Approximately 50% of the grass was offered in the morning immediately after harvesting, with the remaining grass refrigerated at 4 °C and offered in the afternoon.

Data were assessed for normality using PROC UNIVARIATE and the multi-collinearity between the independent variables was completed in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The association between *in vivo* DMI and n-alkane DMI was estimated using a fixed effect model in PROC GLM. A multiple regression model for DMI was also created using a stepwise forward-backward regression where P=0.05 was used as the threshold for entry and exit of the independent variables (Table 1) from the model. The relationships between each of the independent variables, DMI and n-alkane DMI were determined using partial Spearman's rank correlations (PROC CORR) in SAS.

Results and discussion

The average and standard deviation of animal live-weight, *in vivo* DMI, n-alkane DMI and the chemical composition of the feed offered across each of the studies is presented in Table 1. A total of 232 animal records were used to determine the relationship between DMI and n-alkane DMI. There was a 0.09 kg DM d^{-1} difference between *in vivo* DMI and n-alkane DMI.

The r^2 value, calculated between actual DMI and the estimated n-alkane, was 0.75 (P<0.001). When additional fixed effects were included in the multiple regression model the corresponding r^2 value increased to 0.98 (P<0.001). A strong correlation was observed between *in vivo* DMI and n-alkane DMI (Table 2). Furthermore, animal live-weight and DMI kg^{-1} LW were both moderately correlated to DMI (P<0.001; Table 2) and agree with previous research which highlights DMI as a function of live-weight (Jarrige *et al.*, 1986).

Table 1. Mean values for animal traits and chemical composition of grass offered.

Trait	Mean value	Standard deviation	Minimum	Maximum
Liveweight (LW), kg	70	5.89	55.5	77
Dry matter intake (DMI), kg d^{-1}	1.54	0.23	1.00	1.88
In vivo DMI/LW, kg	2.26	0.29	1.72	2.59
N-alkane DMI, kg d^{-1}	1.45	0.26	0.93	1.88
Grass chemical composition				
Dry matter (DM), g kg^{-1}	189	24.3	166	216
In vivo organic matter digestibility, g kg^{-1} DM	811	70	716	902
Ash, g kg^{-1} DM	95	20.2	78	122
Neutral detergent fibre, g kg^{-1} DM	405	57.2	344	467
Acid detergent fibre, g kg^{-1} DM	250	36.8	218	294
Crude protein, g kg^{-1} DM	160	16.1	131	171

Table 2. Spearman rank correlations between independent variables and dry matter intake (DMI).

Trait	*In vivo* DMI	n-alkane DMI
In vivo DMI	1.00	0.71***
n-alkane DMI	0.71***	1.00
Liveweight (LW)	0.45***	0.39***
DMI kg^{-1} LW	0.54***	0.32***
In vivo organic matter digestibility	-0.05	-0.10
Crude protein	-0.16	-0.001
Acid detergent fibre	0.03	0.04

[1] Correlation coefficients are different from zero; *** $P<0.001$.

Conclusions

Identifying the ability of the n-alkane technique in estimating perennial ryegrass DMI in small ruminants will enable the accurate quantification of grass DMI from sheep managed in a rotational grazing system. In a research setting the collection of grass DMI data will enable further investigation into feed efficiency across animals of varying type, genetic strain and merit.

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References

Baumont R., Chenost M. and Demarquilly C. (2004) Measurement of herbage intake and ingestive behaviour by housed animals. In: Penning, P. (ed.) *Herbage intake handbook*. The British Grassland Society, Reading, UK, pp. 121-150.

Beecher M., Baumont R., O'Donovan M., Boland T.M., Aufrere J., Fleming C., Galvin N. and Lewis E. (2018) Effects of harvesting perennial ryegrass at different levels of herbage mass on voluntary intake and *in vivo* digestibility in sheep. *Grass and Forage Science* 73, 553-561.

Boland M.J., Rae A.N., Vereijken J.M., Meuwissen M.P.M., Fischer A.R.H., Van Boekel M.A.J.S., Rutherfurd S.M., Gruppen H., Moughan P.J. and Hendriks W.H. (2013) The future supply of animal-derived protein for human consumption. *Trends in Food Science & Technology* 29, 62-73.

Demarquilly C., Chenost M. and Giger S. (1995) Pertes fecales et digestibility des aliments et des rations. In: Jarrige R., Ruckebusch Y., Demarquilly C. Farce M.H. and Journet M. (eds.) *Nutrition des ruminants domestiques – ingestion et digestion*. Paris, France: INRA Editions, pp. 601-647.

Dillon P. and Stakelum G. (1989) Herbage and dosed alkanes as a grass measurement technique for dairy cows. *Irish Journal of Agricultural Research* 28, 104.

Garry G. (2016) *The effect of animal and sward factors on the* in vivo *organic matter digestibility of perennial ryegrass*. Thesis PhD, University College Dublin, Dublin, Ireland.

Jarrige R., Demarquilly C., Dulphy J.P., Hoden A., Robelin J., Beranger C., Geay Y., Journet M., Malterre C., Micol D. and Petit M. (1986) The INRA Fill Unit system for predicting the voluntary intake of forage-based diets in ruminants: a review. *Journal of Animal Science* 63, 1737-1758.

Mayes R.W., Lamb C.S. and Colgrove P.M. (1986) The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. *Journal of Agricultural Science* 107, 161-170.

The effect of rotational grazing speed on sheep and grassland performance

Meeke T.[1,2], Aubry A.[1] and Gordon A.W.[1]
[1] *Agri-Food and Biosciences Institute, Hillsborough, Co. Down, Northern Ireland, United Kingdom;*
[2] *School of Biological Sciences, Queen's University Belfast, Belfast, Northern Ireland, United Kingdom*

Abstract

The objective of this study was to examine the effect of 4- vs 8-paddock rotational grazing systems on animal and grassland performance. The study was conducted over two consecutive grazing seasons from April to November 2018 and from March to November 2019, using a predominantly *Lolium perenne* sward, at AFBI, Hillsborough. There were two grazing treatments (4- vs 8-paddock rotational grazing system), which were balanced for ewe live weight, body condition score and lamb sire breed. Each system consisted of 1.6 ha which were rotationally grazed at a stocking rate of 14 ewes ha^{-1}. There was no significant effect of grazing treatment on ewe live weight or body condition score. Lambs grazing the 4-paddock system had higher average daily gains from 10 weeks of age to weaning (P<0.001) compared with those grazing the 8-paddock system, which resulted in higher weaning weights (P<0.001) for the 4-paddock lambs. In conclusion, findings demonstrate that in the context of this study the 8-paddock rotational grazing system produced higher levels of herbage utilisation but resulted in lower lamb live weights at weaning.

Keywords: sheep, grazing system, herbage utilisation, average daily gain and carcass output

Introduction

The competitive advantage in ruminant livestock production hinges on maximizing the contribution of grazed grass in the diet. Grass, our cheapest feed resource, can supply up to 95% of the energy requirements of sheep (Davies and Penning, 1996); thus, the efficient production and utilisation of herbage in lamb production systems is the key to profitability. Currently, there are inefficiencies in the level of herbage utilised per ha within sheep production systems, and as utilisation rate affects the cost per kg of grazed grass consumed this needs to be rectified (Earle *et al.*, 2017; Keady *et al.*, 2009). Previous research shows rotational grazing can deliver higher levels of animal performance and more efficient use of grassland compared with continuous stocking (Marley *et al.*, 2007). Rotational grazing has a number of benefits compared with continuous grazing, including (1) increased herbage production (2) it avoids insufficient or excessive levels of herbage availability, (3) it facilitates herbage regrowth, and (4) it allows for the cutting of surplus forage. Rotational grazing is the way forward to improve herbage utilisation and animal performance; however, questions still remain on the optimal number of paddocks to include within a rotational system. The objective of this study was to examine the effect of 4- vs 8-paddock rotational grazing systems on animal performance and herbage production, utilisation, and quality.

Materials and methods

The present study was replicated over two consecutive grazing seasons from April to November 2018 and from March to November 2019, at the Agri-Food and Bioscience Institute (AFBI), Hillsborough, Co. Down, Northern Ireland (54° 27'N; 06° 04'W). The area used for the study consisted of a predominantly perennial ryegrass (*Lolium perenne*) sward. The study comprised two grazing treatments (4- vs 8-paddock rotational grazing systems), which were balanced for ewe live weight, body condition score and lamb sire breed. Each system consisted of 1.6 ha, which were rotationally grazed at a stocking rate of 14 ewes ha^{-1}, which equated to 22 twin-rearing ewes per treatment. The target pre-grazing cover was 2,600 kg dry matter (DM) ha^{-1} for the duration of the experiment and the target post-grazing cover was 1,600 kg

DM ha^{-1} on both treatments. Ewes were weighed and body condition scored at turnout, 10 weeks post-lambing and weaning. Lambs were weighed every 2 weeks from 6 weeks of age using portable electronic scales (Shearwell Data Ltd., Minehead Somerset, UK) and were drafted for slaughter on reaching 45 kg live weight. Lambs were weaned on average at 14 weeks of age, with a leader follower grazing system that operated post-weaning. The lifetime average daily gain (ADG) was calculated as drafting weight minus birth weight divided by number of days to reach slaughter. Carcass conformation was scored using the EUROP grid system and fat score was scored on a 1-5 scoring system in order of increasing fatness. Dressing proportion was calculated as cold carcass weight divided by the pre-slaughter live weight. Pre- and post-grazing compressed sward heights were determined on each paddock before and after grazing by taking 30 measurements across the diagonal of the paddock with a rising plate meter (Jenquip, New Zealand). Pre- and post-grazing herbage mass was determined for each paddock by taking four quadrat (0.5×0.5 m) cuts with Gardena hand shears. All harvested herbage was weighed and a sub-sample was retained for DM and quality analysis. Fresh herbage samples were also analysed at each grazing for crude protein (CP), water-soluble carbohydrate (WSC), acid detergent fibre (ADF) and metabolizable energy (ME) via near infrared reflectance spectroscopy. All statistical analysis was completed using Genstat (16th edition). Lamb live weight, ADG, and carcass traits were analysed using linear mixed models, with ewe and year included as random effects and lamb breed, sex, and the lambs deviation in age from the treatment mean included as fixed effects.

Results and discussion

There was no significant effect of grazing treatment on ewe live weight or body condition score. Lambs grazing the 4-paddock rotational system had higher ADG from birth to weaning ($P<0.001$) compared with those grazing the 8-paddock system and this was mostly driven by higher ADG from 10 to 14 weeks of age ($P<0.001$; Table 1). This resulted in higher weaning weights ($P<0.001$) for lambs on the 4-paddock rotational system and a higher lifetime average daily gain ($P<0.001$) compared to lambs on the 8-paddock system (Table 2). Subsequently, the 8-paddock lambs required more time to reach slaughter ($P<0.001$) but no differences in lamb drafting weight and carcass traits were observed. In terms of drafting pattern, 89% of 4-paddock system lambs were drafted for slaughter by October, in comparison the 8-paddock system only had 60% of lambs drafted. On average, monthly herbage utilisation was 9% higher on the 8-paddock system. Grazing treatment had no significant effect on grass quality in terms of CP, ME, DM, ADF and WSC.

Table 1. Lamb performance from birth to weaning.[1]

Variables	Paddock system		SED	Significance
	4	8		
Birth weight (kg)	4.7	4.7	0.17	NS
ADG (g day^{-1})				
Birth to 6 weeks	276	274	10	NS
6 to 10 weeks	304	279	10.2	*
10 to 14 weeks	261	193	12.2	***
Birth to weaning	279	250	7.6	***
Weaning weight (kg)	32.7	30	0.77	***

[1] ADG = average daily gain; SED = standard error of differences; NS = not significant; * $P<0.05$; *** $P<0.001$.

Table 2. Effect of treatment on lamb lifetime performance and carcass traits.[1]

Variables	Paddock system		SED	Significance
	4	8		
Lifetime ADG (g day^{-1})	220	187	6.6	***
Days to slaughter	183	219	5.9	***
Drafting weight (kg)	46.2	46.9	0.41	NS
Carcass weight (kg)	20.4	20.7	0.29	NS
Carcass conformation	3.4	3.5	0.08	NS
Fat score	2.8	2.7	0.08	NS
Dressing proportion	0.47	0.46	0.006	NS

[1] ADG = average daily gain; SED = standard error of differences; NS = not significant; *** P<0.001.

Conclusions

Lamb performance was higher on the 4-paddock system compared with the 8-paddock rotational grazing system; however, there was no effect on carcass traits between the systems. The findings of this experiment indicate that increasing the number of paddocks within a rotational grazing system results in higher levels of herbage production and utilisation but results in reduced lamb performance. Evidently, there is an inverse relationship between optimising herbage utilisation and high animal performance.

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References

Davies A. and Penning P. (1996) Prospects for improving the efficiency of sheep production from grass. In: *Irish Grassland Association Proceedings 50th Anniversary Programme Sheep Conference* 1996. pp. 11-25.

Earle E., Boland T.M., McHugh N. and Creighton P. (2017) Measures of lamb production efficiency in a temperate grass-based system differing in ewe prolificacy potential and stocking rate. *Journal of Animal Science* 95, 3504-3512.

Keady T.W.J., Hanrahan J.P. and Flanagan S. (2009) An evaluation of two grassland-based systems of mid-season prime lamb production using prolific ewes of two genotypes. *Irish Journal of Agricultural and Food Research* 48, 87-101.

Marley C.L., Fraser M.D., Fisher W.J., Forbes A.B., Jones R., Moorby J.M., Macrae J.C. and Theodorou M.K. (2007) Effects of continuous or rotational grazing of two perennial ryegrass varieties on the chemical composition of the herbage and the performance of finishing lambs. *Grass and Forage Science* 62, 255-264.

Influence of type of N fertiliser on the mineral composition of horse pasture growths with and without herbs

Müller J.[1], Erlinghagen R.[2] and Wolf P.[2]
[1]*Grassland and Forage Science, University of Rostock, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany;* [2]*Nutritional Physiology and Animal Nutrition, University of Rostock, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany*

Abstract

Horse pastures are susceptible to N surpluses due to low nutrient exports and should therefore only receive small amounts of N. Thus, the type of N fertiliser becomes more important for the control of growth and associated nutrient acquisition of the sward. We examined, using CoANOVA, the effects of low-dose calcium ammonium nitrate, ammonium sulphate and calcium cyanamide on the mineral content of pure grass swards and herbaceous grass stands in the first growth and in the second regrowth of a horse pasture. We found significant effects of N fertiliser type, but not of herbs enrichment, on Mg, K, and Zn in the primary growth and Ca and P in the second regrowth. However, Na in both growths and K in the second regrowth were not influenced by the kind of N supply but depended on the kind of grass sward. Se showed no dependence on the factors examined. The interaction 'type of fertiliser'×'type of sward' was not significant in any case. The herb-rich stands did not generally show higher mineral contents. The kind of N-fertiliser used for horse pastures has some potential to affect macro-mineral concentrations in pasture growths. Effects of this factor on micro-element contents are limited.

Keywords: mineral contents, horse pasture, herbs enrichment, nitrogen form

Introduction

The national fertiliser legislation aims to reduce N balance surpluses. This goal is difficult to achieve in pasture-based horse husbandry, since this type of grassland use hardly generates balance-relevant nutrient exports from the area. Zero N fertilisation has a negative effect on the performance of the grassland sward for horses (Szatai and Der, 2007); it promotes nutritionally undesirable species, such as white clover (Singer *et al.*, 2001), and does not allow adaption of the growth rate to the feed demand. On the other hand, permanent annual nutrient surpluses pose the risk of eutrophication and contamination of soil and aquifers (Parvage *et al.*, 2011). A restrained N-fertilisation is therefore required and alternative management options beside the variation of the N-level gain in importance.

While many studies on the influence of the amount of N-fertilisation on the quality of pasture growth are available, there is little information on the possibilities of controlling forage quality by choosing the N-fertiliser type. Therefore, we were interested in the influence of different N formulations on the mineral contents of horse pasture growths. We addressed the following questions: (1) Does the choice of the N-fertiliser type open up a specific way of influencing the mineral pattern in pasture growth? (2) If that is the case, then what role does the functional composition of the grassland sward play in this context?

Materials and methods

To answer the questions, we established a field experiment at a professional horse farm near Rostock, Northeast Germany, in a two-factorial block design (Table 1). The annual amount of 90 kg N applied corresponds to the N-return of a heavily stocked horse pasture. The distribution of the nitrogen application to the individual growths was carried out according to the recommendations of the fertiliser advice to be in line with practitioners.

Table 1. Experimental design – factors, levels and technical specifications.

Factors	Factor levels		
1 Type of grassland sward (S)	1.1 pure grass (mixture 'Country Horse', DSV)		
	1.2 grass & herbs (grass/herb mixture with addition of *Plantago lanceolata*, *Taraxacum officinale*, *Daucus carota*)		
2 Type of N-fertiliser (F)	2.1 no N applied (reference)	0 N	[0/0/0/0/0 kg N ha^{-1}]
	2.2 calcium ammonium nitrate	CAN	[40/30/20/0/0 kg N ha^{-1}]
	2.3 calcium cyanamide	CC	[60/0/30/0/0 kg N ha^{-1}]
	2.4 ammonium sulphate	AS	[60/0/30/0/0 kg N ha^{-1}]

[1] n=4; number of plots=32; plot size=2×10 m; [N-distribution to the five growths in brackets].

The samples for the examination of the mineral contents were taken in the second year of use from the primary growth as well as from the second regrowth. Samples were ground after oven drying at 45 °C and subsequently analysed for their mineral concentrations in duplicate using atomic absorption spectrometry.

We used CoANOVA to examine the effects of the factors S, F and their interaction S×F on the contents of Ca, K, P, Mg, Zn and Se after verification of normal distribution (Shapiro-Wilk-test) and transformation if necessary. The co-variate 'dry matter yield' served as a proxy for the development stage of herbage to gain better control over this potential effect size. All statistical procedures were based on scripts written in R (R Development Core Team, 2016).

Results and discussion

The yield as covariate was able to improve the factor-related effects on the expression of the mineral contents as could be seen from the residual plots. The interaction 'type of sward' × 'type of fertiliser' was not significant in any of the cases. The Se content also did not show any factorial effect. Therefore, these data are not reported in Table 2.

Despite percentages of practicable 7-15% herbaceous dicots in the 'grass & herbs' mixture variant, the factor 'type of sward' was only significant in the case of Na and of K in the second regrowth. Herbs enrichment resulted in significantly higher levels of both cations. This observation could not be traced back to proportions of the known Na-rich perennial ryegrass (*Lolium perenne*) and is therefore very likely a result of adding herbs to the seed mixture. Whether this positive finding from the point of view of horse nutrition can be quickly translated into pasture practice remains questionable, especially as horses are reported to show a preference for grass to herbs (Archer, 1971).

Table 2. Influence of the types of grassland sward and of N-fertiliser on selected mineral contents.

Factor	Effect[1]					
	Ca	P	K	Mg	Na	Zn
Type of grassland sward						
Primary growth	P=0.181^{ns}	P=0.694^{ns}	P=0.305^{ns}	P=0.256^{ns}	P=0.034*	P=0.083^{ns}
2nd regrowth	P=0.207^{ns}	P=0.818^{ns}	P=0.032*	P=0.639^{ns}	P=0.035*	P=0.485^{ns}
Type of N-fertiliser						
Primary growth	P=0.537^{ns}	P=0.071^{ns}	P<0.001***	P=0.022*	P=0.879^{ns}	P=0.026*
2nd regrowth	P=0.007**	P=0.001***	P=0.221^{ns}	P=0.051^{+}	P=0.904^{ns}	P=0.970^{ns}

[1] CoANOVA-results, *P*-values of the F-test. Significant effects indicated with asterisks. ns = not significant; * P<0.05; ** P<0.01; *** P<0.001.

Figure 1 shows how the different fertiliser types influence the P content. In primary growth, the more productive N forms also provided higher P contents, which was reversed in summer regrowths under water stress. Similar effects have been observed in other studies when the N dose was increased.

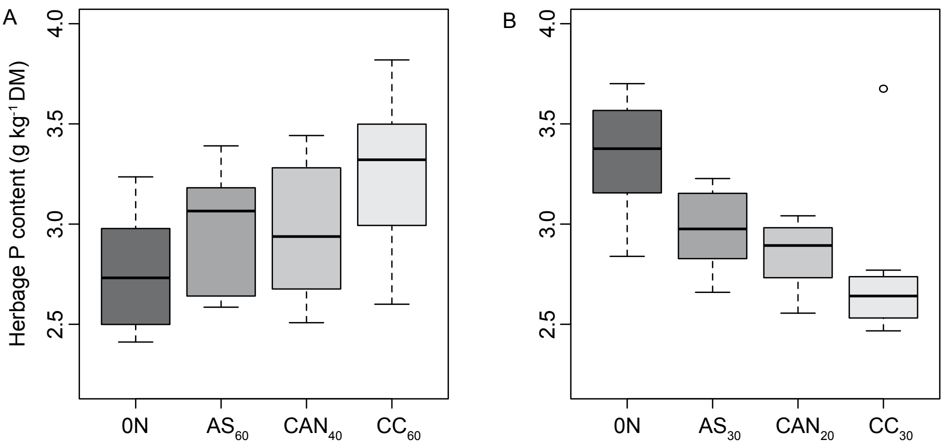

Figure 1. Phosphorus contents of primary growth (A) and the second regrowth (B) as a function of fertiliser type. Boxes were plotted over both sward levels. 0N = no N applied; AS = ammonium sulphate; CAN = calcium ammonium nitrate; CC = calcium cyanamide; DM dry matter.

Conclusions

The kind of N-fertiliser used for horse pastures has some potential to affect macro-mineral concentrations in pasture growths. Effects of this factor on micro-element contents are limited. Ammonium sulphate is a rather slow acting N-fertiliser and did not provide maximum yields, but a harmonious growth and thus for quite balanced mineral concentrations. The addition of herbs had fewer positive effects on the mineral contents of the primary growth than in the summer regrowth.

References

Archer M. (1971) Preliminary studies on the palatability of grasses, legumes and herbs to horses. *Veterinary Record* 89, 236-240.

Parvage M.M., Kirchmann H., Kynkäänniemi P. and Ulén B. (2011) Impact of horse grazing and feeding on phosphorus concentrations in soil and drainage water. *Soil Use and Management* 98, 367-375. https://doi.org/10.1111/j.1475-2743.2011.00351.x

R Development Core Team (2016) R – a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Singer J.W., Bobsin N., Kluchinski D. and Bamka W.J. (2001) Equine stocking density effect on soil chemical properties, botanical composition, and species density. *Communications in Soil Science and Plant Analysis* 32, 2549-2559. https://doi.org/10.1081/CSS-120000390

Szatai Z and Der F. (2007) Effect of zero fertilization on pasture productivity, and draft horse keeping capacity. *Animal Science and Biotechnologies* 63/64, 149-153.

The effect of pre-mowing on the performance of high-production dairy cows

Pollock J.G.[1,2], Gordon A.[3] and McConnell D.A.[2]

[1]Queen's University Belfast, University Road, Belfast, Co. Down, Northern Ireland, United Kingdom; [2]Agri-food and Biosciences Institute, Large Park, Hillsborough, Co. Down, Northern Ireland, United Kingdom; [3]Agri-food and Biosciences Institute, 23a Newforge Lane, Belfast, Co. Down, Northern Ireland, United Kingdom

Abstract

Pre-mowing is a management technique that involves cutting the grazing area prior to *in situ* grazing of herbage. Pre-mowing is gaining interest on dairy farms in temperate regions due to the perceived benefits of improved grass utilisation and cow performance. However, to date limited evidence exists for the potential of pre-mowing to improve the performance of high-production dairy cows, herbage utilisation and herbage quality throughout the season. This 100-day continuous design experiment involved 80 spring-calving Holstein-Friesian lactating dairy cows divided into to four balanced groups and allocated to one of two treatments (1) conventional grazing or (2) grazing a pre-mown area, both in a rotational grazing system. All treatments were offered concentrate at a rate of 7 kg day^{-1}, fed during milking. Herbage utilisation, animal performance and behaviour were measured. Pre-mowing significantly (P<0.05) improved the efficiency of herbage utilisation. However, the performance of lactating dairy cows was not affected, with average values for milk yield, milk fat plus protein yield and change in live weight of 27. 8, 2.04 and -9.57 kg cow^{-1} day^{-1}, respectively. Although pre-mowing a grazing sward can improve pasture utilisation, farmers should consider the labour, fuel and machinery costs associated prior to pre-mowing.

Keywords: pre-mowing, grazing, dairy, milk production, grass utilisation

Introduction

Grass remains the cheapest source of feed available for dairy cows in temperate regions (Dillon, 2007). However, within pasture-based dairy systems, dry matter intake (DMI) is often suboptimal due to the low dry matter content of pasture herbage (Kolver and Muller, 1998). This is particularly evident with high yielding cows where their total energy demand for milk production is significant. Previous management strategies such as increasing daily herbage allowance have improved DMI at pasture but subsequently result in a much lower grass utilisation rate which in turn reduces sward quality (McEvoy *et al.*, 2008). Hence, there is a requirement to develop management strategies for high production dairy cows that improve animal DMI and performance at pasture while maintaining a high pasture utilisation efficiency. Pre-mowing is a management technique which involves mowing the grazing area immediately prior to *in situ* grazing of the herbage. To date, research on this technique is limited and has produced conflicting results. For example, Kolver *et al.* (1999) observed increases in milk solid (MS) production (+0.13 kg MS cow^{-1} day^{-1}) during the summer months following pre-mowing. In contrast, Kay *et al.* (2018) observed no significant animal response; however, improvements in grass quality. The objective of this study was to investigate the effect of two contrasting grass management strategies (pre-mowing vs conventional grazing) on herbage utilisation, the performance and behaviour of high production dairy cattle compared to conventional grazing.

Materials and methods

The 100-day (15/5/19-23/8/19) study was conducted at the Agri-Food and Biosciences Institute (AFBI), Hillsborough, Northern Ireland (54°27'N; 06°04'W). Eighty spring-calving Holstein-Friesian dairy cows (60 multiparous and 20 primiparous) were divided into four balanced groups and assigned to one of two grass management strategies: (1) conventional grazing or (2) grazing a pre-mown area. Treatments were balanced for lactation number, calving date, pre-experimental milk yield, live weight and animal predicted genetic ability for milk yield and milk fat plus protein yield. Primiparous and multiparous animals were offered concentrates at a rate of 6.2 and 7.2 kg cow^{-1} day^{-1}, respectively. Ten primary grazing blocks of perennial ryegrass (*Lolium perenne* L.) were established containing two paddocks of 0.21 ha for each group. Paddocks were rotationally grazed. Pre-mown pasture was mowed between 11am and 1pm using a side-mounted disc mower to a target height of 4.5 cm. All treatments entered a 24-h grazing paddock after evening milking. Target pre- and post-grazing covers were 3,100-3,400 kg dry matter (DM) ha^{-1} and 1,700 kg DM ha^{-1}, respectively. All conventional grazing ground was topped after the second rotation. Pre- and post-grazing sward height was determined using a rising plate meter. In addition, to account for mown pasture herbage on the pre-mown sward, grass left after a 24-h grazing period was calculated by collecting 10 randomly located quadrats (0.5×0.5 m) of surplus grass material. Grass was then weighed and dry matter determined. Herbage mass and compositional quality was analysed twice weekly using an Agria mower (3×4 m strips per paddock) and near infrared spectroscopy (NIRS), respectively. Total herbage utilisation was determined as the difference between available (>4 cm) and consumed herbage. Individual animal milk yields, daily live weight, weekly milk composition and fortnightly body condition score was recorded. Individual animal lying, standing time and motion index (IceRobotics, Edinburgh, UK) was monitored throughout the experiment. Eating and ruminating behaviour (ITIN+HOCH, Bennwil, Switzerland) was monitored during three two-week periods starting on 4 June, 11 July and 12 August. Data were analysed using Genstat 19.1 (VSN International Ltd, 2017) by ReML component analysis.

Results and discussion

Pre-grazing sward height was similar for both treatments, offering animals on average 17 kg DM day^{-1} (Table 1). In the conventional grazing system post-grazing herbage mass was significantly higher (P<0.05) and subsequently total herbage utilisation was significantly lower than the pre-mown treatment (P<0.05) (Table 1). Treatment did not affect herbage compositional quality. However, in later rotations (4th and 5th) water soluble sugars and metabolizable energy were significantly lower compared with the prior rotations.

Management strategy did not significantly (P>0.05) affect milk yield, milk fat plus protein yield or animal live weight (Table 1). Pre-mowing significantly (P<0.05) reduced rumination time, number of rumination chews and length of rumination bout relative to the conventional grazing (Table 1). This was

Table 1. Impact of management strategy on of pre-mowing grass versus conventional grazing on grass utilisation, animal performance and behaviour.[1]

	Conventional	Pre-mown	SED	*P*-value
Pre-grazing herbage mass (kg DM ha^{-1})	3,261	3,208	37.0	NS
Post-grazing herbage mass (kg DM ha^{-1})	2,060	2,010	21.5	0.012
Total herbage utilisation (%)	73.1	75.3	1.26	0.015
Milk yield (kg day^{-1})	27.8	27.7	1.25	NS
Milk fat plus protein yield (kg cow^{-1} day^{-1})	2.02	2.06	0.057	NS
Change in live weight (kg cow^{-1})	-7.6	-11.7	5.02	NS
Eating time (min hr^{-1})	24.8	24.3	0.49	NS
Rumination time (min hr^{-1})	17.3	15.8	0.45	<0.001
Rumination chews (no. day^{-1})	1,087	1,009	37.0	<0.001
Rumination bout length (min hr^{-1})	23.7	22.5	0.44	0.002

[1] DM = dry matter; SED = standard error of differences; NS = not significant.

particularly evident between the hours of 1 am to 7 am and 11 am to 3 pm, after the two main grazing bouts post-milking (Figure 1). Pre-mowing forced animals to take larger mouthfuls which increased physical handling of the of the grass prior to ingestion. This likely increased the breakdown of the grass material and reduced the requirement for rumination.

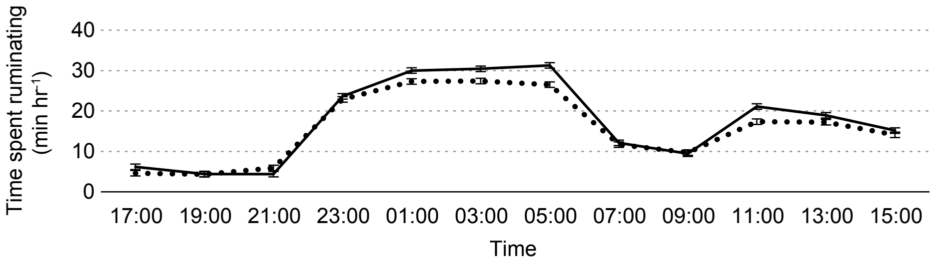

Figure 1. The effect of management strategy on rumination time over the 24hour grazing allocation in three two week periods (pre-mown treatment (dotted line) and conventional grazing (black line), expressed by mean hourly values collected in two hourly periods; standard error represented by error bars).

Conclusions

Pre-mowing of pasture offers the potential to improve the efficiency of herbage utilisation, and with no negative effect on animal performance. However, it is clear animals adapt their grazing behaviour to facilitate their feeding system.

Acknowledgements

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References

Dillon P. (2007) Achieving high dry-matter intake from pasture with grazing dairy cows. In: Elgersma A., Dijkstra J. and Tamminga S. (eds.) *Fresh herbage for dairy cattle.* Frontis, pp. 1-26.

Kay J., Edwards P., Clement A. and Bryant R. (2018) The effect of pre-graze mowing at different pre-graze masses on cow and pasture performance. *Grassland Science in Europe,* 23, 268-270.

Kolver E. and Muller L. (1998) Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. *Journal of Dairy Science* 81, 1403-1411.

Kolver E., Penno J., MacDonald K., McGrath J. and Carter W. (1999) Mowing pasture to improve milk production. *Proceedings of the confernce New Zealand Grassland Assocation.*

McEvoy M., Kennedy E., Murphy J.P., Boland T.M., Delaby L. and O'Donovan M. (2008) The effect of herbage allowance and concentrate supplementation on milk production performance and dry matter intake of spring-calving dairy cows in early lactation. *Journal of Dairy Science* 91, 1258-1269.

Time budget of Konik horses in different nature conservation areas – two case studies

Prończuk M., Chodkiewicz A. and Stypiński P.
Warsaw University of Life Sciences, Faculty of Agriculture and Biology, Warsaw, Poland

Abstract

The abandonment of wetlands and an increase in the surface of uncultivated land has caused a reduction in local biodiversity and become a threat to succession in ecologically valuable habitats. The reintroduction of grazing by Konik horses is one method that may help provide protection of wetland habitats. The time that horses spend at grazing is primarily associated with the availability of various plants on the pastures and the animals' own preferences. This paper presents results of a time-budget analysis of Polish native horses (Koniks) used as a natural management tool within two areas. The studies were carried out in 2009 and 2010 in breeding reserves in the Biebrza National Park, where Koniks were kept throughout the year in fenced enclosure, and in 2018 and 2019 in the Rakutowskie peatbogs, where horses were rotationally grazed on three pastures. In each location, their behaviour was observed throughout three-hour periods in the morning and in the evening in April, June and August. In Biebrza National Park the reserve-bred horses spent approx. 78% of their time on grazing, whereas in the Rakutowskie peatbogs this was about 67%. In both locations, the time spent on grazing varied between months as was highest in April.

Keywords: Konik horse, behaviour, time budget, wetland

Introduction

The total area of grassland communities in Europe has declined in the recent decades as a result of abandonment of less productive sites and changes in hydrological and other natural conditions (European Commission, 2015). Grazing management is a useful and effective tool for the maintenance and protection of nature-valuable habitats. Primitive, robust breeds such as the Polish primitive horses (Koniks) are particularly well adapted to nature-valuable habitats due to their resistance to infections and parasites. These unique abilities allow them to adapt to local environmental conditions, and they are resourceful in the search for food and have modest requirements during periodic feed shortages (Chodkiewicz, 2020).

The objective of this study was to assess the daily behaviour patterns of Konik horses grazing on valuable nature habitats in two different locations in Poland. This study had broader aims in terms of the possibility to predict the usefulness of long-term Konik horse grazing on wetlands, to provide a solution to the problem of overgrowth of vegetation on wastelands, and thereby retain habitats that are important for, among others, birds of order Charadriiformes.

Materials and methods

The observations of grazing Koniks were carried out in two locations: the Biebrza National Park (BNP; in 2009 and 2010) and the Rakutowskie peatbogs (RP; in 2018 and 2019). The main goal of grazing in both of these areas is the protection of wetland habitats. In BNP, ca. 30 adult horses were bred under a whole-year grazing programme. In RP the number of Koniks that stayed on the monitored pastures from January to August and from November to December was subject to change and varied from 2 to 13. The stocking rate was 0.12 LU in BNP and 0.34 LU in RP. In BNP, the grassland communities cover ca. 40 ha (as part of the 210 ha available for horses), whereas in RP it was the entire 12 ha. The observations of grazing Koniks were carried out during a period of three months (April, June and August), in two three-hour time periods (in the morning: 7:00-10:00 am, and in the evening: 16:00-19:00 pm). In BNP, the horses were observed for 6 consecutive days (except for April of the first year of studies; observed for 4 days) and in RP for 2 consecutive days. The Koniks' behaviour was recorded every five minutes, taking into account the following: grazing, resting, moving, drinking and others (playing, social interactions, rolling around). The statistical analysis of collected data was performed by means of the Tukey's Test.

Results and discussion

In general, at both BNP and RP, the Koniks spent most of their time budget on grazing (on average ca. 78 and 67%, respectively). This is consistent with other studies (e.g. Cosyns *et al.* 2001; Dynowski, 2006; Fluerance *et al.,* 2001) that have shown horses kept in nature reserves throughout the day devote ca. 50-70% of their time to grazing, 20-30% to resting and 10% to playing. The higher amount of time committed to grazing at both BNP and RP is probably because the animals spend more time on that activity during morning and evening hours, compared with the middle of the day when high temperatures and insects make them seek shelter in the shade.

At both locations, the Koniks spent higher proportions of their time on grazing during April, compared with other parts of the vegetative season (Table 1 and Table 2). This longer time spent grazing in early spring may possibly because there is a larger amount of available feed (grass) following the winter period, even though in both husbandries the horses are fed with hay during winter. Moreover, as indicated by Naujack *et al.* (2003), the animals devote more of their time to taking bites when vegetation is short.

The grazing time was inversely correlated with the horses' resting time. Slightly lower walking activity, on the other hand, indicated that the animals spend most of the grazing time in one area only. In RP, the Koniks grazed for a significantly longer period of time in the evening than in the morning hours. A

Table 1. Average percentage of time the Koniks spent on different activities in the Biebrza National Park site, for different years, months and observation periods.[1]

	Month			Year		Observation period	
	April	June	August	2009	2010	Morning	Afternoon
Number of observations (days)	8	12	12	14	18	14	18
Grazing	86.8a	71.5b	78.6c	74.4a	80.6b	77.8a	78.0a
Resting	10.9a	28.3b	21.1b	24.9a	18.5b	21.6a	21.0a
Walking	2.0a	0.1b	0.2b	0.5a	0.7a	0.3a	0.9a
Drinking	0.0a	0.1a	0.1a	0.2a	0.0a	0.1a	0.1a

[1] Tests were performed separately for each activity and factor (month, year, observation period). Different letters indicate significant differences ($P \leq 0.05$).

Table 2. Average percentage of time the Koniks spent on different activities in the Rakutowskie peatbog site, for different years, months and observation periods.[1]

	Month			Year		Observation period	
	April	June	August	2018	2019	Morning	Afternoon
Number of observations (days)	4	4	4	6	6	12	12
Grazing	73.3a	66.5b	62.5b	68.8a	66.0a	61.8a	73.0b
Resting	14.2a	17.8b	18.3b	17.3a	16.2a	20.2a	13.3b
Walking	3.7a	7.0b	9.0b	6.0a	7.2b	8.5a	4.6b
Drinking	2.8a	4.0a	4.7a	2.7a	5.0a	3.8a	3.9a
Other	6.0a	4.7a	5.5a	5.2a	5.7a	5.7a	5.2a

[1] Tests were done for each factor (month, year, observation period) and activity separately. Different letters indicate significant differences ($P \leq 0.05$).

possible explanation for this difference is that in the summer the activity of horses is affected by factors such as temperature and abundance of flies (King, 2002; Mayes and Duncan, 1986), which, in turn, makes the Koniks graze less and rest more. In both locations only a small proportion of the time was used for drinking and other behaviour, as was also shown by Cosyns *et al.* (2001).

Conclusions

Koniks kept in two different types of nature conservation areas were observed to have similar time budgets. The results of observations indicate significant differences in the percentage of time that was used for grazing and resting depending on the given month, regardless of location. Horses kept at higher stocking rate (at site RP), however, showed additional significant differences in their time budget at different times of the day.

References

Cosyns E., Degezelle T., Demeulenaere E and Hoffmann M. (2001) Feeding ecology of Konik horses and donkeys in Belgian costal dunes and its implications for nature management. *Belgian Journal of Zoology* 131, 111-118.

Chodkiewicz A. (2020) Advantages and disadvantages of Polish primitive horse grazing on valuable nature areas – a review. *Global Ecology and Conservation* 21, e00879.

Dynowski P. (2006) *Vegetation of the Popielno Peninsula as a food base for the Polish horses*. Ph.D. Thesis, Botany and Nature Protection Department, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland, 114 pp.

European Commission (2015) Report from the Commission to the Council and the European Parliament: The State of Nature in the European Union. Available at: https://www.eea.europa.eu/publications/state-of-nature-in-the-eu.

Fleurance G., Duncan P. and Mallevaud B. (2001). Daily intake and the selection of feeding sites by horses in heterogeneous wet grasslands. *Animal Research* 50, 149-156.

King S.R.B. (2002) Home range and habitat use of free-ranging Przewalski horses at Hustai National Park, Mongolia. *Applied Animal Behaviour Science* 78, 103-113.

Mayes E. and Duncan P. (1986) Temporal patterns of feeding behaviour in free-ranging horses. *Behaviour* 96, 105-129.

Naujack A, Julian H. and Malcolm J.G. (2003) Is diet selection of horses influenced by sward height? *Tropical and Subtropical Agroecosystems* 3, 407-410.

Effect of gradual replacement of barley with oats on methane production in dairy cows

Ramin M., Fant P. and Huhtanen P.

Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, 901 83, Umeå, Sweden

Abstract

The effects of gradual replacement (0, 33, 67 and 100%) on a dry matter (DM) basis of barley with oats were tested using a replicated 4×4 Latin Square design. Sixteen Swedish red cows received a total mixed ration (50:50 forage:concentrate on DM basis). Grass silage was the sole forage with rapeseed meal (10% of diet DM) as a protein supplement. In addition to normal intake and production measurements, methane (CH_4) emissions were measured by the GreenFeed system. The experiment was conducted in 4 periods (28 days each period). The last ten days of each period were used for recording CH_4 emission and feed intake. The study showed that total DM intake did not change by replacing barley with oats in the diet (22.8 vs 22.6 kg d^{-1}). Energy corrected milk did not significantly differ (31.9 vs 31.8 kg d^{-1}). The only significant observation was a linear decline (P<0.01) in CH_4 production by replacing barley with oats in the diet of dairy cows (467 vs 445 g d^{-1}). This study concludes that barley could be replaced by oats in the diet of dairy cows to reduce CH_4 production without having any adverse effect on productivity.

Keywords: barley, oat, methane, dairy cow

Introduction

Methane (CH_4) is a major contributor to total global greenhouse gas (GHG) emissions within the agriculture sector (Wuebbles and Hayhoe, 2002). In addition, CH_4 represents an energy loss to the host animal. For example, the average Y_m (Y_m = CH_4 conversion factor defined as percentage of gross energy intake (MJ head d^{-1})) for dairy cattle has been reported to be 5.4 to 5.7% for North America (Kebreab *et al.*, 2008). There has been intensive research during the last decade, but practical innovations on-farm to lower CH_4 emissions are limited. Plant extracts have been demonstrated to be effective *in vitro* (Calsamiglia *et al.*, 2007), but the amounts required for *in vivo* studies make these supplements too costly. Nitrate supplementation is an effective means for lowering CH_4 emissions (Van Zijderveld *et al.*, 2011), but unless carefully monitored may pose a health risk to the animal, and also increase N emissions. Addition of oils or oilseeds have consistently lowered CH_4 emissions (Beauchemin *et al.*, 2008), but at high levels it lowers feed intake, depresses milk yield, and increases feed costs. In Nordic countries, oats and barley are commonly used grains in the diet of dairy cows. Many studies suggest that replacing barley grain with oat grain in the diet of dairy cows increases milk yield and improves the composition of milk fat by increasing the concentration of unsaturated fatty acids in milk (Ekern *et al.*, 2003; Vanhatalo *et al.*, 2006). We hypothesized that CH_4 emissions will decrease by inclusion of oats in the diet without showing any negative effects on productivity. The objective of this study was to evaluate possibilities to lower enteric CH_4 emissions from dairy cows by substituting barley with oats.

Materials and methods

All animals were cared for according to the rules and guidelines proposed by the Swedish University of Agricultural Sciences Animal Care and Use Committee and the National Animal Research Authority. The production trial was conducted in 2016 at Röbäcksdalen experimental farm of the Swedish University of Agricultural Sciences, Umeå, Sweden (63° 45' N; 20° 17' E). Sixteen Swedish Red dairy cows, with mean days in milk 82±29.6 and producing 31.8±3.50 kg milk d^{-1} at the start of the experiment, were used in a replicated 4×4 Latin square design. Grass silage was the sole forage, with rapeseed meal (10% of diet DM) as a protein supplement. The dietary treatments comprised gradual replacement of barley with oats at levels of 0, 33, 67 and 100% on a DM basis. The resulting four diets were defined as: 100% barley (B100, 300 g kg^{-1} DM barley); 66% barley and 33% oats (B66, 200 g kg^{-1} DM barley); 33% barley and 66% oats (B33, 100 g kg^{-1} DM barley); and 100% oats as (O100, no barley). Feed intake was recorded individually on a daily basis throughout the trial in Roughage Intake Control feeders (Insentec B.V., Markness, the Netherlands), but only the data collected from the last 10 days of each period were used for statistical analysis. Milk samples were collected during the last three days of each period to calculate yield. Methane was measured with the GreenFeed unit (GreenFeed system, C-Lock Inc., Rapid City, SD, USA) and data were collected in the same way as the intake data. The experimental data were analysed by ANOVA for a replicated 4×4 Latin square design using the MIXED procedure of SAS. All data were pooled per cow per period. The statistical model was:

$$Y_{ijkl} = \mu + S_i + P_j + C_k(S_i) + T_l + E_{ijkl},$$

where Y_{ijkl} is a dependent variable and μ is the mean for all observations, S_i is the effect of square i, P_j is the effect of period j, $C_k(S_i)$ is the effect of cow k within square i, T_l is the effect of diet l, and $E_{ijkl} \sim N(0, \sigma_e^2)$ represents the residual error. To compare the effects of gradual replacement of barley with oats in the diet, linear and quadratic contrasts were used. Differences were declared significant at $P \leq 0.05$.

Results and discussion

The indigestible neutral detergent fibre (iNDF) of the O100 diet increased as a result of greater iNDF value in oats compared to the B100 diet (110 vs 81.8 g kg^{-1} DM). The silage fermentation quality was good as indicated by the low pH (3.72) and low concentrations of ammonia N (33.0 g kg^{-1} N). There was no significant effect on total intake by replacing barley with oats. The same trend was observed for nutrient intake, except for iNDF and potentially digestible NDF intake as a result of higher iNDF value in oats (Table 1). Milk yield and energy corrected milk did not change by replacing barley with oats in the diet (Table 1). In contrast to these findings, both Ekern *et al.* (2003) and Vanhatalo *et al.* (2006) reported an increase in milk yield when barley was replaced by oats on a grass silage-based diet. The concentration of milk protein decreased linearly by replacing barley with oats (38.0 vs 36.9 g kg^{-1}). Total methane emission was reduced linearly (22 g d^{-1}) by replacing barley with oats (Table 1).

Table 1. Effect of graded replacement of barley with oats on intake, milk production, digestibility and methane (CH_4) emissions.

Item	Diet[1]				SEM	Contrast[2]	
	B100	B66	B33	O100		Lin	Quad
Total dry matter intake, kg d^{-1}	22.8	22.8	22.6	22.6	0.38	0.46	0.98
Intake of NDF[3]	8.00	8.12	8.18	8.32	0.085	0.01	0.91
Intake of indigestible NDF	1.87	2.07	2.25	2.45	0.037	<0.01	0.98
Milk yield, kg d^{-1}	28.2	28.3	28.0	28.8	0.72	0.28	0.39
Energy corrected milk, kg d^{-1}	31.9	31.5	31.1	31.8	0.77	0.76	0.15
Body weight, kg	622	628	623	626	12.6	0.66	0.64
Methane, g d^{-1}	467	454	449	445	9.5	0.01	0.37
CO_2, g d^{-1}	13,674	13,550	13,291	13,471	228.5	0.05	0.11

[1] Proportions of barley and oats of the diet: B100 = 100% barley, B66=66% barley, B33=33% barley and O100=100% oats.

[2] Lin = linear effect of dietary replacement of barley with oats; Quad= quadratic effect of dietary replacement of barley with oats.

[3] NDF = neutral detergent fibre; SEM = standard error of the mean.

Conclusions

The results of this study demonstrated that it is possible to replace barley with oats without compromising animal performance and at the same time reducing CH_4 emissions.

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References

Beauchemin K.A., Kreuzer M., O'Mara F. and McAllister T.A. (2008) Nutritional management for enteric methane abatement: a review. *Australian Journal of Experimental Agriculture* 48, 21-27.

Calsamiglia S., Busquet M., Cardozo P.W., Castillejos L. and Ferret A. (2007) Invited review: essential oils as modifiers of rumen microbial fermentation. *Journal of Dairy Science* 90, 2580-2595.

Ekern A., Havrevoll Ø., Haug A., Berg J., Lindstad P. and Skeie S. (2003) Oat and barley based concentrate supplements for dairy cows. *Acta agriculturæ Scandinavica. Section A, Animal Science* 53, 65-73.

Kebreab E., Johnson K.A., Archibeque S.L., Pape D. and Wirth T. (2008) Model for estimating enteric methane emissions from US cattle. *Journal of Animal Science* 86, 2738-2748.

Van Zijderveld S.M., Gerrits W.J.J., Dijkstra J., Newbold J.R., Hulshof R.B.A. and Perdok H.B. (2011) Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. *Journal of Dairy Science* 94, 4028-4038.

Vanhatalo, A., Gäddnäs T. and Heikkilä T. (2006) Microbial protein synthesis, digestion and lactation responses of cows to grass or grass-red clover silage diet supplemented with barley or oats. *Agricultural and Food Science* 15, 252-267.

Wuebbles D.J. and Hayhoe K. (2002) Atmospheric methane and global change. *Earth-Science Reviews* 57, 177-210.

Method development for mycotoxin analysis in grass silages

Rämö S., Huuskonen A., Franco M., Manni K. and Rinne M.
Natural Resources Institute Finland (Luke), Finland

Abstract

Mycotoxins are toxic substances produced by filamentous fungi (moulds) that can be found in most feeds, such as cereals, preserved forages, especially silage, and even pasture. Ruminants are known to be more resistant to mycotoxins than monogastric farm animals. However, recently there has been increasing interest to study the incidence of mycotoxins in ruminant feeds. Many articles have been published about mycotoxins in cereals, but less about mycotoxins in maize silage and only few about mycotoxins in grass silage. A method for mycotoxin analysis was developed in Luke's animal feed research in 2017. The QuEChERs extraction and dSPE clean-up was used in the sample pre-treatment. Mycotoxins were separated, identified and quantified by UPLC-MS/MS with the MRM technique using the internal standard method and multi-point matrix matched calibration. Currently, this method contains 31 compounds. The most common mycotoxins, roquefortine C, mycophenolic acid, and zearalenone, were detected in 52 of 155 analysed samples, while deoxynivalenol was detected in 12. Beauvericin and enniatin A1 were detected in almost all of the analysed samples. Possibly low concentrations of enniatins and beauvericin, which have antibiotic features, have prevented mycotoxin production of some fungi in grass silage.

Keywords: roquefortine C, mycophenolic acid, zearalenone, deoxynivalenol, QuEChERs, UPLC-MS/MS

Introduction

Mycotoxins, secondary metabolites produced by fungi, are a worldwide problem in food and animal feed. According to Stead *et al.* (2014), over 400 mycotoxins may be found in feed, and 25% of the world's cereal grains may be contaminated by them. The knowledge of mycotoxin occurrence in animal feed is concentrated primarily on cereal grains and there are fewer studies on maize (*Zea mays*) silage and only few on grass silage (Cheli *et al.,* 2013; Driehuis *et al.,* 2008a,b). Mycotoxins have traditionally been extracted from cereals with acetonitrile-water, while trichothecens have been determined as their trimethylsilyl imidazole derivatives by GC-MS, and other mycotoxins by HPLC-FLD or LC-MS/MS (Hietaniemi *et al.,* 2016). QuEChERs extraction and dSPE clean-up have often been used for feed and highly pigmented samples (Zacharisova *et al.,* 2014, Bourdra *et al.,* 2015). Zearalenone (ZEN), mycophenolic acid (MPA) and roquefortine C (ROC), which are the most common mycotoxins in grass silage according to Driehuis *et al.* (2008 a,b), were detected in some samples, when 20 mycotoxins were first monitored in 17 grass silage samples by UPLC-MS/MS in Luke's laboratory in 2017-2018 (Huuskonen *et al.,* 2018). The method was further developed in this study as follows: (1) caffeine was added as the internal standard, (2) penicillic acid, alternariol monomethyl ether, four enniatins (A, A1, B, B1) and 8 trichothecens were added into the method, and (3) dSPE clean-up was further developed.

Materials and methods

The silage samples (n=138) used in this study originated from Luke's animal feed research in 2017-2018. Most samples (n=108) were silages made of a second cut of a mixed sward of red clover (*Trifolium pratense,* 76%) and timothy (*Phleum pratense*, 24%), ensiled using four additive treatments: (1) control without additive, (2) formic acid, (3) lactic acid bacteria, and (4) salt-based additive (Franco *et al.,* 2019). The silages were sampled immediately after opening the silos and again after a five-day exposure to air. Additionally, 12 grass silage samples from three different cuts were studied as described by Huuskonen *et al.* (2018). Furthermore, six timothy silage samples were compared with six barley (*Hordeum vulgare*) and six triticale (*X Triticosecale)* whole-crop silage samples.

All used solvents, reagents and water were LC-MS grade. The mycotoxin standards were purchased from Sigma-Alrdich (St. Louis, MI, USA), RomerLabs (Tulln, Austria) or Fermentek (Tel Aviv, Israel). The stock solutions of solid standards were made either with methanol or acetonitrile, or commercial standard solutions were used. Different standard mix solutions of mycotoxins were prepared for calibrating the UPLC-MS/MS instrument (Waters Acquity UPLC-Xevo TQ MS Milford, MA, USA).

All mycotoxins were extracted according to Huuskonen *et al.* (2018) with some modifications: Caffeine (100 µl, 1,250 ng ml^{-1}) was added as internal standards both in the calibration and test samples, which were extracted in the same way – the sample was first extracted with 10 ml of water and 100 µl strong acetic acid, then with 10 ml of acetonitrile and 100 µl strong acetic acid by a Vortex blender for 1 min. After that, QuEChERS extraction salt (AHO-9041, Phenomenex, Torrance, CA, USA) was added. The tubes were shaken vigorously until the pressure dissipated, then mixed with a Vortex blender for two min and finally centrifuged (2,000 rpm, 5 min). Two different types of calibration samples and test samples were prepared for UPLC-MS/MS: (1) One ml of either calibration or test sample extracts were filtered in vials; (2) 4 ml either calibration or test sample extracts were transferred into dSPE test tubes (5982-5356, Agilent Technologies, Santa Clara, CA, USA), mixed with a Vortex blender for 2 min and centrifuged (2,500 rpm, 5 min). The cleaned-up extract was transferred into test tubes and evaporated with nitrogen until it was dry. The sample residue was dissolved in 0.1% formic acid in water:acetonitrile (1:1) and filtered in vials. The matrix match calibration samples and the test samples were analysed in the same gradient run with the MRM technique by a UPLC-MS/MS instrument (Huuskonen *et al.*, 2018). The MRM-settings, published by Stead *et al.* (2014), were used for the mycotoxins added in the method.

Results and discussion

The caffeine worked well as an internal standard and the method can be used for quantification. Most mycotoxins could be quantified in the filtered extract. Trichothecenes were quantified with concentrated, dSPE cleaned up extracts, but citrinin and fumonisins were lost in it. The limit of quantification (LOQ) varied between determined mycotoxins: MS/MS-responses of mycotoxins varied, and interference signals from the matrix also affected the LOQ. The lowest LOQ 10 µg kg^{-1}, was achieved for enniatins and ochratoxin A, and 13 µg kg^{-1} for beauvericin. The method did not work for patulin, nivalenol and moniliformin, because of detected matrix interferences or weak MS/MS-responses.

The most common detected mycotoxins in the grass silage samples were ROC, MPA and ZEN, but the concentrations were quite low. Trichothecenes were not detected in grass silage samples, but deoxynivalenol (DON) was quantified both in barley and triticale silage samples. The LOQ, the number of positive samples, and the highest detected concentrations are presented in Table 1. Possibly, the low

Table 1. The limit of quantifications (LOQ) of detected mycotoxins roquefortine C (ROC), mycophenolic acid (MPA), zearalenone (ZEN) and deoxynivalenol (DON) with the number of positive samples in parenthesis and the highest concentration (µg kg^{-1}) in different silage batches.[1]

Mycotoxin	LOQ, µg kg^{-1}	54 fresh red clover grass silages	54 aerobically exposed red clover grass silages	18 grass silages	6 barley silages	6 triticale silages
ROC	25	(8), 240	(16), 3,800	(2), 33	(1), <25	(3), <25
MPA	30	(12), 79	(11), 220	(2), 130	nd	nd
ZEN	30	(4), 37	(3), 1,800	nd	nd	nd
DON	130	nd	nd	nd	(6), 370	(6), 510

[1] nd = not detected.

concentrations of enniatins and beauvericin, which have antibiotic features according to Desjardins and Proctor (2007), have prevented mycotoxin production of some fungi in grass silage.

Conclusions

Further method development and validation will be needed to achieve a lower LOQ for mycotoxins. It is also reasonable to make standard mixtures for matrix matched calibration separately with most common and infrequent mycotoxins. Different UPLC-gradients, analytical columns and MS/MS settings should also be tested for better separation and to avoid detected matrix interferences.

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References

Boudra H., Rouillé B., Lyanc B. and Morgavi D.P. (2015) Presence of mycotoxins in sugar beet pulp silage collected in France. *Animal Feed Science and Technology* 205, 131-135.

Cheli F., Campagnoli A. and Dell'Orto V. (2013) Fungal populations and mycotoxins in silages: From occurrence to analysis. *Animal Feed Science and Technology* 183, 1-16.

Desjardins A.E. and Proctor R.H. (2007) Molecular biology of *Fusarium* mycotoxins. *International Journal of Food Microbiology* 119, 47-50.

Driehuis F., Spanjer M.C., Scholten J.M. and Te Giffel M.C. (2008a) Occurrence of mycotoxins in maize, grass and wheat silage for dairy cattle in the Netherlands. *Food Additives and Contaminants* 1, 41-50.

Driehuis F., Spanjer M.C., Scholten J.M. and Te Giffel M.C. (2008b) Occurrence of mycotoxins in feedstuffs of dairy cows and estimation of total dietary intakes. *Journal of Dairy Science* 91, 4261-4271.

Franco M., Stefanski T., Jalava T., Huuskonen A. and Rinne M. (2019) Manipulation of mixed red clover and grass silage quality through compaction, soil contamination and use of additives. In. Udén P., Eriksson T., Spörndly R., Rustas B-O. and Karlsson J. (eds.) *Proceedings of the 10th Nordic Feed Science Conference.* Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, 302, pp. 143-149.

Hietaniemi V., Rämö S., Yli-Mattila T., Jestoi M., Peltonen S., Kartio M., Sieviläinen E., Koivisto T. and Parikka P. (2016) Updated survey of *Fusarium* species in Finnish cereal grains. *Food additives and contaminants: Part A,* 33, 31-838.

Huuskonen A., Rämö S. and Pesonen M. (2018) Effects of regrowth grass silage on feed intake, growth performance and carcass traits of growing bulls. *Agricultural and Food Science* 27, 232-242.

Stead S., Roberts D., Gledhill A., De Rijk T., Van Poucke C. and De Saeger S. (2014) The development of a sensitive multi-residue LC-MS/MS method for the quantitative determination of mycotoxins in animal feedstuffs and silage using Xevo TQ-S. *Waters: Application Note,* 720004961en.

Zachariasova M., Dzuman Z., Veprikova Z., Hajkova K., Jiru M. Vaclavikova M., Zachariasova A., Pospichalova M., Florian M. and Hajslova J. (2014) Occurrence of multiple mycotoxins in European feeding stuffs assessment of dietary intake by farm animals. *Animal Feed Science and Technology* 193, 124-140.

Automated detection of grazing bites by inertial measurement unit is influenced by sward structure

Rossetto J.[1], Da Silva Neto G.F.[1], Andriamandroso A.L.H.[2], Nunes P.A.A.[1], Monteiro I.M.[1], Bindelle J.[3] and Carvalho P.C.F.[1]

[1]Federal University of Rio Grande do Sul, Av. Bento Gonçalves 7712, 91540-000, Brazil; [2]Yncrea Hauts-de-France, ISA Lille, Agriculture Science Department, 48 Boulevard Vauban, 59046 Lille, France; [3]Université de Liège – Gembloux Agro-Bio Tech, Precision Livestock and Nutrition Unit, Passage des Déportés, 5030 Gembloux, Belgium

Abstract

Precision monitoring of the grazing behaviour of ruminants requires the ability to measure what animals are doing at the individual bite level. The aim of this study was to evaluate whether different sward structures can influence the quality of bite detection by the inertial measurement unit (IMU) and IGER methods and if different sward structures are reflected in detected bites attributes. For this purpose, three cows were equipped with an iPhone 4S which records and stores IMU data (accelerometer, gyroscope, and magnetometer) at a frequency of 100 Hz and equipped with an IGER Behaviour Recorder under grazed swards of *Urochloa brizantha* cv. Marandu at three different sward heights: 20, 40 and 60 cm. The different heights influenced in a linear and negative way the detection quality of the bites (Quality Factor = 94.9 – 0.381x, R^2=0.94). The IMU method was superior to the IGER method in the 20 and 60 cm treatments, with no difference in the 40 cm treatment. The detection of bites in the 60 cm swards was the least accurate in both detection methods applied. The results demonstrate that the sward structure influences the detection quality of the grazing. The IMU method was greater than that of IGER for bite detection in the studied sward structure amplitude.

Keywords: grasslands, precision livestock farming, precision grazing management, cattle behaviour, sensor

Introduction

There are currently many tools for monitoring animals. Such tools are the result of technological advances in the last decade and encompass the use of sensors for individual monitoring of animals (monitoring in real time, from aspects related to health and welfare to location and behaviours including ingestive behaviour), wireless communication tools, internet connections and cloud storage (Berckmans, 2015), supporting the concept of precision livestock farming. Grazing is one of the processes involved in ruminant production, and its fine-scale monitoring constitutes a new management perspective for more efficient management of pastoral ecosystems. Recent research to address the challenges of efficient grazing includes the characterization of the vegetal component in pastures (sward height and biomass) using Unmanned Aerial System (UAS) (Michez *et al.*, 2019) and the identification of the ingestive behaviour of grazing animals (Andriamandroso *et al.*, 2017).

For this, it is necessary to use accurate detection tools and with an adequate resolution level for the parameter to be measured. In the case of grazing, the bite is the 'atom' of this process (Laca and Ortega, 1995) and is reflected in the daily forage intake and performance of the animals. Therefore, monitoring of the grazing process must be carried out at the bite scale and with precision. In this context, the aim of this study was to evaluate whether different sward structures can influence the quality of bite detection, as measured by the inertial measurement unit (IMU) and IGER methods, and if the different sward structures are reflected in detected bites attributes.

Materials and methods

The experiment was conducted at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul, in southern Brazil (30° 05'S, 51° 39'W). It consisted of three sward height treatments (20, 40 and 60 cm) in a pasture of palisadegrass (*Urochloa brizantha*) cv. Marandu. The data were collected in May 2018. The treatments were randomly allocated to six paddocks of approximately 500 m^2 each, with two replications of each area.

Two cows (Angus breed) were equipped with an iPhone 4S which records and stores IMU data (accelerometer, gyroscope, and magnetometer) at a frequency of 100 Hz and equipped with an IGER Behaviour Recorder. The bites performed by the animals were observed in twelve videos of approximately 10 min, using the CowLog 3.0.2 program (Pastell, 2016) to compose the observed bites matrix. The bite was considered as the action performed by the cow when she removes the grass from the canopy structure (Gibb, 1998).

To compose the matrix of bites detected through the iPhone, the rotation rate signal along the y-axis (Ry), filtered between 0.5 and 2 Hz, was used. An automated detection of each hypothetical bite, that is, of each peak recorded in the grazing sequences was detected in Matlab R2015b (Mathworks, Natick, Massachusetts), with the criteria of minimum distance between two peaks of 0.4 seconds and minimum height of peaks of 0.2 radian s^1. In the IGER files, the classification of jaw movements in bites or no bites was performed as described by Rutter (2000). It was essential to ensure the correct alignment of the detected bite sequences to compare the methods. Thus, the observed matrix and detected bite in the different methods were synchronized (adjustments in hundredths of a second) to correct the discrepancy between the times indicated by the devices.

To verify the correspondence between a detected bite and an observed bite matrix, the k-neighbour method was used for both detection methods. When an observed bite corresponded to a detected bite it was classified as true positive. All bites observed with a delay greater than 0.5 seconds were classified as false positives or false negatives. The percentage of correctly detected bites, the Branching Factor and the Miss Factor were considered as described by Porto *et al.* (2015). Analysis of variance within each treatment was performed between the bite detection methods for all variables. The means were compared by Student's t test at 5% significance level, using R (R Development Core Team).

Results and discussion

The different sward heights influenced in a linear and negative way the detection quality of the detected bites by IMU (Quality Factor = 94.9 – 0.381x, R^2=0.94). Herbivores respond to structural changes in pasture through grazing strategies, such as intensifying the processes of searching and prehension of forage in pastures managed with low sward height and forage mass. In pastures with greater sward height (e.g. 60 cm), the animals perform more than just one movement of the tongue and jaw per bite as a strategy to increase the bite area, as the leaves are arranged more sparsely.

The methods of detecting the bites within each treatment were not statistically different for the Miss Factor values (Table 1). However, there was a difference for Branching Factor and the percentage of correctly detected bites (QF), showing that regardless of the sward height, the IMU method presented a higher percentage of correctly detected bites. Both methods overestimate the bite rate. However, the IMU method had a lower proportion than the IGER method in the 40 cm treatment.

In the classification of bites by the IGER method, it was observed that chewing events were erroneously classified as bites, resulting in lower accuracy. This is because secondary peaks associated with bites in

Table 1. Comparison between the bite detection methods, inertial measurement unit (IMU) and IGER Behaviour Recorder (IGER) in the correct detection of bites within the different sward heights.[1]

Sward height (cm)	Method	MF	BF	QF	Detected/observed bites
20	IMU	0.07	0.11	85.33	1.04
	IGER	0.09	0.24	75.27	1.14
		P=0.304	P=0.023	P=0.027	P=0.080
40	IMU	0.09	0.14	81.67	1.05
	IGER	0.07	0.28	73.89	1.20
		P=0.635	P=0.018	P=0.072	P=0.011
60	IMU	0.11	0.32	70.11	1.18
	IGER	0.28	0.63	52.52	1.28
		P=0.055	P=0.004	P=0.003	P=0.287

[1] BF = branching factor; MF = miss factor; QF = percentage of bites correctly detected.

cattle are probably being confused as primary peaks of mandibular movements (Rutter, 2000), mainly in the 60 cm sward height treatment that presented a 52.5% Quality factor. At higher heights, the animal performs larger bites that require more time for manipulation and chewing movements.

Conclusions

The results demonstrate that the sward structure influences the detection quality of the grazing. The IMU method was more efficient than the IGER method for bite detection in the studied sward structure amplitude.

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References

Andriamandroso A.L.H., Lebeau F., Beckers Y., Froidmont E., Dufrasne I., Heinesch B. and Bindelle J. (2017) Development of an open-source algorithm based on inertial measurement units (IMU) of a smartphone to detect cattle grass intake and ruminating behaviors. *Computers and Electronics in Agriculture* 139, 126-137.

Berckmans D. (2015) Smart farming for Europe: value creation through precision livestock farming. In: Halachmi I. (ed.) *Precision livestock farming applications: making sense of sensors to support farm management*. Wageningen, the Netherlands: Wageningen Academic Publishers, pp. 25-35.

Gibb M.J. (1998) Animal grazing/intake terminology and definitions. In: Keane M.G. and O'Riordan E.G. (eds.) *Proceedings of pasture ecology and animal intake workshop for concerted action AIR3-CT93-0947*. Dublin. Ireland. pp. 20-35.

Laca E.A. and Ortega I.M. (1995) Integrating foraging mechanisms across spatial and temporal scales. In: West N.E. (ed.) *Rangelands in a sustainable biosphere*. Denver, Society for Range Management, pp. 129-132.

Michez A., Lejeune P., Bauwens S., Herinaina A.A.L., Blaise Y., Castro Muñoz E., Lebeau F. and Bindelle J. (2019) Mapping and monitoring of biomass and grazing in pasture with an unmanned aerial system. *Remote Sensing* 11, 473.

Pastell, M. (2016) CowLog.– Cross-platform application for coding behaviours from video. *Journal of Open Source Software* 4, 1, 15.

Porto S.M.C. Arcidiacono C., Anguzza U. and Cascone G. (2015) The automatic detection of dairy cow feeding and standing behaviours in free-stall barns by a computer vision-based system. *Biosystems Engineering* 133, 46-55.

Rutter S.M. (2000) Graze: a program to analyze recordings of the jaw movements of ruminants. *Behavior Research Methods, Instruments & Computers* 32, 86-92.

At grazing farmers need to be reactive and flexible

Ruelle E.[1] and Delaby L.[2]
[1]*Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland;* [2]*INRA, AgroCampus Ouest, UMR 1348, Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Elevage, 35590 Saint-Gilles, France*

Abstract

Grazing management should be adapted as required, depending on weather and grass growth. To highlight this variability between years, simulations of 6 weather years from Le Pin au Haras (2007 to 2012) was investigated using the Pasture Based Herd Dynamic Milk (PBHDM) model. A 20 hectare farm divided into 10 paddocks with 36 cows was simulated. Each year had the same grazing management rules applied, such as the date on which grazing started, i.e. when one paddock had reached a grass height 8 cm, or an objective pre-grazing height of 10 cm. The only variation in input was the weather that was impacting the grass growth, forcing the model to adapt to different situations. The main results confirm large grass growth variations between years (6.2 to 14.2 Mg ha^{-1}). The amount of grass intake by the cows was on average 3.1 Mg dry matter (DM)$^{-1}$ cow^{-1} (2.1 to 3.5 Mg DM^{-1} cow^{-1}) and the silage fed to the animals during the grazing season was on average 0.4 Mg DM^{-1} cow^{-1} (0.1 to 1.3 Mg DM^{-1} cow^{-1}). The variation in terms of milk yield was 6.2 to 6.7 Mg cow^{-1}. This paper highlights the challenges faced by farmers and the importance for farmers to be capable of timely reactiveness and flexibility in a changing environment.

Keywords: grazing management, grass growth, adaptation, model

Introduction

Changing weather, and indeed climate change, can disrupt farming. Particularly in a grazing situation the farmer needs to anticipate a possible feed deficit or surplus. Daily grass growth is seasonal, highly dependent on weather and soil fertility, and difficult to control. Farmers need to be reactive to their environment and be able to adapt to the challenges put in front of them. To use grass efficiently and match grass demand of the animal with grass growth, the farmer must anticipate changes in grass availability and react quickly by adjusting factors such as daily herbage allowance (or area allocation), supplementation at grazing, and also by closing paddocks for silage (Delaby and Horan, 2017). In this paper we simulated 6 weather years from Le Pin au Haras and looked at the variation in management that was necessary to highlight the challenges faced each year by farmers.

Materials and methods

Simulation of 6 weather years (2007-2012) observed at the INRA experimental farm in Le Pin au Haras (Normandy, France) was conducted using the Pasture Based Herd Dynamic Milk (PBHDM) (Ruelle *et al.*, 2015) model combined with the MoSt grass growth model (Ruelle *et al.*, 2018). The PBHDM simulates a dairy farm at the paddock- and cow-level. The model simulates each individual animal at grazing and is dependent on the animal characteristics, and also on grass availability and quality, with a decrease in animal intake during the defoliation process included in the model. Within the model, grassland management-based decision rules are included to ensure realistic simulations. Animals move from one paddock to another based on a fixed residency time in the paddocks or based on an objective post-grazing sward height, which can be either fixed or dependent on the pre-grazing height. The farm cover is evaluated daily and is compared with the requirement of the cattle. In situations where there is an excess of farm cover on the farm overall, some paddocks can be allocated for silage conservation rather than grazed, and vice versa if paddocks are closed for silage. In the case of grass deficit (subject to the management rules defined by the user), forage or concentrate supplementation can be added to the diet. In the simulations ran for this paper, concentrate was fixed and only forage supplementation could be fed.

The average rainfall of the 6 years simulated was 724 mm, compared to a total rainfall in 2010 of 579 mm, and 913 mm in 2007. The average temperature of the 6 years was 5.0 °C from January to March and 17.3 °C from June to August. Extreme years have been simulated with years like 2010, which had a relatively cold spring (average temperature of 3.3 °C from January to March) or 2008, which had a relatively warm summer (average temperature of 18.5 °C from June to August) or a year like 2007 which had a colder summer (average temperature of 16.2 °C). Apart from the weather the management rules used in the model were the exact same for each simulation. Simulations covered 10 paddocks of 2 ha each and a herd of 36 cows. Starting biomass of each of the paddocks at the beginning of January was set at 300 kg dry matter (DM) ha^{-1}. Grazing commenced as soon as the grass height of one of the paddocks of the farmlet reached 8 cm. The objective pre-grazing height was 10 cm. In case of a deficit in grass the model fed grass silage during the grazing season up to 12 kg DM^{-1} cow^{-1} day^{-1}. In the case of grass surplus the paddocks were cut for silage. Fertiliser was applied after each grazing or silage cutting event at a rate of 30 kg ha^{-1}. This led to the possibility of different fertilisation levels in each year. Concentrate fed was 500 kg DM^{-1} cow^{-1} per lactation and was independent of grazing management or conditions. The variation between years in terms of grass intake, silage fed and first day of grazing was investigated.

Results and discussion

The results are presented in Table 1 and Figure 1. Large variability between years is shown in grass growth with a variation from 6.2 Mg ha^{-1} in 2010 to 14.2 Mg ha^{-1} in 2007. The year 2010 is marked with two important events, a very cold spring with average herbage growth of 4.6 kg DM ha^{-1} day^{-1} from January to March (compared with an average of 6.3 kg DM ha^{-1}) and a very important drought during the summer with average herbage growth of only 30 kg DM ha^{-1} day^{-1} between June and August (average of 55 kg DM ha^{-1} day^{-1} across years) due to the lower rainfall of that year. Years 2007 and 2012 were similar 'good' years with good grass growth during the spring months (over 7 kg DM ha^{-1} day^{-1}) and the absence of drought in the summer (over 68 kg DM ha^{-1} day^{-1}), and still good growth during the winter of over 33 kg DM ha^{-1} (average of 22 kg DM ha^{-1} over the 6 years).

The day of turnout (when the first paddock reached 8 cm pre-grazing height) ranged from 3 April to 22 April, showing a variability of 19 days between the two extreme years. The start of the grazing season was not directly linked to the amount of grass grown during the year. For example, in for both 2010 and 2009 the turnout date was 19 April, due a cold start of the year; however, 5.2 extra Mg DM were grown in 2009 compared with 2010, which led to an extra ton grazed herbage per cow in 2009 (Table 1). This

Table 1. Yearly variability of diet of the cow, milk produced and grazing management.[1]

Year	Start of the grazing season	Fertiliser applied (kg ha^{-1})	Grass growth (kg DM ha^{-1})	Silage harvested (kg DM ha^{-1})	Number of days without any supplementation (days)	Grass intake (kg DM cow^{-1})	Silage fed (kg DM cow^{-1})	Milk produced (kg cow^{-1})
2007	11 Apr	144	14,215	7,428	228	3,515	60	6,751
2008	22 Apr	120	10,264	3,741	205	3,225	195	6,673
2009	19 Apr	144	11,420	5,338	191	3,038	380	6,582
2010	19 Apr	84	6,246	2,012	113	2,053	1,280	6,213
2011	09 Apr	123	10,921	4,759	206	3,142	283	6,443
2012	03 Apr	150	13,488	6,562	216	3,402	223	6,728

[1] DM = dry matter.

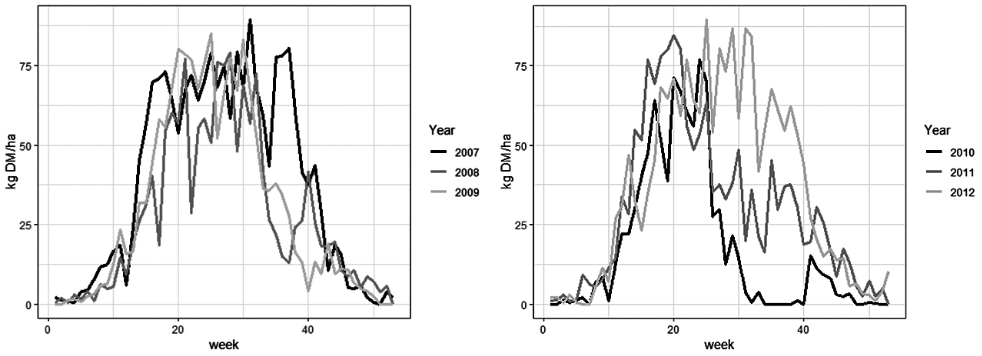

Figure 1. Variation of grass growth between 2007 to 2012 in Le Pin au Haras. DM = dry matter.

shows that even with a similar start of the year the rest of the grazing season could be totally different. The amount of silage which had to be fed during the grazing season (Table 1) ranged from 60 kg DM cow^{-1} in 2007 to 1,280 kg DM cow^{-1} in 2010. This was also reflected in the number of days at grazing without supplementation, 228 days in 2007 and 113 days in 2010. The total grass growth did not always reflect the number of days without supplementation or the amount of silage fed, with a year like 2009 growing 1.2 Mg DM ha^{-1} more than in 2008, this but resulted in feeding 185 kg DM more silage per cow and having 14 fewer days without supplementation. This shows that the amount of grass growth can sometimes be less important than the distribution of the growth. Year 2008 had a higher growth later in the season when the grass growth is normally low (Figure 1); this extra grass at the end of the season is more valuable than extra grass during the main grazing season.

Conclusions

Even with the exact same management rules and site the variation in grass intake, and number of days without silage supplementation was large. This shows the importance of the development of decision support tool around grazing management such as Pâtur'Plan (Delaby *et al.*, 2015) or PastureBase Ireland (Hanrahan *et al.*, 2017), to help the farmer simulate and anticipate grass surplus or deficit and help in his reactivity.

References

Delaby L., Duboc G., Cloet E. and Martinot Y. (2015) Pastur'Plan: a dynamic tool to support grazing management decision making in a rotational grazing system. *Grassland Science in Europe.* 20: 200-2002.

Delaby L. and Horan B. (2017) Improved efficiency in temperate grass based dairy systems. *54. Annual meeting of the Brazilian Society of Animal Science, July 2017, Foz do Iguaçu, Brazil.* 133-145.

Hanrahan, L., Geoghegan A., O'Donovan M., Griffith V., Ruelle E., Wallace M. and Shalloo L. (2017) PastureBase Ireland: a grassland decision support system and national database. *Computers and Electronics in Agriculture.* 136, 193-201. https://doi.org/10.1016/j.compag.2017.01.029

Ruelle, E., Shalloo L., Wallace M., and Delaby L. (2015) Development and evaluation of the pasture-based herd dynamic milk (PBHDM) model for dairy systems. *European Journal of Agronomy* 71, 106-114. https://doi.org/10.1016/j.eja.2015.09.003.

Ruelle, E., Hennessy D., and Delaby L. (2018) Development of the Moorepark St Gilles grass growth model (MoSt GG model): a predictive model for grass growth for pasture based systems. *European Journal of Agronomy* 99, 80-91.

Maize silage as a dairy cow feed in Northern latitudes

Sairanen A. and Kajava S.
Natural Resources Institute Finland (Luke), Maaninka, Finland

Abstract

The experiment examined the effect of partial replacement of high-quality silage by immature maize silage on dry matter (DM) intake (DMI) and milk production of dairy cows. The experiment included 48 dairy cows in a randomized block design for five weeks where the first 2 weeks were used as covariate. The only difference between the treatments was the proportion of maize being 0% in sole grass diet (Grass) and 26% in the forage mixture diet (Maize) on DM basis. The diets consisted of 66% forage, 18% barley, 9% rapeseed meal, 6% commercial concentrate and 1% mineral mixture on DM basis. Replacing the grass silage with the maize silage slightly decreased DMI (24.9 vs 24.2 kg d^{-1}) but had no significant effects on milk yield or milk constituents. Low metabolizable energy (ME) content of the maize silage reduced the energy balance of cows in Maize (-24 MJ ME d^{-1}) compared with Grass (-6 MJ ME d^{-1}). The immature maize silage was equivalent to high quality grass silage in terms of milk production. Strongly negative energy balance of maize diet without decrease in live weight shows that the energy content evaluation of maize is underestimated.

Keywords: maize silage, grass silage, feeding value, dairy cow

Introduction

Maize silage is a new ingredient in the feed ration formulation of dairy cows in Finland. At present the cultivated maize area is only 1000 ha, but is increasing annually. The reason for small cultivated area has been low temperature sum and short growing season in Northern latitudes. Recently, new early maturing maize varieties and the use of plastic film cover during cultivation have improved growth opportunities for maize silage, even in central Finland. Despite this progress, the growth stage of maize usually remains below that of recommendations, and the milk production potential of the ensiled maize is not as high as expected. The aim of this study was to study the possibilities to utilise maize silage in Northern latitudes and assess the milk production potential of northern maize silage compared with high quality grass silage.

Materials and methods

The experimental silages were produced during the growing season of 2018 at the experimental farm of Natural Resources Institute Luke Maaninka. The pre-wilted primary growth grass silage (timothy + meadow fescue, 40:60) was harvested on 12 June and plastic-covered (Samco systems) maize silage (cv P7326 and Ambiente, 75:25) on 2 October. The effective temperate sum was 338 °C for first cut grass silage and 1,600 °C for maize.

The feeding experiment was conducted using 37 Holstein and 11 Nordic red cows (10 primiparous and 38 multiparous cows). Free-stall housed cows averaged 123±65.8 days in milk at the beginning of the experiment. The experimental diets were grass silage (Grass) and the mixture of grass silage (74%) and maize silage (26%, Maize) fed as total mixed ration (TMR). The TMR consisted of 21% concentrate and 10% rapeseed meal, on dry matter (DM) basis. An additional 1.5 kg commercial concentrate was fed with automatic concentrate feeders. The TMR were fed in bins with automatic feed intake measurement. During a 2-wk pretrial period cows were fed a control diet which was a mixture of Grass and Maize (50:50 DM basis). Cows were blocked into three groups according to energy-corrected milk (ECM) and parity at the end of the preliminary period. The treatments were assigned randomly to the cows within a block. The data collected during the last 5 days of the preliminary period was used as a covariate. Cows were switched to the experimental diets for 25 days after the preliminary period and the data from the last 7 days of the experiment were included in the statistical test. A randomised block design with covariates was used to test the treatment differences. The model included covariate, treatment, block and treatment × block interaction.

Results and discussion

Despite an exceptionally warm growing season during 2018, the growth stage of maize was slightly immature according its DM and starch content (Table 1, Khan *et al.*, 2014). The growth stage of grass was early. Both of the experimental silages were preserved well, and aerobic stability was good during the experiment. Maize silage had lower content of crude protein and energy compared with grass silage (Table 1). The differences in nutritive value between the experimental diets were small due to the low proportion of maize in the forage mixture.

The main reason for substituting grass with maize is the expected increase in DMI and consequently in milk production (Khan *et al.*, 2014). The advantageous effect of maize supplementation decreases with increasing quality of substituted silage (Khan *et al.*, 2014). In this study, the early cut grass silage was harvested in good weather conditions and both the digestibility and preservation quality were exceptionally high. There were no differences between Grass and Maize in terms of milk, ECM, fat or protein contents but inclusion of maize slightly decreased intake. Fitzgerald and Murphy (1999) did not observe low DMI due to immature growth stage of maize so the most probable reason for the results in this study is the high quality of the grass silage used as the reference.

Both the metabolizable energy (ME) content and ME intake of Maize was lower compared with Grass. Analysed ME contents were based on pepsin-cellulase solubility of organic matter so the results should be comparable. However, there is no equation available between maize solubility and ME so the equation developed for small-grain whole-crop silages was used. According to milk production the maize silage ME estimation seems to be underestimated.

One reason for maize cultivation is its high forage yield obtained from a single harvest. During 2018 grass yield was 8,000 kg DM ha^{-1} with two cuts, whereas the yield of maize was 12,000 kg DM ha^{-1}. The production costs of plastic film-covered maize were high but the difference of 4,000 kg DM ha^{-1} between the forages resulted in lower production cost per kg DM for maize compared with grass. The production

Table 1. Nutritional values, milk production and feed intake of experimental diets.

		Grass silage	Maize silage	Grass diet	Maize diet
Dry matter (DM), g kg^{-1}		275	288	352	357
	Crude protein, g kg^{-1} DM	143	93	162	152
	Starch, g kg^{-1} DM	0	268	112	161
	Metabolizable protein, g kg^{-1} DM	85	79	94	93
Metabolizable energy (ME), MJ kg^{-1} DM		11.68	10.64	11.80	11.63
		Grass diet	**Maize diet**	**SEM**	***P***
Production, kg	Milk	35.9	35.8	0.36	0.76
	Energy corrected milk	40.8	40.9	0.45	0.85
Intake, kg DM	Silage	15.9	15.7	0.15	0.23
	Concentrate	9.0	8.5	0.09	<0.001
	Total	24.9	24.2	0.22	0.03
	ME, MJ d^{-1}	272	252	2.2	<0.001
ME balance, MJ d^{-1}		-6	-24	2.9	<0.001

cost advantage of maize would disappear if the yield difference between forages had been less than 2,000 kg DM. It has to be kept in mind that temperature sum in this study was 400 °C above the long-term average and the expected maize yield is lower than 10,000 kg DM ha^{-1}. However, the experiment showed that it is possible to achieve reasonable maturity stage of maize even in Central Finland.

Conclusions

The immature maize silage was equivalent to high quality grass silage in terms of milk production. High negative energy balance of maize diet suggests that the energy content evaluation of maize is underestimated when assessed using whole crop equations between *in vitro* cellulase solubility and ME content. The production cost of high yielding maize silage was lower than that of grass silage. The study demonstrated that maize is a reasonable alternative forage, even in Central Finland, when the growing season is favourable.

References

Fitzgerald J.J. and Murphy J.J. (1999) A comparison of low starch maize silage and grass silage and the effect of concentrate supplementation of the forages or inclusion of maize grain with the maize silage on milk production by dairy cows. *Livestock Production Science* 57, 95-111.

Keady T.W.J., Kilpatrick D.J., Mayne C.S. and Gordon F.J. (2008) Effects of replacing grass silage with maize silages, differing in maturity, on performance and potential concentrate sparing effect of dairy cows offered two feed value grass silages. *Livestock Science* 119, 1-11.

Khan N.A., Yu P., Mubarak A., Cone J.W. and Hendriks W.H. (2014) Nutritive value of maize silage in relation to dairy cow performance and milk quality. *Journal of the Science of Food and Agriculture* 95, 238-252.

Nitrogen use efficiency in dairy cows from different diets in north-western Spain

Santiago C., García M.I. and Báez D.
Agricultural Research Center of Mabegondo CIAM-AGACAL, Apto. 10, 15080 A Coruña, Spain

Abstract

This study estimated animal nitrogen use efficiency (NUE) on 19 dairy farms in Galicia (NW Spain) classified by feeding system: ecological and conventional grazing, grass silage (GS), maize and grass silage (MGS) and maize silage (MS). Feed samples were analysed and farmers provided information about milk production and quality and ration supplied to cattle. The proportion of concentrates in diets (21.6% on dry matter basis) and milk yield (17.6 kg milk cow^{-1} day^{-1}) were lowest in the grazing systems. Animal NUE (N secreted in milk per N consumed as feed by lactating cows) was lowest in the ecological grazing system (16.3%), highest in the MS system (34.2%) and intermediate (24.7%) in the other systems. Grazing systems supplied the highest proportion of N through forage, while the other systems supplied higher proportions of N through concentrates (from 52.3% in GS to 69.7% in MS). Considering the high milk yields in GS, MGS and MS and the different dietary origin of N, the GS system was the least dependent on protein intake from feed produced outside the farms. Although animal NUE could be increased on the farms, for a more comprehensive evaluation, the fertiliser/manure NUE and whole farm NUE should also be considered.

Keywords: animal NUE, dairy farm, feeding systems, concentrates, forage

Introduction

Nitrogen use efficiency (NUE) is often used to evaluate how nitrogen (N) inputs are converted into agricultural products and to indicate the environmental risk of N loss. NUE is expressed as the ratio between N outputs and inputs and can be estimated through indices such as crop NUE, animal NUE and whole farm NUE (De Klein *et al.,* 2016). According to De Klein *et al.* (2016), measuring NUE for purposes of optimisation must be based on maximizing N utilisation and minimizing the risk of N loss, without compromising productivity. As a preliminary approach to evaluating the efficiency of N utilisation on livestock farms, in this study we evaluated the animal NUE in dairy systems in Galicia, the main milk producing region in Spain. We estimated N consumption by cows, considering the food sources used in the representative feeding systems for the region (Botana *et al., 2*018).

Materials and methods

Nineteen dairy cow farms were selected in order to represent different feeding systems: ecological grazing (EcoG), conventional grazing (ConvG), grass silage (GS), maize and grass silage (MGS) and maize silage (MS) systems. Between April 2018 and April 2019, the farms were surveyed to determine milk yield, crop management, cattle management and type of feed supplied to cows. Grass intake in the pasture system was estimated through the theoretical dry matter (DM) intake (DMI) and milk yield according to an adapted version of the equation proposed by NRC (2001): DMI = 12 + 0.372 × milk yield. Cows were about 620 kg live weight and at about mid lactation stage. DM and milk yield are expressed in kg cow^{-1} day^{-1} and feeding data were provided by the farmers. In the grazing systems, four feed samples were collected per year. In the more intensive systems (GS, MGS and MS), samples were only collected in spring and autumn because feeding was more homogeneous throughout the year. The percentage of the DM of the feeds was determined in the laboratory, and also the N content using NIR calibrations developed in CIAM (research centre). The proportions of the ingredients of the rations for cows in lactation were determined according to each feeding system, in addition to general parameters: milk yield, stocking rate, agricultural area (AA), DMI and total N consumed cow^{-1} day^{-1} (Table 1). The efficiency of N utilisation was then calculated as the animal NUE = N secreted in milk / N in feed × 100. The data were analysed by one factor ANOVA in Rstudio (2016).

Results and discussion

Of the dairy systems considered, grazing systems used the lowest proportion of concentrates in the diets (21.6% on DM) and also produced the lowest milk yield (17.6 kg milk cow^{-1} day^{-1}). The GM, GS and MGS systems shared characteristics such as relatively high milk production and proportion of concentrates used in the diets, compared to the grazing systems (P<0.001) (Table 1). The animal NUE was lowest in the EcoG system (16.3%) and highest in the MS system (34.2%, P<0.05) (Table 1). In grazing systems it is more difficult to adapt the feed to lactation state and thus achieve a more precise formulation of the ration by optimising the protein and degradable protein contents throughout the year (Powell *et al.*, 2010). In the ConvG, GS and MGS systems, the animal NUE (24.7%) was intermediate between the EcoG and MS systems. However, milk production was similar in the GS, MGS and MG systems (32.7 kg milk cow^{-1} day^{-1}) and significantly higher than in the grazing systems (P<0.001). Furthermore, the mean proportion of concentrates and by-products in the ration were lower in MGS system (40.4%) than in the MS and GS systems (44.9%), although the difference was not significant. The NUE indexes for the systems evaluated in the present study were intermediate between those estimated by other authors for commercial farms (Powell *et al.*, 2006; Roche *et al.*, 2016). The percentage of N supplied by forage (fresh and conserved) relative to the N supplied by concentrates and by-products was higher in grazing systems with N forage: concentrate ratio of 75:25 compared with 37:63 in the other systems. In the more intensive systems, the amount of N in the concentrates and by-products varied from 52.3% in GS system to 69.7% in the MS system (Figure 1). The concentrates used in the GS system supplied less N than those used in the MS system (P<0.001) although a similar proportion of concentrates was included in the ration and N use efficiency was similar.

Table 1. Ingredients of dairy cow diets, milk production data and N use efficiency for milk production (animal NUE) on selected farms, classified by type of feeding system: ecological grazing (Eco-G), conventional grazing (Con-G), grass silage (GS), maize and grass silage (MGS) and maize silage (MS).[1]

	Feeding system					***rsd***	***P*-value**
	EcoG	**ConvG**	**GS**	**MGS**	**MS**		
Number of farms	2	3	4	5	5		
Ingredients from ration (% dry matter):							
Grass	41.4^{b}	39.2^{b}	3.0^{a}	0.0^{a}	0.0^{a}	4.41	***
Grass silage	34.9^{b,c}	23.6^{a,b}	45.5^{c}	27.2^{b}	8.3^{a}	8.15	***
Maize silage	4.1^{a}	5.1^{a}	0.0^{a}	30.7^{b}	43.6^{b}	7.56	***
Dry forage	2.9	5.7	6.4	1.8	3.5	6.29	NS
Concentrates and by-products	16.7^{a}	26.4^{a}	45.2^{b}	40.4^{b}	44.6^{b}	5.46	***
N total (g cow^{-1} day^{-1})	446.0	544.9	576.3	645.4	580.0	74.29	NS
Dry matter intake (kg)	18.5^{a}	20.6^{a}	25.1^{b}	25.8^{b}	26.3^{b}	1.51	***
Milk corrected at 4% fat (kg cow^{-1} day^{-1})	14.4^{a}	20.7^{a}	31.0^{b}	32.0^{b}	35.2^{b}	4.32	***
Animal NUE (%)	16.3^{a}	19.4^{a,b}	28.1^{a,b}	26.7^{a,b}	34.2^{b}	6.17	*
Stocking rate (animal unit ha^{-1})	1.2	1.7	2.0	2.6	2.6	0.64	NS
Agricultural area (ha)	63.1	40.3	49.6	56.0	84.4	45.97	NS

[1] Different letters between numbers in a row implies statistical differences. *** P<0.001, ** P<0.01, * P<0.05, NS = no statistical differences, *rsd* = residual standard deviation.

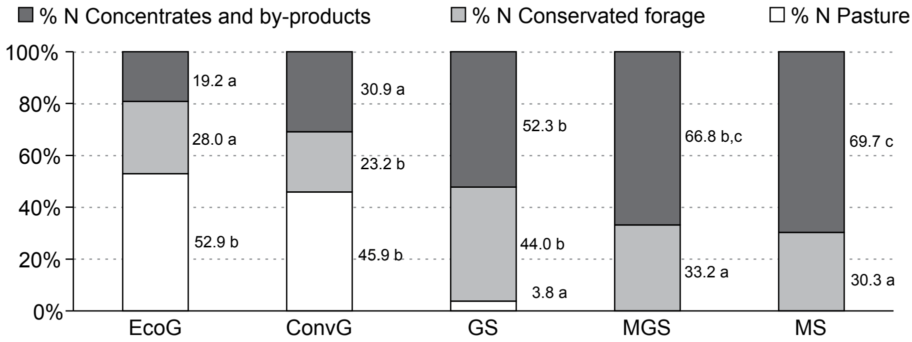

Figure 1. Different N origin in dairy cow diets in different types of feeding systems: ecological grazing (EcoG), conventional grazing (ConvG), grass silage (GS), maize and grass silage (MGS) and maize silage (MS).

Conclusions

Calculation of the animal NUE together with the study of dietary N origin by feed analysis enabled preliminary evaluation of the efficiency of N use in the different systems studied. Comparison of animal NUE revealed acceptable N efficiency on the farms representing the two types of grazing systems. These systems also have the highest N input from forage. Milk production and animal NUE were similar in the GS, MGS and MS systems and higher than in the grazing systems. The N input (from forage) was higher in GS system, and therefore this system was less dependent on protein intake from feeds produced outside the farm. Thus, although the efficiency, in terms of animal NUE in the systems under study, could potentially be increased, other indices such as crop NUE and whole farm NUE should be evaluated.

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References

Botana A., Resch-Zafra C., Pereira Crespo S., Veiga M., González L., Dagnac T., Lorenzana R., Fernández-Lorenzo B. and Flores-Calvete G. (2018) Contrasting diets and milk composition on Galician dairy farms. *Grassland Science in Europe*, 23, 730-732.

De Klein C., Monoghan R.M., Alfaro M., Gourley C., Oenema O. and Powell J.M. (2016) Realistic nitrogen use efficiency goals in dairy production systems: a review and case study examples. *Proceedings of the 2016 Solutions to improve nitrogen use efficiency for the world. International Nitrogen Initiative Conference.*Melbourne, Australia. pp. 4-8.

NRC (2001) Nutrient requirements of dairy cattle. 7th rev. National Academy Press, Washington, DC, USA.

Powell J.M., Gourley C.J.P., Rotz C.A., Weaver D.M. (2010) Nitrogen use efficiency: a potential performance indicator and policy tool for dairy farms. *Environmental Science and Policy* 13, 217-228.

Powell J.M., Jackson-Smith D.B., McCrory D.F., Saam H., Mariola M., (2006) Validation of feed and manure data collected on Wiscosin dairy farms. *American Dairy Science Association* 89: 2268-2278.

Roche J.R., Ledgard S.F., Sprosen M.S., Lindsey S.B., Penno J.W., Horan B., Macdonald K.A., (2016) Increased stocking rate and associated strategic dry-off decision rules reduced the amount of nitrate-N leached under grazing. *Journal of Dairy Science* 99, 5916-5925.

Individual herbage intake estimation of grazing dairy cows, based only on behavioural characteristics

Schori F.[1], Rombach M.[1,2], Münger A.[1] and Südekum K.-H.[2]
[1]*Agroscope, Ruminant Research Unit, 1725 Posieux, Switzerland;* [2]*University of Bonn, Institute of Animal Science, 53115 Bonn, Germany*

Abstract

The objective of the study was to estimate individual herbage dry matter intake (hDMI) of grazing dairy cows based solely on eating and rumination behaviour characteristics as independent variables. Data from four rotational grazing experiments on multi-species pastures were available, including treatments relative to supplementation or herbage mass. Holstein cows of different types (428 to 718 kg body weight and 11.2 to 34.2 kg daily milk production) were grazed around 18 h d^{-1}. At least 105 seven-day measurements of hDMI using the n-alkane double indicator technique constituted the reference data, with an average hDMI of 12.9 kg d^{-1} (4.7 to 20.4 kg d^{-1}). Simultaneously, 27 behavioural characteristics were recorded using the RumiWatch System. For the predictor reduction, the best subset regression approach was applied and equations were validated using bootstrap validation. The following predictors related to grazing were retained in the models: eating time head up or head down, number of other chews, and rumination chews per bolus. Additionally, daily eating time, rumination time and number of other chews were used. Finally, rate of rumination chews were included. The root mean squared prediction errors for the different hDMI estimation models with 4 to 8 predictors were around 1.9 kg d^{-1}.

Keywords: herbage intake, dairy cows, behaviour, pasture

Introduction

The importance of efficient conversion of forage to food through animals is growing in environments where reasonable resource utilisation and significant lowering of emissions play a major role. An efficient conversion of pasture herbage to milk and meat requires a minimization of losses of available biomass at pasture as well as an improved feed efficiency of ruminants. In both cases the knowledge of feed intake is required. The measurement of individual dry matter intake (DMI) for dairy cows kept indoors is already challenging, but for grazing dairy cows it is even more expensive and laborious. Thus, individual herbage DMI (hDMI) measurements are not feasible for a large number of cows under on-farm conditions. As a result, many efforts have been made to indirectly estimate feed intake in general (de Souza *et al.*, 2019) or for grazing dairy cows (Lahart *et al.*, 2019; Rombach *et al.*, 2019).

Although the feeding and rumination behaviour of dairy cows, at first sight, appear promising for intake estimation, a single behavioural characteristic accounts for no more than 35% of hDMI variability (Rombach *et al.*, unpublished data). For a more accurate estimation of individual intake, performance, body size and other characteristics are therefore usually included in equations to estimate total DMI (Lahart *et al.,* 2019) or hDMI (Rombach *et al.,* 2019). The inclusion of performance data in intake estimations may cause interference, because performance (e.g. milk yield) is used in the determination of both input and output. Accordingly, estimation equations independent from output should be developed.

Therefore, in this evaluation, only eating and rumination behaviour data were employed for multivariable regressions for the estimation of hDMI.

Materials and methods

Four rotational grazing experiments on multi-species pastures constituted the data basis, including treatments relative to supplementation (0 to 7.9 kg d^{-1} whole-plant maize silage or 0 to 4 kg d^{-1} concentrate), herbage mass (589 to 2,333 kg DM ha^{-1}) and cow type. Experiments were conducted in western Switzerland in 2014 to 2016, between May and September, and involved a total of 94 dairy cows. Three different types of Holstein cows (body weight, 428 to 718 kg) were grazed around 18 h d^{-1} and produced between 11.2 and 34.2 kg d^{-1} of milk. At least 105 complete seven-day measurements of hDMI (average 12.9 kg d^{-1}, 4.7 to 20.4) with the n-alkane double indicator technique (Rombach *et al.*, 2019) as reference method were available. Behavioural data were collected simultaneously and processed using the RumiWatch System (RWS, Itin & Hoch GmbH, Liestal, Switzerland, Halter V 6.0, Converter 0.7.3.31). The RWS has been validated for grazing dairy cows by Rombach *et al.* (2018, 2019). Behaviour characteristics were averaged either over the day or over the daily length of stay on pasture.

In order to reduce the number of the initially observed 27 behavioural characteristics, the best subset regression approach was applied (R Core Team (2018), package 'leaps'). Finally, a maximum of eight predictors were considered for the hDMI estimation equations. As the sample size was too small to retain an independent validation dataset, the bootstrap cross-validation method was chosen (package 'rms').

Results and discussion

Predictors limited to the daily length of stay on pasture were included, like eating time head up (ETup, 7 to 174 min), eating time head down (ETdown, 348 to 680 min), rumination chews per bolus (RUcb, 37 to 68) and number of other chews (OCnp, 105 to 1,748). On the other hand behavioural characteristics averaged for the whole day were considered, like total eating time (ETtot, 441 to 742 min), number of other chews (OCnd, 189 to 2,816), rumination time (RUT, 303 to 601) and rate of rumination chews (RUrate, 57 to 85 min^{-1}). The root mean squared prediction errors (RMSPE) for the five different hDMI (kg DM d^{-1}) estimation equations with 4 to 8 predictors were similar with 1.93 to 1.89 kg d^{-1}, respectively. For this reason, only the two extreme equations in terms of the number of predictors are presented below:

$$\text{hDMI} = 5.2 + 0.0492 \cdot \text{ETup} + 0.0613 \cdot \text{ETdown} + 0.0017 \cdot \text{OCnp} - 0.0431 \cdot \text{ETtot}$$

$$\begin{aligned}\text{hDMI} = 4.7 &+ 0.0447 \cdot \text{ETup} + 0.0574 \cdot \text{ETdown} + 0.0068 \cdot \text{OCnp} + 0.0121 \cdot \text{RUT} - \\ &0.0507 \cdot \text{RUcb} - 0.06 \cdot \text{RUrate} - 0.0029 \cdot \text{OCnd} - 0.0361 \cdot \text{ETtot}\end{aligned}$$

These RMSPE correspond to a relative prediction error of around 15%. At best and depending on the approach chosen, Rombach *et al.* (2019) obtained a relative prediction error of 11 to 13%. A slightly lower relative prediction error of approximately 10% was reported by Lahart *et al.* (2019). Although error terms less than 10% would be desirable, these values are difficult to achieve and, depending on the objective, up to 20% can be tolerated. In contrast to Lahart *et al.* (2019), who determined total DMI (sum of hDMI and the amount of supplements fed in the barn), the present evaluation as well as Rombach *et al.* (2019) estimated hDMI.

Standardised coefficients (β) allow comparison of the weights of variables in multiple regressions. In both equations above, ETdown reached the highest β (1.24 or 1.70), followed by OCnp (equation with 8 predictors, 0.97) and ETtot (equation with 4 predictors, -0.87). The negative weight of ETtot is likely due to the supplements – maize silage and concentrates – eaten in the barn, which usually have a substitution effect. The significant weight of OCnp may be related to the individual variation in grazing behaviour or to incorrect classifications of chews by the RWS. Presumably, during ETup at pasture, dairy cows mainly process and swallow the grazed herbage. Therefore, even though ETdown and ETup are not sharply separated as cows may repeatedly raise or lower their head during a grazing bout, they are sufficiently independent to be kept in the equations. The inclusion of rumination characteristics like RUT, RUcb and RUrate in the estimation equations can be attributed to the influence of fibre and physical properties of the ration.

Intake estimation exclusively based on behavioural characteristics has the advantage that the input for subsequent efficiency calculations is not calculated on the base of energy sinks, like milk yield, growth or body weight. Further, the inclusion of behavioural characteristics may be beneficial if individual hDMI is impaired, caused for example by health problems, injuries, heat, and other unusual circumstances such as those produced by poor grazing management practice. Of course, the intake estimation should be more precise, accurate and supported by a broader database for individual feed efficiency evaluations.

Conclusions

One eating or rumination behaviour characteristic alone was not sufficient to estimate individual hDMI accurately, but the appropriate combination of several behavioural characteristics reduced the error term to around 15%. An hDMI estimation based exclusively on easily recorded behavioural characteristics has the benefit of an output independent input estimation for subsequent feed efficiency calculations. Other benefits would be the reduced need for measurements related to cows or grazed herbage and the possibility to automate hDMI estimation in future.

References

De Souza R.A., Tempelman R.J., Allen M.S. and VandeHaar M.J. (2019) Updating predictions of dry matter intake of lactating dairy cows. *Journal of Dairy Science* 102, 7948-7960.

Lahart B., McParland D., Kennedy E., Boland T.M., Condon T., Williams M., Galvin N., McCarthy B. and Buckley F. (2019) Predicting the dry matter intake of grazing dairy cows using infrared reflectance spectroscopy analysis. *Journal of Dairy Science* 102, 8907-8918.

Rombach M., Münger A., Niederhauser J., Südekum K.-H. and Schori F. (2018) Evaluation and validation of an automatic jaw movement recorder (RumiWatch) for ingestive and rumination behaviors of dairy cows during grazing and supplementation. *Journal of Dairy Science* 101, 2463-2475.

Rombach M., Südekum K.-H., Münger A. and Schori F. (2019) Herbage dry matter intake estimation of grazing dairy cows based on animal, behavioural, environmental, and feed variables. *Journal of Dairy Science* 102, 2985-2999.

R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.

The effect of fertilisation on the yield and nutritive value of organic lucerne-grass pastures

Tamm U., Meripõld H., Tamm S., Tamm S. and Loide V.
Estonian Crop Research Institute, EE-48309 Jõgeva, Estonia

Abstract

The natural fertility of soils in Estonia provides 1.7-2.3 Mg ha^{-1} of dry matter (DM) with insufficient nutritional value in organic pastures. The majority of forage is produced in the first half of summer. The aim of this study was to examine the effect of manure compost and organic fertilisers on the lucerne-grass pasture to improve forage production and quality by including lucerne in grass-based mixtures. The trials consisted of lucerne (*Medicago sativa*) cv. Artemis and two hybrid lucerne (*Medicago varia* Mart.) cv. Juurlu and cv. Karlu, in mixture with meadow fescue, timothy and meadow grass, three replicates each. The trials were carried out with two treatments: (A) fertiliser Biocat G ('Black pearl') at 420 kg ha^{-1} and fertiliser Kainit at 300 kg ha^{-1}; and (B) 21 Mg ha^{-1} of composted cattle manure. The average DM yields over three years was significantly higher in treatment A (3.9-5.6 Mg DM ha^{-1}). The DM yield of lucerne cv. Karlu with treatment A was exceptionally high (8.89 Mg ha^{-1}) in 2019. The crude protein concentration varied from 87 to 173 g kg^{-1} and metabolizable energy was over 10.5 MJ kg^{-1} DM.

Keywords: lucerne-grass pastures, forage yield, forage nutritive value

Introduction

An optimal combination of suitable grass and legume companion species is needed to obtain high N-use efficiency, high herbage yield and high contents of nutritive compounds in grass-legume mixtures (Elgersma and Søegaard, 2015). Legumes offer important opportunities for sustainable grassland-based livestock production systems (Lüscher *et al.*, 2014). Physiological maturity of lucerne at the time of harvest greatly influences nutritive value of forage (Frame, 2005; Tamm *et al.*, 2011). Hybrid lucerne cv. Karlu and cv. Juurlu show good winter hardiness up to 60°49' N latitude in Finland (Mela *et al.*, 1996). In order to balance the need for nitrogen fertiliser with that from biological nitrogen fixation, it is considered advantageous to grow lucerne in a mixture with grasses. When choosing legumes for lucerne-grass mixtures, the rate of phenological development of the species, persistency and nutritive value should be considered. Earlier results have shown that cultivating lucerne in mixtures with grasses improves the nutritive value and ensiling properties of the crop (Tamm, 2017). The aim of this study was to examine the effect of manure compost and organic fertilisers on the lucerne-grass pasture to improve forage production and quality by including lucerne in grass-based mixtures.

Materials and methods

An experimental field was established in 2016 at Kuusiku Experimental Centre, Estonia (l58°58' N; 24°43' E). The study included data from three years (2017-2019). The trial plots were established on a typical soddy-calcareous soil where the agrochemical indicators were as follows: pH_{KCl} 7.1 (ISO 10390); soil carbon content C_{org} 1.9% (Tyurin method), and concentration of lactate soluble phosphorus (P) and potassium (K) 91 and 95 mg kg^{-1} (Mehlich III method), respectively. An organic fertiliser Biocat G (Black pearl) at 423 kg ha^{-1}, and 300 kg ha^{-1} of fertiliser Kainit (treatment A) were applied after the first cut. The nutrient content in Biocat G was N 42, K 17, S 14 kg ha^{-1}, and in Kainit K 27, Mg 9, S 12, Na 60 kg ha^{-1}. The other three applications were 21 Mg ha^{-1} composted cattle manure. The nutrient content in the manure was N 4, P 23, K 151 kg ha^{-1} and dru matter (DM) was 20.3% (treatment B). The sowing rate of lucerne cv. Artemis *(Ms)*, cv. Karlu (*MvM*) and cv. Juurlu (*MvM*) was 12 kg ha^{-1}, and among the grasses, meadow fescue *(Festuca pratensis* Huds.*)*, timothy *(Phleum pratense* L.) and meadow grass

(Poa pratensis L.) these three grass species together were sown at 20 kg ha^{-1}. The trials were established as a split-plot design in three replicates and the size of the harvested plot was 10.5 m^2. The crop was cut with a forage harvester (Hege 212), and three replicates of samples were taken for analyses. A three-cut system was used. The first cuts were taken between 31 May and 3 June (lucerne budding, grasses shooting), the second cuts were taken in mid-July and the third cuts were taken in early September. Effective temperatures over 5 °C for April – September were 1,246 °C in 2017, 1,754 °C in 2018 and 1,459 °C in 2019. Rainfall in April – September was 488 mm in 2017, 319 mm in 2018 and 412 mm in 2019. The following data were collected in this experiment: DM yield, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), metabolisable energy (ME) content and digestible dry matter (DDM). Statistical analyses (ANOVA and Fisher´s LSD) were carried out by Agrobase 20™.

Results and discussion

The average DM yield in three years was significantly higher in treatment A (3.90-5.64 Mg ha^{-1}). The DM yield of lucerne cv. Karlu with treatment A was exceptionally high at 8.89 Mg ha^{-1} in 2019 (Table 1). Effect of the organic fertilisers was remarkable, especially for lucerne cv. Karlu. The average DM yields in the three years for treatment (A) with organic fertiliser Biocat G and Kainit was 5.64 Mg ha^{-1} for cv. Karlu, 4.87 Mg ha^{-1} for cv. Juurlu and 3.90 Mg ha^{-1} for cv. Artemis. In the treatment with composted cattle manure (treatment B), the DM yield was 1.97 Mg ha^{-1} for Karlu, 1.89 Mg ha^{-1} for Artemis and 1.70 Mg ha^{-1} for Juurlu.

By the year 2019 the pasture of treatment A was tight and full of lucerne. The forage DM yield of treatment A on cuts I and II, respectively, consisted of lucerne proportions: Artemis 58 and 68%, Karlu 63 and 67%, Juurlu 55 and 55%. The forage DM yield of treatment B on cuts I and II consisted of lucerne proportions: Artemis 22 and 48%, Karlu 40 and 58%, Juurlu 28 and 38%. Juurlu was the least competitive compared with other varieties. The fertilisation in treatment (A) on lucerne-grass pastures greatly increased the content of CP and decreased the NDF compared with treatment (B) in cuts I and II. The CP content in DM in treatment (A), cut I was 128-140 g kg^{-1} and in cut II 126-173 g kg^{-1}, which were greater than in treatment (B_. The NDF content in DM of treatment (A) was 487-497 g kg^{-1} in cut I and 421-477 g kg^{-1}, cut II) and significantly less than the NDF in treatment (B) at 528-548 g kg^{-1} in cut I and 500-544 g kg^{-1} in cut II. The lucerne-grass pastures all had high DDM (652-671 g kg^{-1} DM) cut I and II. There was no significant difference in ME and DDM between the treatments A and B (Table 2).

Table 1. The dry matter yields (Mg ha^{-1}) of lucerne-grass pastures in 2017-2019.[1]

Cultivar	Treatment	2017	2018	2019	Average
Artemis	A	2.86^a	3.15^b	5.7^b	3.90^c
	B	1.83^b	2.05^c	1.8c	1.89^d
Karlu	A	3.31^a	4.71^a	8.89^a	5.64^a
	B	2.01^b	2.01^c	1.89^c	1.97^d
Juurlu	A	3.22^a	3.07^b	8.33^a	4.87^b
	B	1.75^b	2.02^c	1.34^c	1.70^d
LSD95%		0.71	0.59	0.95	0.49

[1] Different lowercase letters within a column are significantly different (*P*<0.05; ANOVA, Fisher LSD test).

Table 2. The nutritive value of the lucerne *(Ms)* Artemis, *(MvM)* Karlu and *(MvM)* Juurlu lucerne-grass pastures averaged over 2017 to 2019.[1]

Cultivar	Treatment	First cut				Second cut			
		CP	NDF	ME	DDM	CP	NDF	ME	DDM
(*Ms*) Artemis	A	130^{a}	497^{a}	10.2^{a}	658^{a}	126^{c}	477^{b}	10.3^{a}	649^{b}
	B	87^{b}	548^{b}	10.1^{a}	652^{a}	87^{e}	544^{a}	10.0^{a}	648^{b}
(*MvM*) Karlu	A	140^{a}	487^{a}	10.2^{a}	659^{a}	173^{a}	421^{d}	10.3^{a}	671^{a}
	B	95^{b}	528^{b}	10.1^{a}	654^{a}	103^{d}	500^{b}	10.3^{a}	663^{ab}
(*MvM*) Juurlu	A	128^{a}	495^{a}	10.2^{a}	658^{a}	147^{b}	445^{c}	10.3^{a}	669^{a}
	B	88^{b}	538^{b}	10.1^{a}	654^{a}	92^{de}	531^{a}	10.0^{a}	657^{ab}

[1] Crude protein (CP), neutral detergent fibre (NDF), digestible dry matter (DDM) are expressed in g kg^{-1} dry matter; metabolizable energy (ME) in MJ kg^{-1} DM.

Conclusions

Comparing the DM yield, the lucerne-grass pasture with treatment A (Biocat G ('Black pearl') and Kainit) significantly exceeded that of lucerne-grass pasture with treatment B (composted cattle manure) over the three experimental years. With an accurately timed cut I (when lucerne is budding and grasses are shooting) we managed to harvest forage with high metabolizable energy and digestible dry matter content from both pastures for both treatments A and B. The lucerne cv. Karlu-grasses mixture had the highest yield during all three years of the experiment. There was no significant difference between ME values in treatment A and treatment B in the first and second cuts.

References

Elgersma A. and Søegaard K. (2015) Productivity and herbage quality in two-species grass-legume mixtures under cutting. Grassland and forages in high output dairy farming systems. *Grassland Science in Europe*, 20, 401-403.

Frame J. (2005) *Forage Legumes for Temperate Grasslands.* Plymouth,UK: FAO/ Science Publishers Inc ISBNs 92-5-105043-0, pp 101-115.

Lüscher A., Mueller-Harvey I., Soussana J.F., Rees R.M. and Peyraud J.L (2014) Potential of legume-based grassland-livestock systems in Europe: a review. *Grass Forage Science* 69(2), 206-228.

Mela T., Sormunen-Cristian R. and Niskanen V. (1996) Experiences of the yellow-flowered lucerne (*Medicago falcata* L.) in Finland. *Grassland Science in Europe*, 1, 515-519.

Tamm U., Lättemäe P. and Tamm S. (2011) Estonian Research Institute of Agriculture. Lutserni segukülvi botaanilise koosseisu mõju rohusööda kvaliteedile. The effect of botanical composition of lucerne-grass mixture on nutritive value of forage. – *Agronomy 2010/2011*, Tartu, Estonia, pp. 133-138.

Tamm U. (2017) *Parema toiteväärtusega rohusööt.* Improved nutritional value of forage. http://taim.etki.ee/taim/public/pdf/Trukised/Parema-toitevrtusega-rohust-Tamm.pdf (in Estonian).

Application of the new German protein evaluation system for horses in forage from species-rich meadows

Tüscher T.[1], Vervuert I.[2], Reidy B.[1] and Ineichen S.[1]
[1]*School of Agricultural, Forest and Food Sciences HAFL, Bern University of Applied Sciences, Zollikofen, Switzerland;* [2]*Faculty of Veterinary Medicine, University of Leipzig, Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Leipzig, Germany*

Abstract

The German protein evaluation system for horses is based on the pre-caecal digestible crude protein (pcdCP). Forage contents of pcdCP are available for grass-based swards, while those from species-rich swards are scarce. Grass species are low in tannin contents and interactions during protein digestion are unlikely. However, this may be relevant in forage from species-rich grasslands containing tanniferous forage species. Therefore, contents of pcdCP were quantified in fresh, wilted or ensiled forages from species-rich mountain swards and correlations with the content of condensed tannins (CT) were determined. The forages were obtained from three long-term fertiliser experiments (unfertilised or fertilised with PK or NPK) located at different mountainous sites. Forages were characterized for crude nutrient contents and CT. Ammonia content was additionally analysed in ensiled forages. Average contents of pcdCP (per kg dry matter, DM) accounted for 51.3 g, 80.5 and 52.9 g for fresh, wilted and ensiled forage, respectively. Contents of pcdCP were positively correlated with CT contents; however, this correlation was moderate for forages of the first harvest (R^2=0.344) and weak (R^2=0.085) for forages of the subsequent harvests. The new German protein evaluation system for horses is promising for the prediction of contents of pcdCP adequately in forage from species-rich swards.

Keywords: condensed tannins, equine nutrition, mountain grassland, pre-caecal digestible crude protein, species-rich swards

Introduction

The German protein evaluation system determines the pcdCP content of feeds based on neutral detergent soluble crude protein (NDSCP) with corrections made for silages considering the ammonia-nitrogen (N) content (Kirchhof and Rodehutscord, 2011). This calculation method is primarily based on swards composed of grass or legume species (Meyer and Coenen, 2014), rather than forages from species-rich swards. The latter may contain substantial amounts of CT, which may interact in protein digestion and therefore influence the content of pcdCP (Waghorn, 2008). It is therefore unclear whether the German protein evaluation system can also adequately predict pcdCP contents in forages with moderate CT contents, as in the case of forage from species-rich mountain grasslands.

Materials and methods

The forage samples originated from three different long-term mineral-fertilisation field experiments located at elevations of 930 m a.s.l. (Thomet and Koch, 1993), 1,190 m a.s.l. (Carlen *et al.*, 1998) and 13,40 m a.s.l. (Baumberger *et al.*, 1996) in the Swiss mountains. Swards were either unfertilised (O) or fertilised with phosphorus (P) and potassium (K) PK or NPK and sampled in three or four plots at each site. Due to long-term mineral fertilisation of the mountain grasslands, species-rich swards had been established (Ineichen, 2018). Forage samples including gross nutrient composition, contents of CT and, in the case of silage samples, ammonia (NH_3) concentrations were also available from previous investigations for fresh forage (Ineichen, 2018), wilted and ensiled forage (Seiler, 2018). To determine contents of pcdCP in forages, neutral detergent insoluble crude protein (NDICP) was analysed according to Licitra *et al.* (1996) using an ANKOM fibre analyzer. Prior to analysis, samples were milled on a Retsch mill (MM440) for 35 sec at a frequency of 30/sec and a sample of 0.5 g was weighed in an ANKOM fibre bags in duplicates (F57). Chemical analysis of NDICP followed the protocol by the 'Gesellschaft für Analysetechnik' HLS (2016). Nitrogen content in the NDICP fraction was determined using a C/N-analyser. Contents of pcdCP (g kg^{-1} DM) forage were determined using the following calculations (Kirchhof and Rodehutscord, 2011):

- pcdCP = [CP – NDICP] × 0.9 in fresh and wilted forage
- pcdCP = [CP – NDICP – NH_3-N × 6.25] × 0.9 in ensiled forage.

Analysis of variance was conducted including 'type of fertilisation, *f*' within the first and subsequent harvest using the software (NSCC V9, 2013). Tables display arithmetic means in relation to type of fertilisation and harvest and differences are considered significant at $P<0.05$. Correlations between the content of pcdCP and CT were made for pooled date off all forage samples of the first harvest and the subsequent harvests separately, irrespective of type of conservation of fertilisation.

Results and discussion

On average, contents of pcdCP were 61.6 g kg^{-1} DM for swards of species-rich mountain grasslands (Table 1) and were similar to those reported by Meyer and Coenen (2014) for grass-based forage. From the first to the second harvest, contents of pcdCP increased by +13.2 g kg^{-1} DM in fresh and +5.0 g kg^{-1} DM in wilted foraged and decreased by -1.8 g kg^{-1} DM in ensiled forage. Contents of CP were moderate and ranged from 119 to 133 g kg^{-1} DM, which was also reflected by rather high fibre contents. Within each harvest and type of forage conservation, sward long-term fertilisation for >30 years at each site had little influence on pcdCP, CP or NDF contents.

Contents of CT ranged from 0 g to 19 g kg^{-1} DM with, on average, lower concentrations in the first (6.26±4.01 g kg^{-1} DM; Figure 1A) than in the subsequent harvests (7.38±5.34 g kg^{-1} DM; Figure 1B). The correlations between CT (g kg^{-1} DM) and pcdCP (g kg^{-1} DM) were weak in both the first and subsequent harvests, although positively correlated ($P<0.05$).

- pcdCP = 41.7 + 2.38 3 CT, R^2 = 0.344, n=69 (first harvest, Figure 1A)
- pcdCP = 56.1 + 0.96 3 CT, R^2 = 0.085, n=83 (subsequent harvests, Figure 1B)

Table 1. Contents of pre-caecal digestible crude protein (pcdCP), crude protein (CP) and neutral detergent fibre (NDF) from forage of species-rich mountain grassland swards.[1]

		First harvest					Following harvests				
		0	PK	NPK	SEM	*f*	0	PK	NPK	SEM	*f*
Fresh forage, g kg^{-1} DM	pdcCP	44.3	45.0	44.9	1.86	ns	51.2	53.5	68.2	2.91	ns
	CP	111	108	94.7	3.31	*	130	136	133	2.3	ns
	NDF	488	498	531	5.8	ns	380	385	405	7.5	ns
Wilted forage, g kg^{-1} DM	pdcCP	72.3	82.6	78.9	1.86	ns	74.0	84.2	90.7	3.57	ns
	CP	124	126	115	1.8	ns	125	134	136	3.5	ns
	NDF	505	478	507	12.0	ns	477	425	441	10.5	ns
Ensiled forage, g kg^{-1} DM	pdcCP	58.1	52.8	50.6	2.18	ns	50.2	56.1	49.7	1.98	ns
	CP	129	128	120	2.0	ns	121	131	127	4.0	ns
	NDF	542	501	517	11.1	ns	481	427	428	11.0	ns

[1] Type of fertilisation: unfertilised swards (0), PK or NPK; *f* = fertilisation; SEM = standard error of means; ns = not significant; * $P<0.05$.

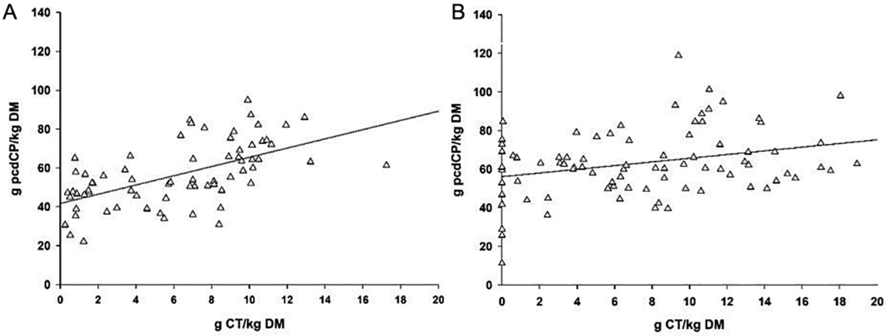

Figure 1. Correlations (P<0.05) of pre-caecal digestible crude protein (pcdCP) and contents of condensed tannins (CT) (g kg^{-1} dry matter, DM) in forage samples from species-rich mountain grasslands for the first harvest (A) or the subsequent harvests (B).

Conclusions

Correlations between CT and pcdCP were moderate in the forage investigated with CT contents below 20 g kg^{-1} DM across harvests. A consideration of the CT content in the calculation of pcdCP contents is therefore unlikely. The new German protein evaluation system for horses appears therefore appropriate for predicting contents of pcdCP adequately in forage from swards rich in herbs and also legume species.

References

Baumberger, C., Koch B., Thomet P., Christ H. and Gex P. (1996) Long-term effects of fertilisation on the botanical composition of an alpine pasture. *Agrarforschung* 3, 275-278.

Carlen C., Darbellay C. and Gex, P. (1998). Long-term effects of fertilisation and cutting frequency on a permanent meadow in mountain regions. *Revue Suisse d'Agriculture* 30, 215-221.

Gesellschaft für Analysetechnik HLS (2016) Neutral detergent fiber (NDF) in Futtermiteln. 'FilterBag'-Technik (ANKOM^{A220}).

Ineichen S. (2018). *Suitability of forage stands with contrasting species richness to improve the nitrogen use efficiency and the milk fatty acid profile of dairy cows*. Doctoral thesis, ETH Zurich, Switzerland. https://doi.org/10.3929/ethz-b-000304897.

Kirchhof S. and Rodehutscord M. (2011) *Erarbeitung von Versorgungsempfehlungen für Pferde und Schafe mit Energie und Nährstoffen sowie Überarbeitung der Schätzformeln für den energetischen Wert von Futtermitteln*. Available at: https://service.ble.de/ptdb/index2.php?de-tail_id=15345&site_key=145&stichw=2&zeilenzahl_zaehler=590&NextRow=200.

Licitra G., Hernandez T.M. and van Soest P.J. (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology* 57, 347-358.

Meyer H. and Coenen M. (2014). Pferdefütterung (5^{th} edition). Enke, Stuttgart, p. 332.

Seiler A. (2018) *Siliereignung artenreicher Pflanzenbestände im Berggebiet. Einfluss von Tannin auf den Proteinabbau*. Bachelor thesis. School of Agricultural, Forest and Food Sciences HAFL, Zollikofen, Switzerland. 75 p.

Thomet P. and Koch B. (1993) Long-term effects of fertilisation and cutting regime on a hay meadow. *Landwirtschaft Schweiz* 6, 107-114.

Waghorn G. (2008) Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production - progress and challenges. *Animal Feed Science and Technology*, 147, 116-139.

Nutritive value of tall fescue (*Festuca arundinacea* Schreb.) silage under practical conditions

Vanden Nest T. and De Vliegher A.
Flanders Research Institute for Agriculture, Fisheries and Food, Merelbeke, Belgium

Abstract

Tall fescue (*Festuca arundinacea* Schreb.) is gaining interest among Belgian dairy farmers due to its higher drought resistance and expected higher dry matter yield compared with perennial ryegrass (*Lolium perenne* L.). Digestibility of tall fescue under fresh conditions is lower than that of perennial ryegrass; however knowledge of digestibility of ensiled tall fescue is sparse, which makes it hard for farmers to assess the impact of replacing perennial ryegrass with tall fescue in part of their grasslands. We have started a small-scale experiment at farm level to test the digestibility of ensiled tall fescue. The management of one monoculture of both grasses in 2017 and 2018 was studied on three farms. Fresh grass samples were taken immediately before every cut, and immediately before the grass was ensiled samples of perennial ryegrass and tall fescue were ensiled in small containers. Digestibility, ash, crude fibre and crude protein content were determined by NIRS and parameters were recalculated to provided *in vivo* digestibility. The results revealed that in 2017 differences in digestibility between fresh tall fescue and fresh perennial ryegrass disappeared after ensiling, but not in 2018. There is no clear explanation for the between-years difference for this effect of the silage process.

Keywords: tall fescue, perennial ryegrass, silage, digestibility, on-farm conditions

Introduction

Many Belgian dairy farms cultivate perennial ryegrass under intensive fertilising conditions and intensive mowing (>5 cuts per year) for silage. Perennial ryegrass is preferred due to its high digestibility and high productivity under Belgian temperate maritime climate conditions. Recent extremely dry summers have led to severe production losses and damage to the ryegrass swards. The change in weather has stimulated some farmers to consider experimenting with alternative crops and alternative grass species, including tall fescue. Tall fescue is well-known for its deeper root system, higher drought tolerance, higher dry matter yields, but for lower digestibility and lower palatability compared to perennial ryegrass (Cougnon *et al.*, 2014). Farmers that started experimenting with tall fescue mostly sowed one or two parcels with tall fescue as a test crop; however, as all mown grasslands are managed and ensiled together those farmers were unable to observe differences in nutrient value and digestibility. The objective of this study was to determine the differences in nutritive value of tall fescue, compared with perennial ryegrass in both fresh herbage and silage products under farm conditions. This information can help farmers in their decision making with regard to choice of grass species, and the relative percentages of their grassland acreage.

Materials and methods

This experiment was carried out on three dairy farms on sandy soils in the Belgian province of East Flanders in 2017 and 2018. On each farm two mown grasslands (aged >1 year after sowing) and located near each other, were selected, one with perennial ryegrass and one with tall fescue. The farmers were allowed to choose their own approach to fertiliser application (amount and time) and harvest (number of cuts and time of cutting) but were required to fertilise and harvest the two fields in exactly the same way and on the same dates. On all three farms, only 4 cuts were taken in 2017 and 3 cuts in 2018 due to the extremely dry conditions in both years. Normally 5-6 cuts are taken per year. Immediately before every cut, fresh grass samples were cut by hand with scissors (3 replicates per field, ca. 2 kg fresh matter per sample). After 2 days of wilting, grass samples (2 replicates per field, grab samples distributed over the whole grassland) were placed in Bokashi containers (15 l, as much grass as could compressed by stepping on the sample in the container), which are used for air-free storage of organic material. After 12 weeks in these containers, the resulting silage was sampled. Fresh forage samples and silage samples were dried at 70 °C for 48 hours to analyse dry matter (DM) and were analysed using near infrared spectrometry (NIRS) to determine ash, DM%, crude fibre (CF), crude protein (CP), and organic matter digestibility (OMD) (De Boever *et al.*, 1988). These parameters were used to predict the net energy for lactation (VEM) according to Van Es (1977); the true protein digested in the small intestine (DVE) and rumen degraded protein balance (OEB) of the fresh forage and silage according to Tamminga *et al.* (1994) using in-house developed regression equations (De Boever, personal communication). The effect of grass species on VEM, DVE and OEB was analysed by ANOVA (Statistica 13.0 package) with factors 'grass species', 'farm', 'cut' and all two-way interactions. The three-way interactions were not significant. The assumptions of normality and homoscedasticity were fulfilled.

Results and discussion

Both VEM and DVE were significantly higher for fresh perennial ryegrass compared to tall fescue (Figure 1). However, in 2017 after 12 weeks of ensiling, differences in VEM and DVE between both grass species diminished and were no longer significant. In 2018, the process of ensiling did not close the gap in VEM and DVE between the two grass species. We observed no differences in OEB between tall fescue and perennial ryegrass in the fresh grass (average OEB = 54 g kg^{-1} DM) and the ensiled grass of 2017 (average OEB = 43 g kg^{-1} DM). The OEB of ensiled tall fescue was significantly lower (60 g kg^{-1} DM) compared with that of ensiled perennial ryegrass (80 g kg^{-1} DM).

VEM, DVE and OEB were calculated based on the chemical composition of the fodder (Table 1). The ash content was lower for tall fescue than perennial ryegrass in both the fresh herbage and silage products. Except for the silage product in 2017, CF was higher for tall fescue than for perennial ryegrass. The OMD for fresh grass and the silage product was in both cases lower for tall fescue than for perennial ryegrass in both years. It seems that the difference in OMD between the two grass species was smaller in 2017 and larger in 2018, which is in accordance to the VEM and DVE.

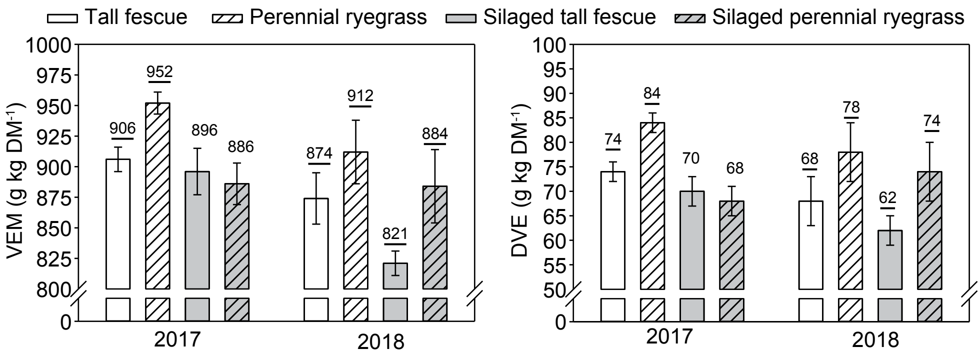

Figure 1. The mean net lactation energy (VEM) (left) and of the mean the true protein digested in the small intestine (DVE) (right) for all three farms and all cuts together in both experimental years. The errors bars refer to standard errors. Significant differences between tall fescue and perennial ryegrass are underlined.

Table 1. The mean composition (± standard error) of the fresh and silage products of both grass species for all three farms and all cuts together.[1]

Year	Product	Grass species	DM, g kg^{-1}	Ash, g kg^{-1} DM	CF, g kg^{-1} DM	CP, g kg^{-1} DM	OMD
2017	Fresh	Tall fescue	236±15	93±3	250±5	191±6	76±1
		Perennial ryegrass	214±13	97±3	231±4	191±6	79±1
		Difference	+22	-4	+19	-	-3
	Silage	Tall fescue	361±41	104±6	276±9	198±9	76±1
		Perennial ryegrass	332±45	117±8	288±9	189±8	77±1
		Difference	+29	-13	-12	+9	-1
2018	Fresh	Tall fescue	182±20	111±7	255±11	194±11	75±1
		Perennial ryegrass	160±13	122±6	243±13	195±11	78±2
		Difference	+22	-11	+12	-1	-3
	Silage	Tall fescue	435±37	113±6	304±12	203±12	72±1
		Perennial ryegrass	385±35	125±3	277±16	224±15	77±2
		Difference	+50	-12	+24	-21	-5

[1] DM = dry matter, CF = crude fibre, CP = crude protein; OMD = organic matter digestibility.

Conclusions

As expected, the digestibility as measured by VEM and DVE was significantly lower for fresh tall fescue than for perennial ryegrass. However, the process of ensiling also had an effect on the difference in digestibility. In 2017 the differences in VEM and DVE disappeared, whereas this was not the case in 2018. There is no clear explanation for differences in these results between the two years.

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References

Cougnon M., Baert J., Van Waes C. and Reheul D. (2014) Performance and quality of tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) and mixtures of both species grown with or without white clover (*Trifolium repens* L.) under cutting management. *Grass and Forage Science* 69, 666-677.

De Boever J.L., Cottyn B.G., Andries J.I., Buysse F.X. and Vanacker J.M. (1988) The use of a cellulase technique to predict digestibility, metabolizable and net energy of forages. *Animal Feed Science Technology* 19, 247-260.

Tamminga S., Van Straalen W.M., Subnel A.P.J., Meijer R.G.M., Steg A., Wever C.J.G. and Blok M.C. (1994) The Dutch protein evaluation system: the DVE/OEB-system. *Livestock Production Science* 40, 139-155.

Van Es A.J.H. (1977) Feed evaluation for ruminants. I. The systems in use from May 1977 onwards in The Netherlands. *Livestock Production Science* 5, 331-345.

Differences in crude protein fractionation content of functional groups in permanent grassland

Wahyuni R.D.[1], Pötsch E.M.[2] and Gierus M.[1]

[1]*Institute of Animal Nutrition, Livestock Products and Nutrition Physiology (TTE), BOKU, Vienna, Austria;* [2]*Agricultural Research and Education Centre (AREC), Raumberg-Gumpenstein, Institute for Plant Production and Cultural Landscape, 8952 Irdning-Donnersbachtal, Austria*

Abstract

Long-term permanent grassland trials are rare but are extremely valuable. From 2014-2016 the forage quality of functional groups (grass, legumes, herbs) were obtained from a long-term experiment, which was established 50 years ago. In this experiment five different levels of N-fertiliser were implemented and applied since 1967, i.e. T1: no fertiliser (control), T2: PK, T3: PK + 80 N, T4: PK + 120 N and T5: PK + 180 N ha^{-1} and year. The cutting frequency was changed from 4 cuts/year, and from 1993 to present days changed to 3 cuts/year. Although the functional groups contribute additively to the dry matter yield, the forage quality may differ among functional groups depending on the fertilisation level. The objective of this study was to investigate the effect of mineral fertilisation administered on the protein fractionation of each functional group in mountainous permanent grassland. Treatments influenced protein fractionation especially the fraction C for all functional groups. Only in grasses was fraction A affected by treatment, the content increasing as N-fertiliser increased. Due to the high proportion of grasses in all treatments, also as a result of the N fertilisation, the protein fractionation reflected the grass proportion in the mixture (P<0.01).

Keywords: grassland, nutritive value, protein fractionation, ruminants

Introduction

Three different functional groups exist in grassland, namely grasses, legumes and herbs. In temperate regions like Europe, legumes are fascinating in terms of economic and political issues aiming to preserve natural resources and reduce the input of mineral fertiliser and concentrates. In addition, the availability of legumes reduces the requirement for N fertiliser, reduces nitrate percolation into groundwater and gives benefits to grass growth. Whereas grasses usually dominate in plant communities in permanent grassland, the contribution of herbs for forage quality is neglected. Nitrogen losses associated with inefficient N utilisation in animals are strongly influenced by the N distribution in forages. Crude protein (CP) alone is no longer an adequate measure to characterise protein supply for ruminants (Krawutschke *et al.*, 2013). The objective of this study was to investigate the effect of the level of N fertiliser administered on the CP fractionation of each functional group in mountainous permanent grassland. It was hypothesised that the CP fractionation for grasses, legumes and herbs as functional groups, after 50 years of similar N-fertilisation level, and consequently, with the botanical composition clearly defined, may show equally the relationship between N-fertilisation levels and CP quality of functional groups.

Materials and methods

The experiment was established at AREC Raumberg-Gumpenstein (N 47°29'38"; E 14°06'10"; 710 m a.s.l.) in 1967 and is one of the oldest still existing grassland experiments in Europe. The data sampling here was from 2014-2016, using randomised complete block design with 5 treatments and 4 replicates in a 3-cut system. The treatments were according to the amount of N-fertiliser applied, as follows: T1: no fertiliser; T2: P_2O_5 (P, phosphorus), K_2O (K, potassium) dynamic; T3: PK dynamic + 80 kg N ha^{-1} $year^{-1}$; T4: PK dynamic + 120 N ha^{-1} $year^{-1}$; and T5: PK dynamic + 180 N ha^{-1} $year^{-1}$. Dynamic means the application rate of P and K was based on the particular removal of PK in the previous year. At sampling, fresh forage was manually separated into functional groups (grass, legume and herb), dried at 58 °C, and ground to pass through a 1 mm sieve. The CP fractionation was determined according to Licitra *et al.* (1996). Crude protein was divided into three fractions: (1) fraction A (non-protein N, NPN), (2) fraction B (true protein, potentially degradable in the rumen), and (3) fraction C (unavailable protein for ruminants). All the data were subjected to analysis of variance. The factors and the interactions were considered as fixed factors: year, cut, treatment (fertilisation) and interactions, with block as replication.

Table 1. Interaction cut vs fertilisation on crude protein (CP) fractionation as g kg^{-1} CP.

	Fraction A				Fraction B				Fraction C			
	Cut 1	Cut 2	Cut 3	Mean	Cut 1	Cut 2	Cut 3	Mean	Cut 1	Cut 2	Cut 3	Mean
Grasses												
T1	227	214	246	229^{C}	724	738	698	720^{A}	$49^{ab,V}$	$47^{b,W}$	$57^{a,X}$	51
T2	262	212	226	233^{C}	686	733	717	712^{A}	$52^{a,V}$	$54^{a,W}$	$57^{a,X}$	54
T3	269	240	263	258^{B}	678	691	670	680^{B}	$53^{b,V}$	$68^{a,V}$	$67^{a,W}$	63
T4	274	250	289	271^{AB}	676	682	635	664^{BC}	$50^{c,V}$	$67^{b,V}$	$76^{a,V}$	64
T5	294	279	294	289^{A}	653	653	633	646^{C}	$53^{b,V}$	$68^{a,V}$	$73^{a,VW}$	65
Mean	265^{v}	239^{w}	264^{v}		683^{w}	700^{v}	670^{w}		51	57	66	
Cut	0.0001				0.0002				<0.0001			
Fertilisation	<0.0001				<0.0001				<0.0001			
Cut×Fertilisation	0.26				0.0857				<0.0001			
SEM	3.78				4.19				0.90			
Legumes												
T1	194	NA	197	NA	722	NA	687	NA	84	NA	116	NA
T2	215	204	200	206	713	676	695	695^{b}	72	120	104	99^{A}
T3	212	202	191	202	721	690	717	710^{a}	67	108	91	88^{B}
T4	197	197	194	196	735	700	706	714^{a}	68	102	100	90^{AB}
T5	206	208	213	209	727	682	690	700^{ab}	67	110	94	91^{A}
Mean	205	NA	199		723^{v}	NA	699^{w}		72^{w}	NA	102^{v}	
Cut	0.3069				<0.0001				<0.0001			
Fertilisation	0.0582				0.0166				<0.0001			
Cut×Fertilisation	0.23				0.4535				0.7090			
SEM	2.41				3.44				2.10			
Herbs												
T1	$167^{a,V}$	$142^{a,V}$	$96^{b,W}$	136	$715^{b,V}$	$716^{b,V}$	$770^{a,V}$	733	117	142	134	131^{AB}
T2	$183^{a,V}$	$155^{ab,V}$	$138^{b,V}$	158	$719^{a,V}$	$720^{a,V}$	$727^{a,VW}$	722	98	125	135	119^{B}
T3	$165^{a,V}$	$119^{b,V}$	$130^{ab,VW}$	138	$738^{a,V}$	$759^{a,V}$	$738^{a,VW}$	745	97	122	132	117^{B}
T4	$167^{a,V}$	$123^{b,V}$	$137^{ab,V}$	142	$702^{a,V}$	$740^{a,V}$	$712^{a,W}$	718	131	137	150	139^{A}
T5	$172^{a,V}$	$131^{b,V}$	$139^{a,V}$	147	$699^{a,V}$	$738^{a,V}$	$721^{a,W}$	719	129	131	140	133^{AB}
Mean	171	134	128		715	735	734		114^{w}	131^{v}	138^{v}	
Cut	<0.0001				0.0017				<0.0001			
Fertilisation	0.0060				0.0034				0.0003			
Cut×Fertilisation	0.0085				0.0013				0.3041			
SEM	3.24				3.59				2.50			

[1] ^{A,B} Different superscripts in a column show significance; ^{v,w} Different superscripts in a row show significance; ^{a,b} LSMeans differ between cutting within treatment; ^{V,W} LSMeans differ between treatment within cutting; NA = not available, it means not enough sample for analysis due to low amount of dry matter yield production; SEM = standard error of the mean.

Results and discussion

Permanent grassland after 50 years with constant mineral N fertiliser application, without any reseeding, provides well defined botanical composition, which is stable for measurements on the nutritive value in the present sampling years. The plant community in permanent grassland was dominated by grasses in all treatments, ranging from 40 to 90%. The absence of N and P application (T1) produced low numbers of tall grasses but plenty of herbs and legumes in that treatment. The dominant species of grass were *Trisetum flavescens*, *Anthoxanthum odoratum* and *Festuca rubra*. There were three dominant species of legumes, i.e. *Trifolium pratense*, *T. repens* and *Vicia cracca*. Furthermore, *Leontodon hispidus* and *Aegopodium podagraria* were the most frequent herb species. Year had a strong effect on all fractions for all functional groups (data not shown). The interaction cut×fertilisation was significant for some fractions and functional groups (Table 1). The mineral N fertiliser application increased fraction A and fraction C in grasses, and consequently reduced fraction B content. Cut 3 of grasses had the highest proportion of fraction C. In legumes the proportion of fraction A remained stable at all fertilisation levels. Fraction C of legumes tended to decrease with higher N-fertilisation rate. Fractions A and B of herbs showed cut×fertilisation interaction, with the third cut showing most variation. Herbs had the lowest content of fraction A, but the highest fraction C among the functional groups. There are limited studies regarding the potential of the contribution of herbs for dry matter yield and nutritive value of permanent grassland. Fraction A was highest in grasses, supporting that excess of N can be accumulated as NO_3, free amino acid and amides, indicating that the Fraction A is poorly converted into proteins at higher fertilisation levels. The N application level did not affect the concentration of fraction A in legumes and herbs. Herbs had the lowest amount of fraction A, compared with grasses and legumes. Low content of fraction A and increasing content of fraction C may result from secondary compounds available in legumes and herbs, which may support higher N use efficiency in ruminant nutrition (Krawutschke *et al.*, 2013).

Conclusions

Due to the high proportion of grasses in all treatments, also as a result of the N fertilisation, the crude protein fractionation reflected the grass proportion in the mixture.

References

Krawutschke M, Kleen J, Weiher N, Loges R, Taube F and Gierus M (2013) Changes in crude protein fractions of forage legumes during the spring growth and summer regrowth period. *Journal of Agricultural Science*, 151, 72-90.

Licitra G., Hernandez T.M. and Van Soest, P.J. (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology* 57, 347-358.

Ensilability and silage quality of grass from intensive permanent grasslands of contrasting botanical composition

Wyss U.[1], Probo M.[2] and Huguenin-Elie O.[3]

[1]*Agroscope, Ruminant Research Unit, 1725 Posieux, Switzerland;* [2]*Agroscope, Grazing Systems, 1260 Nyon, Switzerland;* [3]*Agroscope, Forage Production and Grassland Systems, 8046 Zürich, Switzerland*

Abstract

Grasslands cover nearly 80% of the agriculturally used area in Switzerland. Their botanical composition is highly diverse, and so is the quality of the forage they produce. Within this project, 154 forage samples were collected on intensively managed permanent grasslands across Switzerland to investigate the influence of botanical composition and regrowth cycle on silage quality. Specifically, samples were collected in 2018 and 2019 from the first to the fifth regrowth cycle of the year on 20 different grasslands, which were each assigned to one of four types of botanical composition. Forage was prewilted, chopped and ensiled in laboratory silos. Before ensiling, subsamples were taken for determination of dry matter (DM) and nutrient contents. After a storage period of 90 days, DM, nutrient contents, pH and acid contents were analysed to determine silage quality. Results showed that forage samples were ensiled with an average DM content of 33%. From spring to autumn, the crude protein and nitrate contents increased. Some samples had butyric acid, and the silage quality was generally moderate, particularly for those types of grasslands with a higher proportion of ryegrass.

Keywords: permanent grassland, botanical composition, nutrient content, silage quality

Introduction

In Switzerland, permanent grasslands cover more than 70% of the agriculturally used area. Their botanical composition and growth conditions vary widely (Lüscher *et al.*, 2019). Herbage silages from Swiss farms often have low nutrient values and poor fermentation characteristics (Augsburger *et al.*, 2019). The question arises if this is caused by the inherent characteristics of the forage in the course of the year or by the method of ensiling. To answer the first part of this question, forage samples were collected on diverse intensively managed permanent grasslands to investigate the influence of botanical composition and regrowth cycle of the year on silage quality following a standardised ensiling process.

Materials and methods

The permanent grasslands were located at altitudes ranging from 440 to 1000 m a.s.l. They had been frequently defoliated and fertilised. The relative abundance of grass species in the plant community ranged from 30 to 96%. The relative abundance of legumes and forbs ranged from 1 to 42 and 1 to 50%, respectively. The grasslands were classified into four classes (G, G_r, A, A_r) according to their botanical composition (Agroscope, 2016). In particular, the proportion of grasses was between 71 and 100% in the grassland types G and G_r and between 50 and 70% in types A and A_r. The letter 'r' means that ryegrass biomass was more than 50% of total grass biomass.

Forage samples were collected at 14 grassland sites in 2018, and at 19 grassland sites in 2019. Sampling was performed on three plots (replicates) per grassland and sampling event. The number of samples collected per regrowth cycle, respectively per grassland type, is given in Table 1. The forage was pre-wilted, short chopped and ensiled, each repetition separately, in laboratory silos each having a volume of 1.5 litres. No silage additives were used. Before ensiling and after a storage time of 90 days, dry matter (DM) and nutrient contents of samples were analysed by near infrared spectroscopy (NIRS) (Ampuero Kragten and Wyss, 2014). Fermentability coefficients (FC) were calculated (Weissbach and Honig,

Table 1. Dry matter (DM), nutrient contents and fermentability coefficient (FC) of fresh forage at ensiling from different grassland sites in Switzerland.[1]

		n	DM	Ash	Crude protein	Nitrate	ADF	NDF	Lignin	WSC	FC
			%	g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM	
Regrowth cycle	1st (spring)	72	29.8	87	143	0.37	285	505	28	123	49
	2nd+3rd (summer)	49	36.1	98	141	1.00	384	490	33	95	50
	4th+5th (autumn)	33	35.3	130	177	3.21	231	400	34	103	46
Botanical composition	A	70	33.7	97	149	0.67	265	457	34	109	48
	A_r	6	32.1	127	161	3.79	244	417	34	110	48
	G	51	33.5	94	140	0.85	296	522	29	106	49
	G_r	27	30.1	111	169	2.54	255	461	25	120	48
Significance	SEM		0.77	1.9	2.5	0.19	3.4	5.6	0.7	2.7	0.8
	Regrowth cycle		**	**	**	**	**	**	**	**	ns
	Botanical composition		ns	**	*	**	**	**	**	*	ns

[1] n = number of samples; ADF = acid detergent fibre; NDF = neutral detergent fibre; WSC = water-soluble carbohydrates; A, A_r = proportion of grasses between 50 and 70%; G, G_r = proportion of grasses between 71 and 100%; A_r, G_r = ryegrass biomass >50% of total grass biomass; SEM = standard error of the mean; ns = not significant; * P<0.05; ** P<0.01.

1992). The FC summarizes the potential effects of DM and of the ratio of sugar content to buffering capacity on the fermentation process. Additionally, fermentation parameters (pH, acids, ethanol and NH_3) were analysed in the silages. Silage quality was classified by using the point scoring system of the 'Deutsche Landwirtschafts-Gesellschaft' (DLG) (Pahlow and Weissbach, 1999). This method considers concentrations of butyric and acetic acids together with pH in relation to DM content. The maximum value is 100 DLG points for the highest fermentation quality.

Data were evaluated by a general linear model both on the fresh material before ensiling and on the silages, with 'regrowth cycle' (with three categories: a) first cycle, b) second and third cycles and c) fourth and fifth cycles) and 'botanical composition' as fixed effects. Differences among arithmetic means were considered significant at P<0.05.

Results and discussion

The forage was wilted on the field to an average DM content of 33%, with a range from 18.2 to 67.7%. From spring to autumn, the crude protein and nitrate contents (NO_3) increased in the fresh forage (Table 1). 91% of the samples were characterized as free of NO_3 (i.e. nitrate content under 4.4 g kg^{-1} DM). These results agree with those of Weiss *et al.* (2006), who also found 90% of the fresh forage under practical conditions was free of NO_3.

The contents of ash and water-soluble carbohydrates (WSC) were different among regrowth cycles and among botanical compositions. In general, forage from the first regrowth cycle and forage with a high proportion of ryegrass (G_r and A_r) had higher WSC contents. The FC showed that 6% of the samples were difficult, 26% moderately difficult and 69% easy to ensile. All the silages showed relatively high pH values and differed among regrowth cycles (Table 2). The butyric acid content was influenced by the grassland botanical composition. In particular, forage from the first regrowth cycle with a high ryegrass proportion showed the highest butyric acid content. The silages reached on average 70 DLG points, which were significantly influenced by botanical composition. This value indicates a moderate to good silage quality.

Table 2. Dry matter (DM), fermentation parameters and DLG points of silages from different grassland sites in Switzerland.[1]

		n	DM %	pH	Lactic acid g kg^{-1} DM	Acetic acid g kg^{-1} DM	Propionic acid g kg^{-1} DM	Butyric acid g kg^{-1} DM	Ethanol g kg^{-1} DM	NH_3-N/ N total, %	DLG points
Regrowth cycle	1st (spring)	72	28.8	4.7	53	21	1	9	16	8.9	64
	2nd+3rd (summer)	40	36.9	5.0	44	12	0	7	9	7.8	74
	4th+5th (autumn)	33	34.8	5.2	60	20	0	4	9	9.5	78
Botanical composition	A	64	33.4	4.9	49	17	0	4	13	7.7	79
	A_r	6	31.2	5.1	65	25	1	11	10	10.8	59
	G	48	33.1	4.9	44	18	1	11	14	8.8	62
	G_r	27	29.0	4.8	71	22	1	10	10	10.6	65
Significance	SEM		0.80	2.6	1.2	0.2	1.1	0.5	0.4	2.3	0.80
	Regrowth cycle		**	**.	ns	ns	ns	ns	**	ns	*
	Botanical composition		*	ns	**	ns	ns	*	ns	*	**

[1] n = number of samples; NH_3-N/N total = ammonia nitrogen per total nitrogen; DLG = Deutsche Landwirtschafts-Gesellschaft; A, A_r = proportion of grasses between 50 and 70%; G, G_r = proportion of grasses between 71 and 100%; A_r, G_r = ryegrass biomass >50% of total grass biomass; SEM = standard error of the mean; ns = not significant; * $P<0.05$; ** $P<0.01$.

The correlation between FC and DLG points was 0.47, indicating that other factors such as ash and fibre content affected silage quality. Furthermore, forage with high NO_3 content showed low butyric acid content with a correlation of -0.12, as described also by Weiss *et al.* (2006).

Conclusions

The nutrient contents of forage with different botanical composition varied markedly, and differences among regrowth cycles were detected. This finding confirms results from analyses of silages produced on Swiss farms. The quality of the silages varied, and some samples contained butyric acid. The average silage quality was moderate for grasslands with a high proportion of grasses. This was also the case for the silage from grasslands with a high proportion of ryegrass, even if this could not be expected from the higher WSC contents.

References

Agroscope (2016) Fütterungsempfehlungen für Wiederkäuer (Grünes Buch). Available at: https://www.agroscope.admin.ch.

Ampuero Kragten S. and Wyss U. (2014) Futtermittel im Nah-Infrarotlicht (NIRS). Agrarforschung Schweiz 5, 204-211.

Augsburger C., Bracher A. and Boessinger M. (2019) *Gras- und Maissilagen* (2018) Agridea, Switzerland.

Lüscher A., Grieder C., Huguenin-Elie O., Klaus V.H., Reidy B., Schneider M.K., Schubiger F., Suter D., Suter M. and Kölliker R. (2019) Grassland systems in Switzerland with a main focus on sown grasslands. *Grassland Science in Europe* 24, 3-16.

Pahlow G. and Weissbach F. (1999) New aspects of evaluation and application of silage additives. *Landbauforschung Voelkenrode SH* 206, 141-158.

Weiss K., Kaiser E. and Taube F. (2006) Content of nitrate in green forage and its effect on silage quality. *Grassland Science in Europe* 11, 56-58.

Weissbach F. and Honig H. (1992) Ein neuer Schlüssel zur Beurteilung der Gärqualität von Silagen auf Basis der chemischen Analyse. 104. VDLUFA-Kongress Göttingen. *VDLUFA-Schriftenreihe* 35, 489-494.

Effect of grazing system on dairy cow performance and nitrogen use efficiency

Zom R.L.G. and Holshof G.

Wageningen University and Research, Wageningen Livestock Research, De Eng 1, 6708 WB Wageningen, the Netherlands

Abstract

In the Netherlands, the proportion of dairy farms that apply grazing has declined since the 1990s. A major reason for this is the perceived complexity of grazing management, in particular for intensive dairy farms with high stocking rates. Easily implemented grazing systems may stimulate farmers to start or maintain grazing. However, these easy grazing systems should not compromise milk production and nitrogen use efficiency (NUE, N_{milk}/N_{intake}). Therefore, we studied the effects of grazing system on dairy cow performance and NUE with conventional strip-grazing (SG), easy rotational (ER), and compartmented continuous (CC) grazing with 20 dairy cows per grazing and stocking rates of 6 cows ha^{-1}. Individual intake of grass and supplemental forage was measured during two periods (spring, autumn). Over the whole grazing period, there were no differences in the cumulative milk and milk solids yield between SG, ER and CC. In spring NUE was lower for SG than for ER and CC, due to a greater total dry matter intake (TDMI) and hence a greater N-intake with ER and CC. In autumn, NUE was similar because the absolute differences in grass dry matter intake (GDMI) and TDMI intake of grass between the grazing systems were less than in spring. In conclusion there is no evidence that EG systems compromise dairy cow production and NUE.

Keywords: grazing system, nitrogen use efficiency, dairy cows

Introduction

In the Netherlands grazing on dairy farms has declined since the 1990s. This is driven by an increased complexity of grazing management with larger herds, limited possibilities for pasture access, and high stocking rates. Easily implemented grazing systems (EG) which require less mental and physical efforts for planning, control and management may lure dairy farmers to maintain or to reintroduce grazing. Such potential EG systems could be easy rotational (ER) and compartmented continuous (CC) grazing (see Materials and methods). However, these EG systems should not have any negative trade-offs in terms of milk production and nitrogen use efficiency (NUE). Therefore, an experiment was conducted to study the effects of EG systems (ER and CC) on grass intake, milk production and NUE in comparison with conventional strip-grazing with flexible fencing (SG).

Materials and methods

The experiment ran from 29/4 to 29/10 2015 at Dairy Campus, the Netherlands. Sixty spring calving Holstein-Friesian dairy cows were assigned to blocks of 3 cows based on parity, stage of lactation, milk constituent yield, fat and protein corrected milk yield (FPCM) and body weight. The cows from one block were randomly allocated to SG, ER and CC, creating 3 groups, which were managed separately, on 2.33 ha (6 cows ha^{-1}). During each milking (at 5 am and 4 pm) the cows received 2.5 kg dry matter (DM) d^{-1} of a concentrate (5 kg DM d^{-1}; 7.7 MJ net energy of lactation and 190 g crude protein per kg DM). In all systems, grass intake and grass growth were balanced with supplementary forage fed indoors. The first 6 kg DM d^{-1}, of supplemental forage consisted of maize silage. If more supplementary forage was needed, then grass silage was included in the forage mixture on top of the maize silage. The cows of SG and CC were kept indoors between the am and pm milkings. The cows of ER were rotationally stocked in a fixed-order 24 one-day paddocks of 970 m^2, which were grazed down to <5 cm residual sward height, in order to maintain high quality pasture herbage. Once a paddock was depleted, the cows were supplemented indoors with supplementary forage. As grass growth and sward height decreased during the season, the access time to pasture was reduced and supplementary forage was increased. The grazing area of CC was divided in 6 paddocks of 3,880 m^2. The cows on CC rotationally grazed 5 one-day paddocks (resting period 4 days). The paddock, with the poorest sward condition (tall patches) was set aside for cutting in order to maintain good pasture quality. After sufficient regrowth (sward height 10 cm) the paddock was added rotation while another paddock was set aside for cutting. The average sward height was maintained at 8-10 cm. The cows of the SG system were grazing strips from which the size was adjusted to match the intake and target residual pasture mass. To maintain pasture herbage of good quality, the regrowth after two consecutive grazings was cut for silage.

Individual concentrate intake and milk yields were recorded at each milking. Milk composition was recorded weekly during four consecutive milkings. During two periods in spring (May 22-28) and autumn (October 23-28), individual grass dry matter intake (GDMI) and supplemental forage intake were determined using the n-alkane technique.

For 14 days the cows were dosed with 0.42 kg of a concentrate containing 910 mg kg^{-1} C32 n-alkane at each milking. Supplementary forage was labelled with C36 n-alkane labelled soybean meal (SBM; 4,680 mg C36 n-alkane kg DM^{-1}; 4.7 kg SBM per 1000 kg fresh weight forage) similar as described by Hameleers and Mayes (1998). From day 7 to 14 of the alkane-dosing period, samples of grass, supplemental forage, concentrates and faeces (per cow after each milking). These samples were analysed for the concentrations of odd chain (C27 to C35) and dosed n-alkanes (C32 and C36) as described by Abrahamse *et al.* (2009). The proportions of grass, concentrates, and supplementary forage in the diet of individual cows were estimated using a non-negative least-squares procedure (Dove and Moore, 1995). The total dry matter intake (TDMI) and GDMI were calculated from the known intake of the concentrate and their proportion in the diet according to Dove and Charmley (2008). The feed samples were analysed for chemical composition and feeding value at Eurofins Agro (Wageningen, the Netherlands). The NUE was calculated as N in milk/N intake. Statistical analysis was performed using the statistical programme GenStat 18. A mixed model with repeated measurements was used to analyse the effect of grazing system on the weekly mean milk yield and milk constituent yield and fat and protein corrected milk (FPCM) yield with grazing system and period as fixed effects and block and cow as random effects. The data on cumulative milk, fat and protein yield, and GDMI, TDMI, N in milk and N-intake, NUE recorded during intake measurements periods were analysed using ANOVA.

Results and discussion

There were significant week and grazing system×week interactions (P<0.001), on milk yield, fat and protein concentrations and FPCM yield. However, milk production and composition and cumulative yields of milk, fat, protein and FPCM were not significantly different between the grazing systems (Table 1). The experiment was carried out in a continuous design. Consequently, stage of lactation, changes in pasture quality and quantity, and level of supplemental forage are confounded, which causes the effects of week or week×system interactions. In spring there were no significant differences in GDMI between the treatments, but TDMI was lower in ER than in SG and CC (Table 2). Differences in GDMI and TDMI and pasture herbage composition (Table 3) resulted in differences in N-intake between the treatments, with N intake in SG > ER > CC (P<0.001). However, the milk-N output was not significantly different between treatments. Consequently, NUE was different between treatments, with NUE of CC > ER > SG (P<0.001). In spring, cows on ER and CC grazed previously grazed swards, whereas cows on SG grazed previously cut swards. A higher grass quality (leaf:stem ratio) may explain the relatively high GDMI in SG. Moreover, CC contained more tall patches, with a lower crude protein and organic matter digestibility % of the pasture (Table 3). In autumn, GDMI was significantly greater for CC than for SG

Table 1. Effect of grazing system (S) and week (W) and S×W interactions on average daily milk production and composition and cumulative milk and milk constituent yields, fat and protein corrected milk (FPCM) during the grazing season 29 April to 29 October.[1]

	SG	ER	CC	SED	S	W	S×W
Milk (kg d^{-1})	23.7	24.7	24.5	0.91	NS	0.001	0.001
Milk fat (%)	4.42	4.25	4.4	0.1	NS	0.001	0.001
Protein (%)	3.46	3.54	3.53	0.04	NS	0.001	0.001
FPCM (kg d^{-1})	24.8	25.3	25.6	0.95	NS	0.001	0.001
Cumulative milk constituent yield 29 April – 29 October							
Milk (kg)	4294	4463	4450	140	NS	-	-
Fat (kg)	186	185	191	6.3	NS	-	-
Protein (kg)	146	155	154	4.9	NS	-	-

[1] SG = strip grazing, ER = easy rotational grazing, CC = compartmented continuous grazing, SED = standard error of the difference, NS = not significant.

Table 2. Effect of grazing system on grass (GDMI) and total (TDMI) dry matter intake, N- intake, -milk and NUE (N-milk/N-intake) in spring and autumn.[1]

	SG	ER	CC	SED	Significance
Spring (22-28 May)					
GDMI (kg d^{-1})	14.4	14.8	12.8	0.95	NS
TDMI (kg d^{-1})	25.5[a]	20.1[b]	23.7[c]	0.77	<0.001
N-intake (g d^{-1})	651[a]	611[b]	541[c]	20.4	<0.001
N-milk (g d^{-1})	147	158	159	6.1	NS
NUE	0.22[a]	0.26[b]	0.29[c]	0.008	<0.001
Autumn (23-28 October)					
GDMI (kg d^{-1})	2.7[a]	2.9[a]	3.7[b]	0.38	0.05
TDMI (kg d^{-1})	15.1	14.6	15.1	0.51	NS
N-intake (g d^{-1})	344[a]	389[b]	379[b]	14.3	0.01
N-milk (g d^{-1})	107	133	113	4.6	NS
NUE	0.31	0.29	0.3	0.015	NS

[1] Different superscripts within rows indicate significant differences. SG = strip grazing, ER = easy rotational grazing, CC = compartmented continuous grazing, SED = standard error of the difference, NS = not significant.

Table 3. Chemical composition (g kg^{-1} DM) and feeding value of pastures.[1]

	Spring			Autumn		
	SG	ER	CC	SG	ER	CC
Crude protein	177	191	148	161	243	205
Sugars	153	163	191	183	156	123
NDF	499	468	467	448	439	457
NEL MJ kg^{-1} DM	6.86	7	6.72	6.44	7.24	6.25
OMD (%)	83.4	84	82.3	80.3	88.3	80.1

[1] DM = dry matter, NDF = neutral detergent fibre, NEL = net energy of lactation, OMD = organic matter digestibility, SG = strip grazing, ER = easy rotational grazing, CC = compartmented continuous grazing (one pooled sample per treatment, per period).

and ER (P<0.001), but not TDMI. The low intakes compared to spring measurement could be related to a lower energy requirement and a relatively high level of supplementary feeding. The N-intake was higher for SG compared with ER and CC. Milk-N outputs were not significantly different between the grazing systems, resulting in similar NUE for the systems. The week×system interactions and the absence of a clear effect of grazing system on GDMI and NUE in both intake-measurement periods can be explained by the fact that grazing management decisions (e.g. post-grazing pasture mass, cutting strategy) implemented on a certain time point, will inevitably have an effect on sward structure (i.e. sward height, leaf:stem ratio, DM yields) later on in the grazing season.

Conclusions

Easy grazing systems did not compromise milk and milk constituents yields or NUE, compared with strip grazing.

References

Abrahamse P.A., Tamminga S and Dijkstra J. (2009) Effect of daily movement of dairy cattle to fresh grass in morning or afternoon on intake, grazing behaviour, rumen fermentation and milk production. *Journal of Dairy Science* 147, 721-730.

Dove H. and Charmley E. (2008) Using the alkanes and long-chain alcohols of plant cuticular wax to estimate diet composition and the intakes of mixed forages in sheep consuming a known amount of alkane-labelled supplement. *Animal* 2, 1474-1485.

Dove H. and Moore A.D. (1995) Using a least-squares optimization procedure to estimate botanical composition based on the alkanes of plant cuticular wax. *Australian Journal of Agricultural Research* 46, 1535-1544.

Hameleers A. and Mayes R.W. (1998) The use of n-alkanes to estimate supplementary grass silage intake in grazing dairy cows. *Journal of Agricultural Science* 131, 205-209.

Theme 3.
Grasslands and environment

Grassland soil organic carbon stocks as affected by management intensity and climate change

Poeplau C.
Thünen Institute of Climate-Smart Agriculture, Bundesallee 68, 38116 Braunschweig, Germany

Abstract

Grasslands are a major terrestrial ecosystem type and store large amounts of soil organic carbon (SOC) per unit of area. Much of the permanent grassland area is managed, especially in countries with industrialized agriculture, but quantitative and mechanistic knowledge on management effects on SOC stocks in grasslands is limited. Climate change can also have an indirect anthropogenic impact on SOC stocks, and warming effects on grassland SOC are certainly understudied. Here, several studies investigating management and climate change effects on SOC stocks, with a central to northern European focus, are summarised. According to analyses, SOC sequestration increased with management intensity, i.e. cutting frequency and mineral fertilisation under cold temperate and humid climates, and this was partly explicable by increased productivity and increased microbial anabolism, respectively. At the same time, soil warming depleted SOC, and it can thus be expected to counterbalance efforts of SOC build-up to some extent. Intensifying grassland use is also accompanied by increased greenhouse gas emissions. However, including perennial grasses in agricultural crop rotations is multi-beneficial and has been proven to be a highly efficient measure to increase SOC stocks.

Keywords: fertilisation, cutting frequency, perennial grasses, soil warming

Introduction

Soil organic matter is the largest terrestrial carbon pool. Globally, it stores more carbon than vegetation and atmosphere combined (Ciais, 2013). Small relative changes in soil organic carbon (SOC) stocks can thus have strong impacts on the atmospheric CO_2 concentration. For this reason, accumulating SOC is considered as a potential negative emission technology to achieve international climate mitigation goals. Furthermore, soil organic matter content is a key soil quality indicator, due to the numerous positive effects of organic matter on soil properties. Loss of organic matter and SOC is thus considered as soil degradation (Global Soil Partnership, 2017).

In research and policy, there is a strong focus on cropland soils as potential carbon sinks or sources. This might be related to the fact that croplands are acknowledged to store the lowest amounts of SOC per unit of land area and they are the most intensively managed system. For example, Debreczeni and Körschens (2003) listed more than 600 agricultural long-term experiments globally, of which only 49 were initiated on pasture or meadow soils. However, globally grasslands cover 68% of the total agricultural area (Leifeld *et al.*, 2015) and store an estimated 245 Pg SOC (Bolin *et al.*, 2000). In Europe, 21% of the total land area is covered by grassland and 22% by cropland. In urban areas also, grassland is a major land cover type (Ignatieva *et al.*, 2015). Especially in Europe, grasslands are intensively managed ecosystems. Accordingly, management-induced SOC changes need to be better understood in order to develop climate-smart farming and management solutions, as well as to feed earth-system models. Management interventions such as grazing, cutting, fertilisation, reseeding, species selection or irrigation have been found to affect SOC to some extent (Conant *et al.*, 2001; Rumpel *et al.*, 2015). For most of these mentioned interventions the existing literature is limited and controversial, and has involved mechanisms that are insufficiently understood. In addition, SOC sequestration potentials as derived from scattered field trials may not be representative for a wide range of soils and climate zones, and not always be applicable to real-world agriculture. Recently, Batjes (2019) underlined the large discrepancy between potential

SOC sequestration and technically achievable SOC sequestration. For example, the implementation of best management practices on degraded grasslands has the potential to sequester 2.3-6.8 Pg C in 20 years globally, while the currently achievable range was estimated at 0.12-1.02 Pg C. However, even the latter number can play a considerable role in climate change mitigation – total annual anthropogenic C emissions amount to approximately 10 Pg C (Ciais, 2013).

Apart from direct management interventions, also human-induced climate change is likely to be a threat to SOC. Such as, increasing temperatures are acknowledged to catalyse microbial activity and thus SOC mineralisation, inducing a climate-carbon cycle feedback loop (Davidson and Janssens, 2006). However, warming and CO_2 fertilisation will also affect plant production, which could counterbalance SOC mineralisation. Thus, how the ecosystem carbon balance and subsequent SOC will respond to climate change is highly uncertain (Conant *et al.*, 2011). In this work, we aimed to summarise some of the recent studies analysing management and warming effects on SOC dynamics in European grassland soils.

Effect of cutting frequency on soil organic carbon dynamics in urban lawns

The cutting frequency of grasslands can vary strongly due to differences in climate and vegetation cover, thereby affecting growth patterns, total net primary production (NPP) and aboveground-belowground carbon allocation of grasslands (Wohlfahrt *et al.*, 2008). Mechanistic and quantitative understanding of cutting frequency effects on SOC stocks is, however, limited. This might be related to the fact that experiments including cutting frequency are often combined with varying fertiliser additions. Kramberger *et al.* (2015) tested cutting frequency effects at equal fertiliser rates and did not find any effect on SOC stocks. The cutting interval varied between 2 and 12 weeks and clippings were removed.

In the course of the Swedish LAWN project, we conducted a cutting frequency experiment in three Swedish cities (Gothenburg, Malmö and Uppsala) in order to analyse the differences between two contrasting managed urban lawn types in residential areas: utility lawns and meadow-like lawns. In all three cities, the unfertilised lawns were established along with multi-family housing in the beginning of the 1950s and were managed similarly since then. On average, the short utility lawns were mown every eighteen days (eight times per growing season), while meadow-like lawns were cut only once a year. Accordingly, utility lawns were dominated by small, fast-growing, defoliation-tolerant species such as *Poa annua* L., *Agrostis capillaris* L. and *Lolium* spp., whereas the most abundant grass species in meadow-like lawns were tall, slow-growing species, such as *Phleum pratense* L., *Alopecurus pratense* L. and *Arrhenaterum* spp. In each of the three cities, three test sites were selected. At each test site, three plots were established on each lawn type. Soil samples for assessing SOC, total nitrogen, soil pH, soil texture and bulk density were taken with a thin auger to a depth of 20 cm, and NPP was determined by harvesting regrowth several days after each cutting event and fitting a climate-driven vegetation model to the cumulative growth curve.

We found significantly higher SOC stocks in utility lawns than in meadow-like lawns (Poeplau *et al.*, 2016). After an average of 62 years, the different lawn management caused an SOC stock difference of 7.8 Mg ha^{-1} (12%). Across nine sites, the mere change of the cutting regime and induced species assembly had thus led to SOC sequestration at a rate of 0.13 Mg C ha^{-1} yr^{-1} during six decades. This increase in SOC stocks correlated well with the increase in NPP, which amounted to 0.7 Mg C ha^{-1} yr^{-1} (24%) (Figure 1). In this case, the combination of plant functional groups and cutting led to an increase in aboveground biomass production and carbon inputs, since the clippings were not removed. The study results highlighted that NPP, i.e. the amount of carbon that is assimilated by plants, is a primary driver of soil carbon dynamics. Other studies have found unchanged or even decreased SOC stocks as a result of increased cutting frequency with biomass removal (Balasubramanian *et al.*, 2020; Kramberger *et al.*, 2015), which points to the fact that the SOC sequestration amount found here was

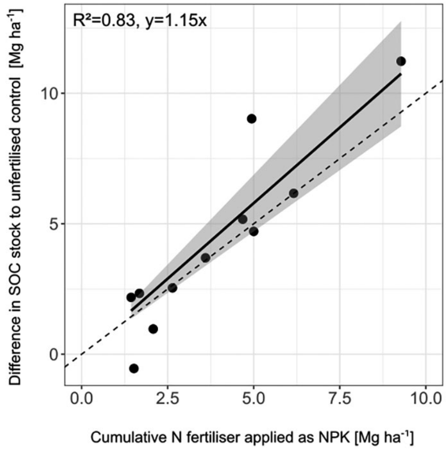

Figure 1. Soil organic carbon stock changes (0-30 cm depth) in response to nitrogen fertilisation in seven Central European grassland fertilisation experiments over a period of up to 58 years. Regression (black line) with 95% confidence interval is forced through origin; dashed line represents the 1:1 line (adapted from Poeplau *et al.,* 2018b).

achieved only by leaving the clippings on site. Leaving the clippings on site, however, might in turn stimulate N_2O emissions, which could potentially lead to a greenhouse gas trade-off against increased SOC sequestration.

Mineral fertilisation effects on soil organic carbon stocks

Besides cutting frequency, fertilisation with major plant nutrients is the most effective means of increasing net primary productivity. Accordingly, mineral fertilisation might have positive effects on SOC stocks. In long-term cropland fertilisation experiments, Kätterer *et al.* (2012) found that approximately 1 kg ha^{-1} of nitrogen fertiliser sequestered 1 kg ha^{-1} SOC within 4-5 decades, which they related to increased C inputs. In a synthesis of results from 40 different studies, Conant *et al.* (2001) also found slightly positive fertilisation effects on grassland SOC. At the same time, the major carbon input in managed and harvested grasslands is root-derived, and mineral fertilisation is acknowledged to shift plant root:shoot ratios towards shoots (Hermans *et al.*, 2006; Olff *et al.*, 1994). Symbioses with mycorrhizae are also reduced when plants are supplied with sufficient nutrients (Sochorová *et al.*, 2016). The fertilisation effect on C input and thus SOC might thus also be negligible or negative (Sochorová *et al.*, 2016). Another important driver of SOC dynamics is microbial anabolism, which depends to a large extent on nutrient availability (Manzoni *et al.*, 2012). Species richness and functional diversity also change in response to fertilisation, which might have further effects on SOC (Lange *et al.*, 2015). In consequence, SOC changes are reported in both directions, as the mechanisms involved are diverse and not easily quantifiable at the same time. Taken together, this hampers the identification of key processes driving SOC dynamics in grasslands as a consequence of changes in nutrient availability. For this reason, we identified and sampled a total of seven different long-term (>10 years) mineral fertilisation experiments to study effects on SOC stocks, potential litter decomposition, above- and belowground biomass and microbial metabolism (Poeplau *et al.*, 2018, 2019). Most grassland experiments did not included investigations of belowground changes at all – neither biomass nor soil properties – but had a strong focus on quality and quantity of aboveground biomass.

The experiments were located in central Europe, Germany (n=6) and the Netherlands (n=1), and they comprised both shallow mountain grasslands as well as fertile floodplain grasslands. They covered a wide range of abiotic site properties and thus differed in productivity. Cutting frequency varied between two and four times and length of the experiments ranged between 16 and 58 years. Soils were sampled to a maximum depth of 100 cm or until the bedrock using a power-driven soil auger. Soil cores were split along fixed-depth increments and vertically, to derive samples for both root biomass quantification and for soil analyses.

For the topsoil (0-30 cm), we detected significant effects of PK, NPK and NPK+ (+ stands for enhanced NPK fertilisation) on SOC stocks, with sequestration rates of 0.28 (PK), 0.13 (NPK) and 0.37 (NPK+) Mg C ha^{-1} yr^{-1} in 34 (PK, NPK) and 20 (NPK+) years. In contrast to Fornara *et al.* (2013), we did not detect a significant effect of N-only fertilisation on SOC stocks. In the case of NPK fertilisation, we found a significant correlation between the amount of N fertiliser and the amount of C sequestered: 1.15 kg N was needed to sequester 1 kg C (Figure 1). Notably, sequestered C corresponded to the amount of C (1.16 kg C or 4.25 kg CO_2) that is emitted during N fertiliser production, and not accounting for any onsite (field) N_2O emissions (Edwards *et al.*, 2017). In view of this, SOC sequestration may widely counterbalance NPK fertilisation-related emissions, although this cannot considered as a climate mitigation measure. This might be different for PK fertilisation, which was found to have comparably positive effects on SOC stocks. The CO_2 emissions related to production of P (0.54 kg CO_2 kg^{-1}) and K (0.42 kg CO_2 kg^{-1}) are much lower than for N, and PK fertilisation also favours the abundance of legumes, which supply the grassland with extra nitrogen. In the investigated experiments, the proportion of legumes increased by 12% as a result of PK fertilisation.

The positive effect of fertilisation on grassland biomass and thus C inputs appears to be the most obvious explanation for increased SOC stocks. However, root biomass tended to be lower in fertilised than in unfertilised plots and much of the aboveground biomass was harvested. We concluded that changes in input might not be the only driver of SOC sequestration associated with fertilisation, and we hypothesised that fertilisation increased microbial carbon use efficiency (CUE); i.e. growth over C uptake, being a major pathway of SOC stabilisation.

To test this hypothesis, we conducted an incubation experiment with two out of seven experiments (Poeplau *et al.*, 2018a). Soils from the unfertilised and NPK-fertilised treatments were labelled with ^{18}O water and incubated for 24 hours, after which respiration and microbial biomass was determined and the microbial DNA was quantified and analysed for the ^{18}O content. In this way, it was possible to determine microbial growth under steady state conditions (Spohn *et al.*, 2016). Specific respiration was significantly decreased by fertilisation, while microbial growth doubled. In consequence, microbial CUE was 53±21% higher in fertilised soils as compared to unfertilised soils. Furthermore, CUE correlated negatively with substrate C:N ratio (R^2=0.48), which provided evidence that N availability is strongly driving microbial metabolism. This is related to microbial stoichiometry: microbes have relatively fixed stoichiometric demands (Manzoni *et al.*, 2012), with bacteria especially having narrow C:nutrient ratios (Cleveland and Liptzin, 2007). This implies that the more N and P is available, the more C can be taken up by microbes. Once C is taken up, and not respired, it can be further stabilised in the soil. A modelling exercise with all the seven fertilisation experiments revealed that C input alone cannot explain SOC stock changes. We conclude that the microbial anabolic pathway is likely to have directly contributed to increased SOC stocks.

Increasing the proportion of leys and perennial cover crops in crop rotations

In the previous sections it was shown that increasing NPP, either by increasing the cutting frequency or fertilisation, has positive effects on SOC stocks. In croplands, another option to increase NPP is to maximize the duration of active vegetation cover (i.e. time of photosynthesis) and to minimise the bare fallow period. This can be obtained by introducing perennial grasses into the crop rotation, either as a main crop or as cover crop between two cash crops. By this means, the additional root-derived C input is specifically beneficial for SOC build-up (Rasse *et al.*, 2005; Bolinder *et al.*, 2010; Sokol *et al.*, 2019). In Swedish agriculture, the proportion of perennial grasses (leys) and green fallows in crop rotations increased from 32% in 1988 to 48% in 2014 (Poeplau *et al.*, 2015a). In the same time period, three agricultural soil inventories have been conducted with SOC as a key target variable. In the first inventory, between 1988 and 1997, 3146 locations were sampled to a depth of 20 cm, whereas this number was reduced to 2034 in the second and third Inventory (2001-2007 and 2010-2018). In this whole period, the average SOC content of Swedish agricultural soils increased from 24.8 to 26.7 g kg^{-1}, which equals a relative increase of 7.7%. From the first to the third inventory, SOC increased in 18 out of the 21 counties of Sweden. The increase in ley per county was the most important explanatory variable, confirming the importance of perennial grasses for SOC storage. The doubling of the Swedish horse population since 1981, which mainly happened in the course of Swedish lifestyle changes, was identified as the most likely driver of increasing leys (R^2=0.72). Owning a horse for recreational purposes increased the demand for hay and established a new market for farmers, who could sell hay to the often wealthy horse owners at a good price. Approximately 1 ha of ley is needed to feed one horse per year, which means that 13% of the current Swedish agricultural land (cropland) is used for horses alone.

Another option to integrate grasses into agricultural crop rotations is the use of cover crops, also known as catch crops, which reduce nitrogen losses and soil erosion in regions where high autumn precipitation and intensive agriculture coincide. In recent years, the subsequent reduction in the fallow period has gained in importance, and has been shown to increase SOC stocks in arable systems (Poeplau and Don, 2015b). In Nordic countries, grass species like perennial ryegrass (*Lolium perenne* L.) or timothy (*P. pratense* L.) are often used as cover crops, undersown in the main crop. In three Swedish long-term experiments (16-24 years), we investigated the effect of undersown ryegrass on SOC stocks in the 0-20 cm soil depth. We found an average annual SOC sequestration rate of 0.32±0.28 Mg C ha^{-1} across experiments, which matches the value of a global meta-analysis on cover crop effects on SOC (Poeplau and Don, 2015b). The aboveground ryegrass biomass produced in the Swedish experiments ranged from 0.5 to 1 Mg drymass (DM) ha^{-1} yr^{-1}. A literature review revealed, that growth of grass cover crops strongly depends on temperature, having an upper limit of about 7 Mg DM ha^{-1} yr^{-1} at a mean annual temperature (MAT) of 16 °C and a lower limit of around 1 Mg DM ha^{-1} yr^{-1} in regions with MAT <7 °C. Nonetheless, even at low MAT of 4 °C on a test site in Southern Finland, undersown ryegrass and timothy had considerable yields and reduced N leaching (Känkänen and Eriksson, 2007). In consequence, in intensive cropping systems, perennial grasses have a great potential to improve soil fertility and enhance SOC stocks, while the opposite is true for forage-based cropping systems, which are changed into more cereal-based rotations (Bolinder *et al.*, 2010).

Experimental evidence for negative warming effects on grassland soil organic carbon stocks and soil structure

Studying and quantifying potential warming effects at an ecosystem scale is challenging and costly. Temperature manipulation studies are thus often short-term and restricted to one or two warming treatments, threshold values, or new steady state situations unknown. This in turn hampers accurate model predictions on long-term warming effects (Conant *et al.*, 2011).

In 2008, an earthquake shifted a geothermal channel to a mountain slope covered with unmanaged grassland and a small spruce plantation in the east of Reykjavik, Iceland. To analyse the effect of soil warming, research plots of different warming intensities were setup at these grasslands to study ecosystem-warming responses. In 2014, approximately six years after the earthquake, the grassland soil was sampled to study changes in bulk soil SOC and SOC fractions. These fractions were isolated following the notion that certain stabilization mechanisms in the soil, such as organo-mineral interactions, might lead to C pools that show a distinct response to warming. Five transects of six different warming intensities were sampled: ambient temperature, +0.6, +1.8, +3.9, +9.9, +16.3, and +~40 °C. The unwarmed grassland was dominated by common bent (*A. capillaris* L.), common meadow grass (*Poa pratensis* L.), meadow horsetail (*Equisetum pratense* L.) and meadow buttercup (*Ranunculis acris* L.). Interestingly, species composition changed only gradually in relation to warming intensities >10 °C, with increasing abundance of mosses. The +40 °C plots were entirely vegetated by mosses, so that C input can be considered negligible at this warming intensity. Soil samples in 0-10 cm (topsoil) and 20-30 cm depth (subsoil) were analysed (Poeplau *et al.*, 2017). Soil warming of 0.6 °C tended to increase topsoil and subsoil SOC contents by 22±43 and 27±54% respectively, while further warming led to exponential depletion of SOC. As much as 79±14% (topsoil) and 74±8% (subsoil) of SOC was lost in just six years (Figure 2). Drastic changes in SOC were also observed in less unrealistic warming intensities: +3.9 °C reduced SOC by 27±46 and 29±40%, without strong changes in species composition. Results demonstrate that potential changes in NPP could not buffer increased microbial activity and associated SOC decay. Indeed, soil warming resulted in neither increased plant productivity nor changes in ecosystem stoichiometry (plants and soil C:N ratios). This suggested that nitrogen was lost at a similar rate as carbon. However, under climate change, this might have been slightly different, since (1) warming comes from above and thus plants are less favoured than microbes, (2) global warming is associated with increased CO_2 and therefore with increased CO_2 fertilisation of plants, which was not given here, and (3) warming happened at once, not gradually. However, the very strong and comparatively long soil warming revealed interesting features of SOC response to warming; e.g. SOC fraction associated with stable aggregates, as well as the aggregates themselves were almost entirely depleted (Figure 2). We thus hypothesised that warming might have devitalised biotic aggregate-binding mechanisms such as fine roots, mucilage or arbuscular mycorrhizal fungi, leading to decreased SOC stabilisation. However, the opposite may also be the case; loss of SOC might have caused aggregate break-down, since organic matter as such is acknowledged to stabilize aggregates (Tisdall and Oades, 1982). In any case, the extreme warming treatments proved that loss of SOC is associated with strong soil structural changes, which in turn might feedback to plant productivity. Despite the fact that the geothermal experiment is located in Iceland on a very specific soil (Andosol), it is of high value for monitoring ecosystem changes in relation to warming.

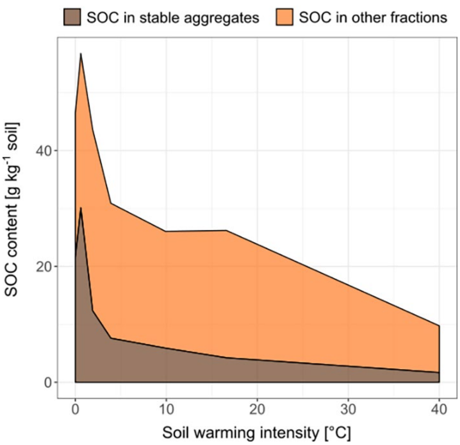

Figure 2. Response of soil organic carbon content (0-10 cm depth) to six years of geothermal soil warming on a subarctic grassland (adapted from Poeplau *et al.* (2017)).

Modelling climate change effects on German grassland soil organic carbon stocks

Climate change will also affect ecosystems and agriculture in temperate regions. To estimate SOC stock development in Southern German grassland soils, we applied the RothC model to a total of 30 permanent grassland sites in Bavaria (Wiesmeier *et al.*, 2016). Current SOC stocks and fraction (pool) distribution were known and carbon inputs were estimated by combining regional allocation coefficients and management information from each individual site. For each point, we run a climate model ensemble to predict temperature increase until the end of the century (A1B scenario, +3.3 °C).

Assuming unchanged management and carbon inputs, the model projected an average decrease in SOC stocks of 7.5 Mg C ha^{-1} (11%) until the end of the 21^{st} century. This was in line with other modelling studies (Senapati *et al.*, 2013; Xu *et al.*, 2011). To compensate for C losses caused by increased SOC mineralization, the required additional C input would be +26% compared to current C inputs, which highlights that SOC sequestration under climate change will be a challenge.

Conclusions

Growth of perennial grasses is associated with high net primary productivity, especially belowground, which favours sequestration of soil carbon. It has multiple benefits to include perennial grasses used in agricultural crop rotations. However, increasing SOC stocks in permanent grassland soils (that are not severely degraded) is a challenge, since this is often accompanied by climate-relevant trade-offs such as high CO_2 emissions during fertiliser production or *in situ* greenhouse gas emissions. In this respect, increasing the share of legumes to fix atmospheric nitrogen and increase microbial anabolism might be a way forward. Other multi-beneficial options, such as planting trees or hedges on grasslands and grassland renewal remain understudied in terms of their effects on SOC stocks. Finally, any effort to increase SOC stocks to mitigate climate change will be hampered by the effects resulting from climate change.

References

Balasubramanian D., Zhou W.-J., Ji H.-L., Grace J., Bai X.-L., Song Q.-H., Liu Y.-T., Sha L.-Q., Fei X.-H., Zhang X., Zhao J.-B., Zhao J.-F., Tan Z.-H. and, Zhang Y.-P. (2020) Environmental and management controls of soil carbon storage in grasslands of southwestern China. *Journal of Environmental Management* 254, 109810.

Batjes N.H. (2019) Technologically achievable soil organic carbon sequestration in world croplands and grasslands. *Journal of Land Degradation & Development* 30, 25-32.

Bolin B., Sukumar R., Ciais P., Cramer W., Jarvis P., Kheshgi H., Nobre C., Semenov S. and Steffen, W. (2000) Global perspective. In: Watson R.T., Bolin B., Ravindranath N.H., Verardo D.J. and Dokken D.J. (eds.) *Special report on land use, land-use change and forestry*. Cambridge, UK: Cambridge University Press.

Bolinder M.A., Kätterer T., Andrén O., Ericson L., Parent L.E. and Kirchmann H. (2010) Long-term soil organic carbon and nitrogen dynamics in forage-based crop rotations in Northern Sweden (63-64°N). *Agriculture, Ecosystems & Environment* 138, 335-342.

Ciais P. (2013) *Climate change 2013: the physical science basis*. Cambridge University Press.

Cleveland C.C. and Liptzin D. (2007) C: N: P stoichiometry in soil: is there a 'Redfield ratio' for the microbial biomass? *Biogeochemistry* 85, 235-252.

Conant R.T., Paustian K. and Elliott, E.T. (2001) Grassland management and conversion into grassland: effects on soil carbon. *Ecological Applications* 11, 343-355.

Conant R.T., Ryan M.G., Ågren G.I., Birge H.E., Davidson E.A., Eliasson P.E., Evans S.E., Frey S.D., Giardina C.P. and Hopkins F.M. (2011) Temperature and soil organic matter decomposition rates-synthesis of current knowledge and a way forward. *Global Change Biology* 17, 3392-3404.

Davidson E.A. and Janssens I.A. (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440, 165-173.

Debreczeni K. and Körschens M. (2003) Long-term field experiments of the world. *Archives of Agronomy and Soil Science* 49, 465-483.

Edwards R., Padella M., Giuntoli J., Koeble R., O'Connell A., Bulgheroni C. and Marelli L., 2017. *Definition of input data to assess GHG default emissions from biofuels in EU legislation*, Version 1c – July 2017. EUR28349 EN.

Fornara D.A., Banin L. and Crawley M.J. (2013) Multi-nutrient vs. nitroge-only effects on carbon sequestration in grassland soils. *Global Change Biology* 19, 3848-3857.

Global Soil Partnership (2017) *Global soil organic carbon map.* Leaflet. FAO, Rome, Italy.

Hermans C., Hammond J.P., White P.J. and Verbruggen N. (2006) How do plants respond to nutrient shortage by biomass allocation? *Trends in Plant Science* 11, 610-617.

Ignatieva M., Ahrné K., Wissman J., Eriksson T., Tidåker P., Hedblom M., Kätterer T., Marstorp H., Berg P. and Eriksson T. (2015) Lawn as a cultural and ecological phenomenon: a conceptual framework for transdisciplinary research. *Urban Forestry & Urban Greening* 14, 383-387.

Känkänen H. and Eriksson C., (2007) Effects of undersown crops on soil mineral N and grain yield of spring barley. *European Journal of Agronomy* 27, 25-34.

Kätterer T., Bolinder M., Berglund K. and Kirchmann, H. (2012) Strategies for carbon sequestration in agricultural soils in northern Europe. *Acta Agriculturae Scandinavica, Section A- Animal* Science 62, 181-198.

Kramberger B., Podvršnik M., Gselman A., Šuštar V., Kristl J., Muršec M., Lešnik M. and Škorjanc D. (2015) The effects of cutting frequencies at equal fertiliser rates on bio-diverse permanent grassland: soil organic C and apparent N budget. *Agriculture, Ecosystems & Environment* 212, 13-20.

Lange M., Eisenhauer N., Sierra C.A., Bessler H., Engels C., Griffiths R.I., Mellado-Vázquez P.G., Malik A.A., Roy J. and Scheu S. (2015) Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications* 6, 6707.

Leifeld J., Meyer S., Budge K., Sebastia M.T., Zimmermann M. and Fuhrer J. (2015) Turnover of grassland roots in mountain ecosystems revealed by their radiocarbon signature: role of temperature and management. *PLoS one* 10, e0119184.

Manzoni S., Taylor P., Richter A., Porporato A. and Ågren G.I. (2012) Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist* 196, 79-91.

Olff H., Berendse F. and De Visser W. (1994) Changes in nitrogen mineralization, tissue nutrient concentrations and biomass compartmentation after cessation of fertilizer application to mown grassland. *Journal of Ecology*, 611-620.

Poeplau C., Bolinder M.A., Eriksson J., Lundblad M. and Kätterer T. (2015a) Positive trends in organic carbon storage in Swedish agricultural soils due to unexpected socio-economic drivers. *Biogeosciences* 12, 3241-3251.

Poeplau C. and Don A. (2015b) Carbon sequestration in agricultural soils via cultivation of cover crops – a meta-analysis. *Agriculture, Ecosystems & Environment* 200, 33-41.

Poeplau C., Aronsson H., Myrbeck Å. and Kätterer T. (2015c) Effect of perennial ryegrass cover crop on soil organic carbon stocks in southern Sweden. *Geoderma Regional* 4, 126-133.

Poeplau C., Marstorp H., Thored K. and Kätterer T. (2016) Effect of grassland cutting frequency on soil carbon storage – a case study on public lawns in three Swedish cities. *SOIL* 2, 175-184.

Poeplau C., Kätterer T., Leblans N.I. and Sigurdsson B.D., (2017) Sensitivity of soil carbon fractions and their specific stabilization mechanisms to extreme soil warming in a subarctic grassland. *Global Change Biology* 23, 1316-1327.

Poeplau C., Zopf D., Greiner B., Geerts R., Korvaar H., Thumm U., Don A., Heidkamp A. and Flessa H., (2018) Why does mineral fertilization increase soil carbon stocks in temperate grasslands? *Agriculture, Ecosystems & Environment* 265, 144-155.

Poeplau C., Helfrich M., Dechow R., Szoboszlay M., Tebbe C.C., Don A., Greiner B., Zopf D., Thumm U., Korevaar H. and Geerts R., 2019. Increased microbial anabolism contributes to soil carbon sequestration by mineral fertilization in temperate grasslands. *Soil Biology and Biochemistry* 130, 167-176.

Rasse D.P., Rumpel C. and Dignac M.F., (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil* 269, 341-356.

Rumpel C., Crème A., Ngo P., Velásquez G., Mora M. and Chabbi A., (2015) The impact of grassland management on biogeochemical cycles involving carbon, nitrogen and phosphorus. *Journal of Soil Science and Plant Nutrition* 15, 353-371.

Senapati N., Smith P., Wilson B., Yeluripati J., Daniel H., Lockwood P. and Ghosh S.J., (2013) Projections of changes in grassland soil organic carbon under climate change are relatively insensitive to methods of model initialization. *European Journal of Soil Science* 64, 229-238.

Sochorová L., Jansa J., Verbruggen E., Hejcman M., Schellberg J., Kiers E.T. and Johnson N.C. (2016) Long-term agricultural management maximizing hay production can significantly reduce belowground C storage. *Agriculture, Ecosystems & Environment* 220, 104-114.

Sokol N.W., Kuebbing S.E., Karlsen-Ayala E. and Bradford M.A. (2019) Evidence for the primacy of living root inputs, not root or shoot litter, in forming soil organic carbon. *New Phytologist* 221, 233-246.

Spohn M., Klaus K., Wanek W. and Richter, A. (2016) Microbial carbon use efficiency and biomass turnover times depending on soil depth – implications for carbon cycling. *Soil Biology and Biochemistry* 96, 74-81.

Tisdall J.M. and Oades J.M. (1982) Organic matter and water-stable aggregates in soils. *European Journal of Soil Science* 33, 141-163.

Wiesmeier M., Poeplau C., Sierra C.A., Maier H., Frühauf C., Hübner R., Kühnel A., Spörlein P., Geuß U. and Hangen E. (2016) Projected loss of soil organic carbon in temperate agricultural soils in the 21st century: effects of climate change and carbon input trends. *Scientific Reports* 6, 32525.

Wohlfahrt G., Anderson-Dunn M., Bahn M., Balzarolo M., Berninger F., Campbell C., Carrara A., Cescatti A., Christensen T., Dore S., Eugster W., Friborg T., Furger M., Gianelle D., Gimeno C., Hargreaves K., Hari P., Haslwanter A., Johansson T., Marcolla B., Milford C., Nagy Z., Nemitz E., Rogiers N., Sanz M., Siegwolf R.W., Susiluoto S., Sutton M., Tuba Z., Ugolini F., Valentini R., Zorer R. and Cernusca A. (2008) Biotic, abiotic, and management controls on the net ecosystem CO2 exchange of European mountain grassland ecosystems. *Ecosystems* 11, 1338-1351.

Xu X., Liu W. and Kiely G. (2011) Modeling the change in soil organic carbon of grassland in response to climate change: effects of measured versus modelled carbon pools for initializing the Rothamsted Carbon model. *Agriculture, Ecosystems & Environment* 140, 372-381.

Biodiversity in intensive grasslands: is a compromise possible?

Bullock J.M.[1], Woodcock B.A.[1], Herzon I.[2] and Pywell R.F.[1]
[1]UK Centre for Ecology and Hydrology, Benson Lane, Wallingford, Oxfordshire, OX10 8BB, United Kingdom; [2]Department of Agricultural Sciences and HELSUS, P.O. Box 27, 00014 University of Helsinki, Finland

Abstract

Intensive grasslands are managed for production, while semi-natural grasslands provide biodiversity. Can we bridge the divide, given declines in biodiversity and the sustainability agenda? It is clear that very intensively managed grasslands are damaging to biodiversity, likely as much as intensive arable land. There are, however, methods to improve biodiversity on intensive grasslands. These include relatively 'light touch' approaches, e.g. small changes in management, such as decreasing cutting or grazing frequency, or reducing fertiliser use. Alternatively, field margins might be taken out of production. All these approaches have been shown to enhance plant, invertebrate and bird diversity. Adding a few plant species can enhance yields as well as biodiversity in low intensity grasslands. The most radical intervention is to restore semi-natural grasslands onto intensive grassland sites. These approaches will all likely come at a cost to production, but recent research is showing how compromises might be achievable. Increasing plant diversity, even in intensive grasslands, can benefit yield and revenues. Furthermore, considering a wider range of public benefits from grasslands, such as carbon storage, suggests mechanisms whereby farming for profit will also allow farming for biodiversity.

Keywords: management, production, restoration, semi-natural grasslands, sustainability

Introduction

There is a great divide in grassland research. Intensive grasslands (also agriculturally-improved, or production grasslands) are generally studied in terms of enhancing their agricultural production and developing efficient livestock systems. Natural and semi-natural grasslands (also conservation, or species-rich, traditional, or High Nature Value grasslands) are studied in terms of their biodiversity and wider environmental benefits (Bengtsson *et al.*, 2019; Bullock *et al.*, 2011). Individual researchers and research teams rarely cross over this divide. However, there is increasing policy interest in developing grassland systems that provide both production and environmental benefits (Pe'er *et al.*, 2019). There has been interest in the production potential of semi-natural grasslands, with the idea that increasing plant richness can boost production (Bullock *et al.*, 2007, Isselstein *et al.*, 2005; Schaub *et al.*, 2020). However, the relatively low production in non-intensive grasslands remains an issue and is the driver for the conversion of semi-natural grasslands to more productive agricultural systems (Bullock *et al.*, 2011).

It is much more rare to consider the biodiversity of intensive grasslands, or their potential to host biodiversity. There is scope for integrating at least some aspects of more traditional grazing management systems into productive agriculture to support not only productivity but also biodiversity. For example, the use of more complex legume-dominated swards to support yields under reduced fertiliser regimes. Here, we consider these issues within a wider discussion of: (1) intensive grasslands as a cause of biodiversity loss; (2) whether intensive grasslands provide any biodiversity benefit; (3) what can be done to improve biodiversity with no/little effect on production in intensive grasslands; and (4) approaches to restoring semi-natural grasslands on intensive grasslands.

Intensive grasslands as a cause of biodiversity loss

The conversion of traditional forms of agricultural grassland – semi-natural grasslands, and occasionally natural grasslands – to intensive grasslands leads to loss of biodiversity almost by definition. While European semi-natural grasslands are among the most floristically diverse habitats in the World (Wilson *et al.*, 2012), intensive grasslands are characterised by having very few plant species, several of which are often non-native cultivars. This plant biodiversity loss extends to other taxa. Reduced prey availability and more frequent cutting or grazing regimes is linked to declines in farmland bird species across Europe (Donald *et al.*, 2002; Strebel *et al.*, 2015; Vickery *et al.*, 2001). In general, semi-natural grassland hosts more bird species than intensive grassland (Barnett *et al.*, 2004; Woodhouse *et al.*, 2005). Similarly, more intensively managed grasslands have lower arthropod diversity than semi-natural grasslands, across a wide range of taxa (Attwood *et al.*, 2008; Simons *et al.*, 2015). This can be due to a reduction in floristic diversity, but also as a result of swards tending to be structurally homogenous and lacking many floristic structures upon which arthropods depend, e.g. flowers or seed heads (Woodcock *et al.*, 2007b). Frequent cutting regimes supported by inorganic fertiliser inputs also vastly decreases arthropod abundance and diversity in these swards (Humbert *et al.*, 2010). Below-ground taxa suffer as well. Changed soil characteristics in intensive grasslands, such as low organic matter and changed pH, decrease microbial biomass and growth (Malik *et al.*, 2018) and earthworm species richness (Johnston, 2019).

Is the semi-natural vs intensive grassland a fair comparison? That is, are intensive grasslands located where traditionally there were semi-natural grasslands? Our work in Dorset, a rural county on the south coast of England suggests the situation is more complex that this simple characterisation. In the 1930s, Dorset had ca. 152,000 ha of semi-natural grassland (Hooftman and Bullock, 2012). By 2000, there was less than 11,000 ha remaining. About 58,500 ha (39%) had been converted to intensive grassland, while ca. 65,500 ha (43%) had become arable land. This loss continues; between 1990 and 2015, 15% of semi-natural grasslands in Dorset were converted to arable and 65% to intensive grassland (Ridding *et al.*, 2020). Other major conversions over both time periods were to urbanisation and tree planting. Overall, conversion of this and other semi-natural habitats (heathland, broad-leaved woodland) in Dorset to intensive agriculture massively increased agricultural production, from an estimated £33M p.a. in the 1930s to £219M in 2000 (using prices for the year 2000) (Jiang *et al.*, 2013). The improved grassland contributed most to this, with an increased agricultural production over the whole of Dorset worth an extra £141M p.a. From a biodiversity point of view, any conversion of these species-rich habitats is a loss. In Dorset we found that plant species richness decreased from a mean of ca. 393 species per 2×2 km grid cell in the 1930s to 289 species in 2000 (Jiang *et al.*, 2013). But it is worth asking the question whether conversion to intensive grassland is the 'worst' that can happen to semi-natural habitats in terms of biodiversity.

Do intensive grasslands provide any biodiversity benefit?

Improved grasslands are not a wildlife desert. They generally have very low plant diversity, but they can provide habitat and resources for other taxa including invertebrates and birds. In England, Barnett *et al.* (2004) found twice the number of birds on improved than semi-natural grasslands, although this was due to large numbers of corvids and gulls. Species including snipe *Gallinago gallinago*, blackbird *Turdus merula,* and redwing *Turdus iliacus* were more common on semi-natural grassland. Jackdaw *Corvus monedula*, rook *Corvus frugilegus*, carrion crow *Corvus corone*, and starling *Sturnus vulgaris* were found more on intensive grassland, possibly because manure addition had led to an increase in invertebrate food quantity. Rutgers *et al.* (2016) found a higher abundance and no difference in diversity of earthworms in European intensive grasslands compared with semi-natural grasslands.

Intensive grasslands may, in some cases, be better for biodiversity compared to alternative agricultural land uses. Across Europe, Tsiafouli *et al.* (2015) found that species richness of earthworms, *Collembolans*, and oribatid mites was lower in arable systems than in intensive grasslands, as did Rutgers *et al.* (2016) for earthworms across Europe. A global meta-analysis by Attwood *et al.* (2008) showed that intensive grass has higher overall and decomposer arthropod species richness than cropped systems. But it should not be assumed that intensive grassland will always be the best intensive form of agriculture for biodiversity. For example, in a study across four European countries, farmed areas with flowering crops enhanced species richness of wild bees, in contrast to livestock systems (Le Feon *et al.*, 2010). The meta-analysis by Attwood *et al.* (2008) showed no difference between intensive grasslands and arable lands in arthropod predator species richness. Furthermore, *Miscanthus* bioenergy crops increased earthworm diversity compared to intensive grasslands (McCalmont *et al.*, 2017).

What can be done to improve biodiversity in intensive grasslands?

European agri-environment schemes have focused mostly on improving biodiversity in arable systems and fields. Interventions mostly implement a 'land-sparing' approach; removing some land from arable production – from field margins to whole fields – to provide resources and habitat for wild species (Rey Benayas and Bullock, 2012). Improving biodiversity and associated ecosystem services on intensive grassland is less well discussed or researched. Here, we consider various approaches that have been researched. To be clear, we focus on management of intensive grasslands and exclude management of semi-natural grasslands.

Changed management

As we showed above, low biodiversity and low plant species richness in intensive grasslands is linked to the intensity of management through cutting and/or grazing and fertiliser additions. So, can altered management enhance biodiversity, and what does this mean for production? A study on agriculturally-improved upland permanent pastures in Wales found that hay cutting alone or combined with aftermath grazing created more diverse plant swards with greater numbers of flowers than grazing alone, and this enhanced diversity of foliage-dwelling arthropods (Garcia and Fraser, 2019). Buckingham *et al.* (2011) showed that reducing the number of silage cuts and stopping aftermath grazing greatly increased the amount of ryegrass seed available. Use of Italian rather than perennial ryegrass also increased seed yield. In both cases, increased seed enhanced bird use of the grasslands. In general delaying mowing or grazing onset tends to benefit plants, invertebrate and birds, as shown by a Conservation Evidence review (https://www.conservationevidence.com/actions/131).

Reducing fertiliser or pesticide inputs can also enhance biodiversity in intensive grasslands. A Conservation Evidence review (https://www.conservationevidence.com/actions/139) considered 38 studies into this approach. Of these, 34 showed benefits to plant, invertebrate and bird abundance or species richness, but the others showed no, or slow effects. The aridity of intensive grassland soils is also an issue. Onrust *et al.* (2019) suggest replacing slurry- and slit injection-based management, with the use of farmyard manure to enhance surface soil moisture to benefit earthworms, and the birds that feed on them.

At larger scales, heterogeneity across grasslands has been emphasised as the best approach to benefit biodiversity. Research in England suggested that the diversity of bird species in intensive grassland landscapes through the winter would be enhanced by mosaics of fields managed as short-term leys and permanent pastures with low-intensity cattle grazing over the autumn and winter (Perkins *et al.*, 2000). Also, incorporation of some arable cropping into grassland-dominating landscapes is known to benefit mobile taxa (Robinson *et al.*, 2001).

While all above studies took place in intensive grasslands, the consequences for agricultural production and the farm economy in general were not covered. A few studies that looked at the farm economy reported costs associated with such modified management, which can be considerable for some production systems (e.g. Gottwald and Stein-Bachinger, 2017). It therefore remains unclear whether farmers can apply management to improve biodiversity without negative economic consequences. Indeed, many of these actions might be considered as leading to 'de-intensified' grassland (Isselstein *et al.*, 2005), with positive consequences for biodiversity, but having negative impacts on production. The effect, however, depends on the intensity of the baseline grassland management. Klaus *et al.* (2013) compared conventional grasslands in Germany with those managed organically, having lower fertiliser inputs and cutting frequency, although there were no differences in grazing intensity. Vegetation biomass (mean 3 Mg dry matter ha^{-1} for both conventional and organic grasslands) and nutrient content (used to indicate fodder quantity and quality) and soil fertility did not differ between management types, while arthropod diversity was increased by organic management. However, both grassland types can be regarded extensive in terms of their production.

Grassland margins

Similar to using field margins on arable land, field margins in intensive grassland can be used to enhance biodiversity. By contrast to arable systems, grassland field margins are not commonly used to enhance biodiversity, and can be complicated to manage. For example, livestock may need to be fenced off from these margins. Margins also represent land lost to production. While such margins have been shown to enhance production in arable systems by providing pest control, pollination and other services (Pywell *et al.*, 2015), the potential for such benefits has not been studied in intensive grasslands, and demand for some (pest control) is likely to be small.

There are, however, clear biodiversity benefits. In a large project in England, 10 m wide margins were created in intensive livestock farms and managed to increase sward architectural complexity through combinations of fertiliser, cattle grazing, and timing and height of cutting (Woodcock *et al.*, 2007a). The absence of inorganic fertiliser, combined with a reduction in the intensity of both cutting and grazing regimes, promoted floral species richness and sward architectural complexity. Beetle abundance and species richness was higher in the extensively managed margins compared the more intensively managed margins (Woodcock *et al.*, 2007a), as were these measures for a broader group of arthropods including bugs, planthoppers and spiders (Blake *et al.*, 2011; Woodcock *et al.*, 2009). These same treatments, along with treatments in which extra grass and herb species were sown, were assessed in terms of their ability to provide forage and structural resources for pollinators (Potts *et al.*, 2009). Bumblebees were most abundant, species-rich and diverse in the sown treatments and virtually absent from the grass-based treatment. Butterflies showed similar responses, albeit also being found in the more extensively managed margins. Cattle grazing in these margins had negative effects on biodiversity, and fencing was required to exclude livestock during the flowering period.

Other studies have found similar benefits. Fencing off grassland field margins alongside watercourses enhanced densities of bugs, harvestmen, sawflies and slugs (Cole *et al.*, 2012). In Ireland, Anderson *et al.* (2013) fenced off grassland field margins and found a greater diversity of a wide range of arthropods – beetles, bugs, flies, spiders and hymenopterans – in the margins compared to the intensively-managed field. These benefits increased if the margins were cultivated and sown with a wildflower and grass mixture. Birds can also benefit, and Wiggers *et al.* (2016) found that grass field margins contained more large aerial insects – which are fed on by waders – than in-field, and additional management of the grass field margin including the cessation of fertilisers and exclusion of grazers increased the number of aerial insects. Margins use a relatively small part of the field and so will have relatively limited effects on production (Pywell *et al.*, 2015). But, there can be impacts on farming operations, such as the need to fence margins to prevent access by livestock (Potts *et al.*, 2009).

Introducing plant species

There is increasing interest in the establishment of relatively biodiverse and productive grasslands, sometimes called herbal leys. This might offer a compromise between economically viable forage production and modest plant species richness gains using agricultural legumes and robust herbaceous species known to provide pollen and nectar resources. What evidence is there for the success of such approaches? A recent meta-analysis found that, globally, grassland net primary productivity (NPP) is higher in grasslands with legumes relative to grass-only controls (Ashworth *et al.*, 2018). However, this is a poor argument for increasing plant diversity *per se*, especially as adding one legume led to 52% increase in NPP, additional legume species added only a further 6%.

More biodiversity-focused studies give more promising results. We focus here on adding plant species to intensive grassland rather than restoration of semi-natural grassland, which is covered in the next section. Hofmann and Isselstein (2005) stopped fertiliser additions in a grassland and found that over-sowing with wildflower mixtures increased dry matter yield (by 23% on average) and crude protein concentrations, although digestibility was decreased. Woodcock *et al.* (2012) sowed an intensive, species-poor grassland in central England with treatments contrasting a mix of five productive grass species, a mix comprising the same grasses with the addition of seven agriculturally bred legume species, and finally a mix comprising the same grasses and legumes with the addition of six competitive native forb species. Here, again, fertiliser additions were stopped. The increasingly plant species-rich swards had higher diversity of phytophagous beetles (Woodcock *et al.*, 2012), predatory beetles and spiders (Woodcock *et al.*, 2013), and bees, butterflies and hoverflies (Woodcock *et al.*, 2014). The addition of only legumes had some benefit, although on their own the cover of these species (originating from agricultural seed stock) declined rapidly, so forb addition was important for maintaining biodiversity gains. In this and a parallel study in south-west England, the sowing of legumes and forbs gave higher yields of silage and increased nutrient quality of the herbage (Defra, 2013). Although the additional forb component resulted in slightly higher herbage production and quality over simply the presence of legumes, this effect was comparatively small when compared to the benefits of including legumes over a sward sown with just grasses. In addition, while the inclusion of legumes and forbs did not increase the daily rate of increase in livestock weight, they did result in swards that could be grazed for longer periods of time.

Approaches to restoring semi-natural grasslands on intensive grasslands

A radical approach to improving biodiversity in intensive grasslands is to restore them to semi-natural grassland vegetation. While there is a long history of establishing semi-natural grasslands on arable land (Pywell *et al.*, 2002; Torok *et al.*, 2011), there has been less work on restoring from intensive grasslands. Indeed, in general it seems easier to achieve such restoration on arable than intensive grassland sites, where the intact vegetation provides poor opportunities for introduced species to establish and survive (Kiehl *et al.*, 2010). Furthermore, high soil fertility in intensive grasslands can limit success in introducing plant species (Janssens *et al.*, 1998).

Studies into methods to introduce species to intensive grasslands have found similar results across Europe. In an experiment in England, severe disturbance involving turf removal followed by seed addition by sowing was the most effective and reliable means of increasing plant diversity (Pywell *et al.*, 2007). Disturbance by multiple harrowing was moderately effective and was enhanced by molluscicide application to reduce seedling herbivory and by sowing the hemiparasite *Rhinanthus minor* to reduce competition from grasses. Low-level disturbance by grazing or slot-seeding was ineffective, and fertiliser addition had no effect. In Germany, introduction of seed in hay taken from semi-natural grasslands was most successful when combined with deep ploughing, but shallow tillage also gave good establishment of target species, and no soil disturbance resulted in poor establishment (Bischoff *et al.*, 2018). In essence, all studies have found successful restoration on intensive grasslands requires adding seeds, for example by sowing or hay from donor sites, along with severe soil disturbance, for example by rotovating, harrowing, or ploughing, to decrease competition (Schmiede *et al.*, 2012; Sullivan *et al.*, 2020).

Using *R. minor* or related species to reduce competition from grasses has been found to be beneficial in other projects (Bullock and Pywell, 2005; Pywell *et al.*, 2004), and this could be a less extreme approach to reducing competition. But establishment of this species is erratic, leading to variable outcomes (Hellström *et al.*, 2011; Mudrak *et al.*, 2014), and it has not been tested across a range of countries.

Implications

It is clear that intensification of both grassland and arable systems has led to biodiversity loss (Stoate *et al.*, 2009). Furthermore, because in some parts of Europe a high proportion of biomass from arable crops is being fed to animals, both grassland and arable can be regarded as parts of the same livestock production systems. In high yielding production systems, grassland and arable management is intensive. But, recent scenarios demonstrate that under a reduced share of animal protein in human diets, it is feasible to achieve reduction of either management intensity (e.g. organic management, Muller *et al.* (2017)) or the amount of land under crops and intensive grass needed for food production (Berners-Lee *et al.*, 2018).

We have shown there are a number of well-demonstrated approaches to enhance biodiversity in intensive grasslands, not only for plants but also for other taxa including birds and invertebrates. These range from changing management intensity such as cutting frequency or fertiliser application, to setting small parts aside such as field margins, to adding plant species across the whole grassland from a few to many.

But, increasing biodiversity in grasslands is likely to come at a cost to production. At the extreme, production will decline greatly when restoring intensive grasslands to a semi-natural state, mirroring the large differences in production between semi-natural and intensive grasslands (Bullock *et al.*, 2011). Production aside, it is clear that semi-natural grasslands provide high levels of many ecosystem services compared to intensive grasslands (Bengtsson *et al.*, 2019; Bullock *et al.*,2011). It is therefore worth considering how to bring semi-natural, or more biodiverse, grasslands into the mainstream of agriculture. As we have pointed out, the grassland production and the grassland conservation communities tend to work separately. As a result, there is little work contrasting production in intensive grasslands to production in grasslands where biodiversity is enhanced. As it is assumed there will be a shortfall in production and so income, agri-environment schemes, which compensate farmers for income foregone, are the standard mechanism to achieve this integration of production and biodiversity conservation. Another and more forward-thinking approach would be to consider better how to utilise semi-natural grasslands for production in sustainable livestock systems, which avoid competition for arable land between human food and animal forage (Röös *et al.* 2016).

To this end, an increasing number of studies are demonstrating that increased plant diversity can benefit grassland production. For example, a long-term study contrasting non-fertilised, restored grasslands with seven grasses *vs* those sown with 11 grasses and 28 forbs found after eight years, that the species-rich treatment had an average 43% higher hay yield than the species-poor treatment (Bullock *et al.*, 2007). In a broader scale study, Schaub *et al.* (2020) contrasted extensive high-diversity systems and intensive low-diversity systems, considering both biomass yield and forage quality to assess revenues for milk production. Independent of management intensity, they found a positive relationship between plant diversity and potential revenues from milk production. Unsurprisingly however, revenues were lowest in the extensive systems, and highest in the most intensive systems.

As the sustainability agenda increases in importance, economic considerations other than production might become critical. For example, considering the economic benefits of both forage yield and carbon storage, Binder *et al.* (2018) calculated that even a profit-maximizing farmer would favour a diverse mix of species, with optimal richness falling between the low levels found in intensive grasslands and the high levels found in semi-natural grasslands. Finally, as well as in-field approaches, it may be possible to seek compromises between production and biodiversity by extensifying management of a proportion of grasslands in a landscape, while maintaining or even increasing intensity of management in other grasslands (Qi *et al.*, 2018). Supporting farmers with public payments for multiple public goods from grasslands, beyond their production values, remains a promising development agenda (Bengtsson *et al.* 2019).

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References

Anderson A., Carnus T., Helden A.J., Sheridan H. and Purvis G. (2013) The influence of conservation field margins in intensively managed grazing land on communities of five arthropod trophic groups. *Insect Conservation and Diversity* 6, 201-211.

Ashworth A.J., Toler H.D., Allen F.L. and Auge R.M. (2018) Global meta-analysis reveals agro-grassland productivity varies based on species diversity over time. *PloS One* 13, e0200274.

Attwood S.J., Maron M., House A.P.N. and Zammit C. (2008) Do arthropod assemblages display globally consistent responses to intensified agricultural land use and management? *Global Ecology and Biogeography* 17, 585-599.

Barnett P.R., Whittingham M.J., Bradbury R.B. and Wilson J.D. (2004) Use of unimproved and improved lowland grassland by wintering birds in the UK. *Agriculture Ecosystems & Environment* 102, 49-60.

Bengtsson J., Bullock J.M., Egoh B., Everson C., Everson T., O'Connor T., O'Farrell P.J., Smith H.G. and Lindborg R. (2019) Grasslands—more important for ecosystem services than you might think. *Ecosphere* 10, e02582.

Berners-Lee M., Kennelly C., Watson R. and Hewitt C.N. (2018) Current global food production is sufficient to meet human nutritional needs in 2050 provided there is radical societal adaptation. *Elementa-Science of the Anthropocene* 6(1), 52.

Binder S., Isbell F., Polasky S., Catford J.A. and Tilman D. (2018) Grassland biodiversity can pay. *Proceedings of the National Academy of Sciences of the USA* 115, 3876-3881.

Bischoff A., Hoboy S., Winter N. and Warthemann G. (2018) Hay and seed transfer to re-establish rare grassland species and communities. How important are date and soil preparation? *Biological Conservation* 221, 182-189.

Blake R.J., Woodcock B.A., Ramsay A.J., Pilgrim E.S., Brown V.K., Tallowin J.R. and Potts S.G. (2011) Novel margin management to enhance Auchenorrhyncha biodiversity in intensive grasslands. *Agriculture Ecosystems & Environment* 140, 506-513.

Buckingham D.L., Bentley S., Dodd S. and Peach W.J. (2011) Seeded ryegrass swards allow granivorous birds to winter in agriculturally improved grassland landscapes. *Agriculture Ecosystems & Environment* 142, 256-265.

Bullock J.M., Jefferson R.G., Blackstock T.H., Pakeman R.J., Emmett B.A., Pywell R.F., Grime J.P. and Silvertown J. (2011) *Semi-natural grasslands*. The UK National Ecosystem Assessment Technical Report. UNEP-WCMC, Cambridge, UK.

Bullock J.M. and Pywell R.F. (2005) Rhinanthus species, a tool for restoring diverse grassland? *Folia Geobotanica* 40, 273-288.

Bullock J.M., Pywell R.F. and Walker K.J. (2007) Long-term enhancement of agricultural production by restoration of biodiversity. *Journal of Applied Ecology* 44, 6-12.

Cole L.J., Brocklehurst S., McCracken D.I., Harrison W. and Robertson D. (2012) Riparian field margins, their potential to enhance biodiversity in intensively managed grasslands. *Insect Conservation and Diversity* 5, 86-94.

Defra (2013) *Widescale Enhancement of Biodiversity*. http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=17734.

Donald P.F., Evans A.D., Muirhead L.B., Buckingham D.L., Kirby W.B. and Schmitt S.I.A. (2002) Survival rates, causes of failure and productivity of Skylark *Alauda arvensis* nests on lowland farmland. *Ibis* 144, 652-664.

Garcia R.R. and Fraser M.D. (2019) Impact of management on foliage-dwelling arthropods and dynamics within permanent pastures. *Scientific Reports* 9, 11090.

Gottwald F. and Stein-Bachinger K. (2017) 'Farming for Biodiversity'—a new model for integrating nature conservation achievements on organic farms in north-eastern Germany. *Organic Agriculture*. DOI 10.1007/s13165-017-0198-2

Hellström K., Bullock J.M. and Pywell R.F. (2011) Testing the generality of hemiparasitic plant effects on mesotrophic grasslands – a multi-site experiment. *Basic and Applied Ecology* 12, 235-243.

Hofmann M. and Isselstein J. (2005) Species enrichment in an agriculturally improved grassland and its effects on botanical composition, yield and forage quality. *Grass and Forage Science* 60, 136-145.

Hooftman D.A.P. and Bullock J.M. (2012) Mapping to inform conservation – a case study of changes in semi-natural habitats and their connectivity over 70 years. *Biological Conservation* 145, 30-38.

Humbert J.-Y., Ghazoul J., Sauter G.J. and Walter T. (2010) Impact of different meadow mowing techniques on field invertebrates. *Journal of Applied Entomology* 134, 592-599.

Isselstein J., Jeangros B. and Pavlu V. (2005) Agronomic aspects of biodiversity targeted management of temperate grasslands in Europe – A review. *Agronomy Research* 3, 139-151.

Janssens F., Peeters A., Tallowin J.R.B., Smith R.E.N., Bakker J.P., Bekker R.M., Verweij G.L., Fillat F., Chocarro C., and Oomes M.J.M. (1998) Relationship between soil chemical factors and grassland diversity. *Plant and Soil* 202, 69-78.

Jiang M., Bullock J.M. and Hooftman D.A.P. (2013) Mapping ecosystem service and biodiversity changes over 70 years in a rural English county. *Journal of Applied Ecology* 50, 841-850.

Johnston A.S.A. (2019) Land management modulates the environmental controls on global earthworm communities. *Global Ecology and Biogeography* 28, 1787-1795.

Kiehl K., Kirmer A., Donath T. W., Rasran L. and Holzel N. (2010) Species introduction in restoration projects - Evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. *Basic and Applied Ecology* 11, 285-299.

Klaus V.H., Kleinebecker T., Prati D., Gossner M.M., Alt F., Boch S., Gockel S., Hemp A., Lange M., Müller J., Oelmann Y., Pašalić E., Renner S.C., Socher S.A., Türke M., Weisser W.W., Fischer M. and Hölzel N. (2013) Does organic grassland farming benefit plant and arthropod diversity at the expense of yield and soil fertility? *Agriculture, Ecosystems & Environment* 177, 1-9.

Le Feon V., Schermann-Legionnet A., Delettre Y., Aviron S., Billeter R., Bugter R., Hendrickx F. and Burel F. (2010) Intensification of agriculture, landscape composition and wild bee communities – a large scale study in four European countries. *Agriculture Ecosystems & Environment* 137, 143-150.

Malik A.A., Puissant J., Buckeridge K.M., Goodall T., Jehmlich N., Chowdhury S., Gweon H.S., Peyton J.M., Mason K.E., Van Agtmaal M., Blaud A., Clark I.M., Whitaker J., Pywell R.F., Ostle N., Gleixner G. and Griffiths R.I. (2018) Land use driven change in soil pH affects microbial carbon cycling processes. *Nature Communications* 9, 3591.

McCalmont J.P., Hastings A., McNamara N.P., Richter G.M., Robson P., Donnison I.S. and Clifton-Brown J. (2017) Environmental costs and benefits of growing Miscanthus for bioenergy in the UK. *Global Change Biology Bioenergy* 9, 489-507.

Mudrak O., Mladek J., Blazek P., Leps J., Dolezal J., Nekvapilova E. and Tesitel J. (2014) Establishment of hemiparasitic Rhinanthus spp. in grassland restoration, lessons learned from sowing experiments. *Applied Vegetation Science* 17, 274-287.

Muller A., Schader C., El-Hage Scialabba N., Brüggemann J., Isensee A., Erb K.-H., Smith P.,. Klocke P., Leiber F., Stolze M. and Niggli U. (2017) Strategies for feeding the world more sustainably with organic agriculture. *Nature Communications* 8, 1290.

Onrust J., Wymenga E., Piersma T. and Olff H. (2019) Earthworm activity and availability for meadow birds is restricted in intensively managed grasslands. *Journal of Applied Ecology* 56, 1333-1342.

Pe'er G., Zinngrebe Y., Moreira F., Sirami C., Schindler S., Müller R., Bontzorlos V., Clough D., Bezák P., Bonn A., Hansjürgens B., Lomba A., Möckel S., Passoni G., Schleyer C., Schmidt J. and Lakner S. (2019) A greener path for the EU Common Agricultural Policy. *Science* 365, 449-451.

Perkins A.J., Whittingham M.J., Bradbury R.B., Wilson J.D., Morris A.J. and Barnett P.R. (2000) Habitat characteristics affecting use of lowland agricultural grassland by birds in winter. *Biological Conservation* 95, 279-294.

Potts S.G., Woodcock B.A., Roberts S.P.M., Tscheulin T., Pilgrim E.S., Brown V.K. and Tallowin J.R. (2009) Enhancing pollinator biodiversity in intensive grasslands. *Journal of Applied Ecology* 46, 369-379.

Pywell R.F., Bullock J.M., Hopkins A., Walker K.J., Sparks T.H., Burke M.J.W. and Peel S. (2002) Restoration of species-rich grassland on arable land – assessing the limiting processes using a multi-site experiment. *Journal of Applied Ecology* 39, 294-309.

Pywell R.F., Bullock J.M., Tallowin J.B., Walker K.J., Warman E.A. and Masters G. (2007) Enhancing diversity of species-poor grasslands, an experimental assessment of multiple constraints. *Journal of Applied Ecology* 44, 81-94.

Pywell R.F., Bullock J.M., Walker K.J., Coulson S.J., Gregory S.J. and Stevenson M.J. (2004) Facilitating grassland diversification using the hemiparasitic plant *Rhinanthus minor*. *Journal of Applied Ecology* 41, 880-887.

Pywell R.F., Heard M.S., Woodcock B.A., Hinsley S., Ridding L., Nowakowski M. and Bullock J.M. (2015) Wildlife-friendly farming increases crop yield – evidence for ecological intensification. *Proceedings of the Royal Society B – Biological Sciences* 282, 20151740.

Qi A., Holland R.A., Taylor G. and Richter G.M. (2018) Grassland futures in Great Britain – productivity assessment and scenarios for land use change opportunities. *Science of the Total Environment* 634, 1108-1118.

Rey Benayas J. and Bullock J. (2012) Restoration of biodiversity and ecosystem services on agricultural land. *Ecosystems* 15, 883-899.

Ridding L.E., Watson S.C.L., Newton A.C., Rowland C.S. and Bullock J.M. (2020) Ongoing, but slowing, habitat loss in a rural landscape over 85 years. *Landscape Ecology* 35, 257-273.

Robinson R.A., Wilson J.D. and Crick H.Q.P. (2001) The importance of arable habitat for farmland birds in grassland landscapes. *Journal of Applied Ecology* 38, 1059-1069.

Röös E., Patel M., Spångberg J., Carlsson G. and Rydhmer L. (2016) Limiting livestock production to pasture and by-products in a search for sustainable diets. *Food Policy*, 58, 1-13. DOI 10.1016/j.foodpol.2015.10.008

Rutgers M., Orgiazzi A., Gardi C., Römbke J., Jänsch S., Keith A.M., Neilson R., Boag B., Schmidt O., Murchie A.K., Blackshaw R.P., Pérès G., Cluzeau D., Guernion M., Briones M.J.I., Rodeiro J., Piñeiro R., Cosín D.J. D., Sousa J.P., Suhadolc M., Kos I., Krogh P.-H., Faber J.H., Mulder C., Bogte J.J., Van Wijnen H.J., Schouten A.J. and De Zwart D. (2016) Mapping earthworm communities in Europe. *Applied Soil Ecology* 97, 98-111.

Schaub S., Finger R., Leiber F., Probst S., Kreuzer M., Weigelt A., Buchmann N. and Scherer-Lorenzen M. (2020) Plant diversity effects on forage quality, yield and revenues of semi-natural grasslands. *Nature Communications* 11, 768.

Schmiede R., Otte A. and Donath T.W. (2012) Enhancing plant biodiversity in species-poor grassland through plant material transfer - the impact of sward disturbance. *Applied Vegetation Science* 15, 290-298.

Simons N.K., Gossner M.M., Lewinsohn T.M., Lange M., Turke M. and Weisser W.W. (2015) Effects of land-use intensity on arthropod species abundance distributions in grasslands. *Journal of Animal Ecology* 84, 143-154.

Stoate C., Báldi A., Beja P., Boatman N.D., Herzon I., Van Doorn A., De Snoo G.R., Rakosy L., Ramwell C. (2009) Ecological impacts of early 21st century agricultural change in Europe - A review. *Journal of Environmental Management* 91, 22-46.

Strebel G., Jacot A., Horch P. And Spaar R. (2015) Effects Of Grassland Intensification On Whinchats Saxicola Rubetra And Implications For Conservation In Upland Habitats. *Ibis* 157, 250-259.

Sullivan E., Hall N. and Ashton P. (2020) Restoration of upland hay meadows over an 11-year chronosequence – an evaluation of the success of green hay transfer. *Restoration Ecology* 28, 127-137.

Torok P., Vida E., Deak B., Lengyel S. and Tothmeresz B. (2011) Grassland restoration on former croplands in Europe – an assessment of applicability of techniques and costs. *Biodiversity and Conservation* 20, 2311-2332.

Tsiafouli M.A., Thebault E., Sgardelis S.P., De Ruiter P.C., Van der Putten W.H., Birkhofer K., Hemerik L., De Vries F.T., Bardgett R.D., Brady M.V., Bjornlund L., Jorgensen H.B., Christensen S., Hertefeldt T.D., Hotes S., Hol W.H.G., Frouz J., Liiri M., Mortimer S.R., Setala H., Tzanopoulos J., Uteseny K., Pizl V., Stary J., Wolters V. and Hedlund K. (2015) Intensive agriculture reduces soil biodiversity across Europe. *Global Change Biology* 21, 973-985.

Vickery J.A., Tallowin J.R., Feber R.E., Asteraki E.J., Atkinson P.W., Fuller R.J. and Brown V.K. (2001) The management of lowland neutral grasslands in Britain – effects of agricultural practices on birds and their food resources. *Journal of Applied Ecology* 38, 647-664.

Wiggers J., Van Ruijven J., Berendse F. and De Snoo G.R. (2016) Effects of grass field margin management on food availability for Black-tailed Godwit chicks. *Journal for Nature Conservation* 29, 45-50.

Wilson J.B., Peet R.K., Dengler J. and Pärtel M. (2012) Plant species richness – the world records. *Journal of Vegetation Science* 23, 796-802.

Woodcock B.A., Bullock J.M., Nowakowski M., Orr R., Tallowin J.R.B. and Pywell R.F. (2012) Enhancing floral diversity to increase the robustness of grassland beetle assemblages to environmental change. *Conservation Letters* 5, 459-469.

Woodcock B.A., Potts S.G., Pilgrim E., Ramsay A.J., Tscheulin T., Parkinson A., Smith R.E.N., Gundrey A.L., Brown V.K. and Tallowin J.R. (2007a) The potential of grass field margin management for enhancing beetle diversity in intensive livestock farms. *Journal of Applied Ecology* 44, 60-69.

Woodcock B.A., Potts S.G., Tscheulin T., Pilgrim E., Ramsey A.J., Harrison-Cripps J., Brown V.K. and Tallowin J.R. (2009) Responses of invertebrate trophic level, feeding guild and body size to the management of improved grassland field margins. *Journal of Applied Ecology* 46, 920-929.

Woodcock B.A., Potts S.G., Westbury D.B., Ramsay A.J., Lambert M., Harris S.J. and Brown V.K. (2007b) The importance of sward architectural complexity in structuring predatory and phytophagous invertebrate assemblages. *Ecological Entomology* 32, 302-311.

Woodcock B.A., Savage J., Bullock J.M., Nowakowski M., Orr R., Tallowin J.R.B. and Pywell R.F. (2013) Enhancing beetle and spider communities in agricultural grasslands – the roles of seed addition and habitat management. *Agriculture Ecosystems & Environment* 167, 79-85.

Woodcock B.A., Savage J., Bullock J.M., Nowakowski M., Orr R., Tallowin J.R.B. and Pywell R.F. (2014) Enhancing floral resources for pollinators in productive agricultural grasslands. *Biological Conservation* 171, 44-51.

Woodhouse S.P., Good J.E.G. Lovett A.A., Fuller R.J. and Dolman P.M. (2005) Effects of land-use and agricultural management on birds of marginal farmland, a case study in the Llyn peninsula, Wales. *Agriculture Ecosystems & Environment* 107, 331-340.

A comparison of dairy cow methane losses from grazed and confined diets

Fitzpatrick E.[1,2], O'Donovan M.[1], Condon T.[1], Gilliland T.J.[2] and Hennessy D.[1]
[1] Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland; *[2] Institute for Global Food Security, Queen's University Belfast, N. Ireland, United Kingdom*

Abstract

The chemical composition of a dairy cow's diet influences enteric methane (CH_4) emissions due to the interactions of feed material and various integrated microbial species in the rumen. Agriculture is responsible for 13.5% of global greenhouse gas (GHG) emissions. Methane gas from enteric CH_4 fermentation accounts for 13.9% of total Irish GHG emissions. The objective of this experiment was to examine the effect of dairy cow diet on enteric CH_4 emissions. A farm scale experiment was carried out in 2019, comprising three treatment groups: 'TMR' (grass silage, maize silage and concentrate), 'GR250' grazed grass only receiving 250 kg N ha^{-1} and 'CL150' grazed grass-white clover receiving 150 kg N ha^{-1}. Methane emissions were measured in July and September using the calibrated sulphur hexafluoride (SF_6) tracer gas technique on 10 spring-calving dairy cows in each group. The differences in CH_4 emissions from the three diets changed between the July and September samplings, and given the associated differences in cow body weight and condition it was concluded that factors, such as dry matter intake, forage quality and lactation stage were likely determinant drivers that required further examination.

Keywords: white clover, grass, total mixed ration, methane emissions, diet

Introduction

Methane (CH_4) is a potent GHG which has a global warming potential 28 times greater than carbon dioxide (CO_2) on a 100-year time frame. Mitigation of enteric CH_4 emissions from ruminant production has been under scrutiny recently due to CH_4 enrichment of the atmosphere and its contribution to climate change (Munoz *et al.*, 2016). Methane is produced as a by-product of anaerobic respiration within the rumen and this is strongly influenced by the chemical composition of the diet (Beauchemin *et al.*, 2008). Fermentation of carbohydrates in the rumen from the integrated activities of various microbial species causes the conversion of feed material to CH_4. Methane represents a loss of 5 to 7% of dietary energy depending on the proportion of forage consumed in the diet (Hristov *et al.*, 2013). Forage is a vital component of a dairy cow diet; however, the high neutral detergent fibre (NDF) concentration associated with forage accelerates acetic acid production in the rumen and results in CH_4 production (Brask *et al.*, 2013). Many countries, including Ireland, have a commitment to substantially decrease their total greenhouse gas (GHG) emissions including CH_4 emissions from livestock. Therefore, CH_4 must be reduced on a per animal basis to improve the environmental sustainability of milk production. Improving the digestibility of the diet results in a fermentation profile within the rumen that causes conditions which are unfavourable for CH_4 production (Munoz *et al.*, 2016). Other benefits of improving feed quality are greater intakes, improved animal production, reduced absolute maintenance energy requirements and less CH_4 per unit of animal production (Hristov *et al.*, 2013). The inclusion of white clover improves the feed value of the herbage; however there is contradicting literature regarding its ability for reducing dairy cow CH_4 emissions. The aim of this experiment was to measure the effect of dairy cow diet on enteric methane emissions.

Materials and methods

A full lactation farm-scale experiment was undertaken at Teagasc, AGRIC, Moorepark, Fermoy, Co. Cork, Ireland from February 2019 to November 2019. Three treatments we imposed: total mixed ration

(TMR), grass only swards receiving 250 kg N ha^{-1} (GR250) and grass-clover swards receiving 150 kg N ha^{-1} (CL150). The GR250 swards were a 50:50 perennial ryegrass (*Lolium perenne* L.) mixture of AstonEnergy (tetraploid) and Tyrella (diploid) and the CL150 swards had the same grasses plus a 50:50 blend of Chieftan and Crusader medium leaf white clovers (*Trifolium repens* L.). The TMR treatment involved cows housed indoors and fed 8.5 kg of concentrate, 4.5 kg of maize silage and 9 kg of grass silage on a dry matter (DM) per cow basis, using an electronic controlled Roughage Intake Control system feed bins (Hokofarm Group B.V., the Netherlands). This was fed daily at 08:00 h. The GR250 and CL150 treatments were stocked at 2.74 cows ha^{-1} and comprised a daily herbage allowance of 17 kg DM per cow and an individual concentrate allocation of 1 kg per cow per day. Target pre-grazing herbage mass was 1,400-1,600 kg DM ha^{-1} and the target post grazing height was 4 cm. Thirty spring-calving dairy cows (10 for each treatment) were selected and balanced based on breed (Friesian and Friesian×Jersey dairy cows), mean calving date (03/02/2019), lactation number (2.63), milk yield (25 kg) and milk solids (fat+protein) production (2.34 kg). Enteric CH_4 emissions were measured using the calibrated sulphur hexafluoride (SF_6) tracer gas technique (Deighton *et al.*, 2013). Cows were orally dosed with permeation tubes containing 2.5 g of SF_6 seven days before the initial gas sampling commended. Each cow carried a 2.14 L gas collection canister at an initial pressure of 900 kPa. An ambient air sample was also collected in an identical canister to measure CH_4 and SF_6 beside the grazed areas and in the confinement shed. Gas measurements were taken continuously for 6 days and were measured at 2 different stages throughout lactation (July and September). The canisters were changed daily after morning milking and the concentration of SF_6 and CH_4 were determined using gas chromatography with an Ailgent 3800 GC. Milk yield was recorded daily (Dairymaster, Causeway, Co. Kerry, Ireland) and milk composition (fat and protein concentrations) was measured weekly using MilkoScan 203 (DK-3400, Foss Electric, Hilerod, Denmark). Data were analysed in SAS using Proc Mixed with terms for treatment, time (measurement period) and associated interactions. Data were estimated using LSMEANS and differences were compared at a 5% significance level.

Results and discussion

Daily milk yield and milk solids yield were significantly greater (P<0.001) for the TMR treatment (24.0 and 2.00 kg cow^{-1}, respectively) compared to the CL150 (19.6 kg cow^{-1} and 1.66 kg cow^{-1}, respectively) and GR250 (19.4 kg cow^{-1} and 1.66 kg cow^{-1}, respectively) treatments, which did not differ from each other. The TMR treatment had a lower (P<0.05) milk protein content (36 g kg^{-1}) compared to CL150 and GR250, which both had a protein content of 37.5 g kg^{-1}. There was a significant (P<0.001) interaction between treatment and the measurement period for all emission values. In July (week 23 of lactation) there were no treatment differences in CH_4 emissions per kg milk or milk solids produced but in September (week 32 of lactation) cows grazing CL150 swards had significantly (P<0.05) greater emissions per kg milk and milk solids (MS) produced compared to the other two diets (Table 1). On both sampling occasions the TMR diet resulted in significantly (P<0.001) greater daily CH_4 emissions per cow than CL150 and GR250; however, the ranking of the CL150 and GR250 diets reversed between the two sampling occasions. It was also notable that body weight was greatest for the TMR cows, intermediate for CL150 cows and least for GR250 cows (548 kg, 518 kg and 538 kg, respectively, P<0.05) and likewise for body condition score (3.15, 3.13 and 3.08, respectively, P<0.001).

The differing responses between the two sample dates indicates that there are additional underlying parameters of these dietary systems influencing cow performance. The significantly greater daily CH_4 emissions per cow from TMR clearly reflected the higher productivity of these animals, particularly in July. Given the differences in body weight and condition score, one possible factor would be whether the diets were equally meeting the energy needs of the cows. Hence, further examination of factors such as DM intake, forage quality and stage of lactation are needed to provide a full understanding of the effect of diet on enteric CH_4 emissions.

Table 1. Methane emissions from spring-calving dairy cows on different forage diets.[1]

		CL150	GR250	TMR	SE	Treatment
July 2019	g CH_4 kg^{-1} MS	257	271	292	20.0	NS
	g CH_4 kg^{-1} MY	20.7	23.0	23.1	1.79	NS
	g CH_4 cow^{-1} day^{-1}	396^a	416^a	536^b	24.1	<0.001
September 2019	g CH_4 kg^{-1} MS	367^a	288^b	298^b	19.4	<0.05
	g CH_4 kg^{-1} MY	33.8^a	27.3^b	26.23^b	2.25	<0.05
	g CH_4 cow^{-1} day^{-1}	520^a	443^b	578^c	22.7	<0.001

[1] Total mixed ration (TMR) diet: grass, silage, maize and concentrate; GR250 diet: grazed grass receiving 250 kg N ha^{-1}; CL150 diet: grazed grass-white clover receiving 150 kg N ha^{-1}. Within rows, treatment values with differing lowercase superscript differ significantly (P<0.05); NS = not significant; SE = standard error; MS = milk solids; MY = milk yield.

Conclusions

Methane emissions per kg MS and milk yield were similar across treatments in July, but greater on the CL150 treatment compared to GR250 and TMR in September. Methane emissions per cow were greatest for the TMR treatment reflecting the greater milk production per cow compared to the other two treatments.

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References

Beauchemin K.A., Kreuzer M., O'Mara F. and McAllister T.A. (2008) Nutritional management for enteric methane abatement: a review. *Australian Journal of Experiemntal Agriculture* 48, 21-27.

Brask M., Lund P., Hellwing A.L.F., Poulsen M. and Weisbjerg M.R. (2013) Enteric methane production, digestibility and rumen fermentation in dairy cows fed different forages with and without rapeseed fat supplementation. *Animal Feed Science and Technology* 184, 67-79.

Deighton M.H., O'Loughlin B.M., Williams S.R.O., Moate P.J., Kennedy E., Boland T.M. and Eckard R.J. (2013) Declining sulphur hexafluoride permeability of polytetrafluoroethylene membranes causes overestimation of calculated ruminant methane emissions using the tracer technique. *Animal Feed Science and Technology* 183, 86-95.

Hristov A.N., Ott T., Tricarico J., Rotz A., Waghorn G., Adesogan A., Dijkstra J., Montes F., Oh J., Kebreab E. and Oosting S.J. (2013) Mitigation of methane and nitrous oxide emissions from animal operations: I.A review of enteric methane mitigation options. *Journal of Animal Science* 91, 5045-5069.

Muñoz C., Letelier P.A., Ungerfeld E.M., Morales J.M., Hube S. and Pérez-Prieto L.A. (2016) Effects of pregrazing hergage mass in late spring on enteric methane emissions, dry matter intake, and milk production of dairy cows. *Journal of Dairy Science* 99, 7945-7955.

The CO_2 exchange dynamics and carbon sequestration on two contrasting grasslands in Finland

Kulmala L.[1,2], Lohila A.[1,3], Heimsch L.[1], Vekuri H.[1], Nevalainen O.[1], Fer I.[1], Viskari T.[1], Vira J.[1], Joki-Tokola E.[4], Liimatainen M.[5], Aalto T.[1], Laurila T.[1] and Liski J.[1]

[1]Finnish Meteorological Institute, Finland; [2]Institute for Atmospheric and Earth System Research/Forest Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Finland; [3]Institute for Atmospheric and Earth System Research / Physics, Faculty of Science, University of Helsinki, Finland; [4]Natural Resources Institute Finland (LUKE), Finland; [5]University of Oulu, Finland

Abstract

We are developing a reliable and practical methodology for verifying the climatic effects of grasslands and their management practices. For this purpose, we studied the carbon dioxide (CO_2) exchange dynamics, the fluxes of other greenhouse gases, and vegetation characteristics on a grassland on southern coastal clay soil, and on a more northly site on drained peatland. Eddy covariance technique was used to study momentary dynamics of bigger target areas but, in addition, both sites were accompanied with experimental treatments with plot designs. Both sites acted as carbon sinks through the growing season. The observed photosynthetic capacity followed the changes in vegetation indices from remote sensing data. Accurate modelling of soil carbon dynamics is still underway.

Keywords: agriculture, climate, remote sensing, modelling, GHG emissions

Introduction

Agricultural soils are at present generally considered to be sources of carbon, but so-called *climate-smart* practices have potential for storing atmospheric carbon in soil (Paustian *et al.,* 2016). In addition to climate change mitigation, an increasing soil carbon content provides other benefits; for example, increased water retention (Rawls *et al.*, 2003). Nevertheless, the practices that enable soil C sequestration may have other climatic impacts: they alter soil moisture, temperature and the quantity of organic matter, which possibly change the production and consumption of nitrous oxide and methane.

The effects of different management practices vary because grasslands differ in terms of age, cultivation history, soil properties and climate. While it is not possible to use laborious and instrument-intensive measurements on all fields, it is necessary to develop a general and inexpensive yet reliable method to estimate the climatic impacts of management practices. In this study, our objectives were to (1) quantify the dynamics of carbon sequestration on grasslands in Finland, (2) test the climatic effects of different management practices, and (3) develop a reliable and practical method for verifying the climatic effects of these practices.

Materials and methods

We studied the dynamics of greenhouse gas exchange, vegetation indices and environmental factors on two different grasslands. The southern Qvidja site (60.29550°N, 22°39281°E) is on clay soil, and in 2019 it grew a mixture of *Phleum pratense* L. and *Festuca pratensis* Huds. sown in 2017. The continuous CO_2 flux measurements with Eddy covariance technique were conducted since spring 2018. More information on the site is found in Heimsch (2019).

The northern Ruukki site (64.68399°N, 25.10632°E) consists of two fields on peat with depth 30-70 cm. In 2019, one field grew *P. pratense* and *F. pratensis* sown in 2018 whereas the other site was sown with the same species and barley (*Hordeum vulgare* L.) as a cover crop in 2019. An eddy covariance tower was located between these fields in June 2019, i.e. the momentary target depended on prevailing wind direction. More information on the site is found in Vekuri *et al.* (2019).

The momentary photosynthetic capacity derived from the eddy covariance measurement was compared with multiple vegetation indices, such NDVI (normalised difference vegetation index), derived from Sentinel-2 data. In addition, we tested simple models for photosynthesis which were driven just by vegetation indices from Sentinel-2 together with the momentary intensity of photosynthetically active radiation.

The effects of different management practices were studied using an experimental design in which different plots varied in terms of cutting height, number of species and fertilisation. On these plots, we regularly measured CO_2, N_2O and CH_4 fluxes using opaque chambers on permanently installed collars. In addition, we measured light response of net CO_2 exchange using transparent chambers which were gradually shaded by netted fabrics. In all plots, the height, as well as chlorophyll content of the most common species were regularly measured together with leaf area index and soil moisture. Soil temperature was recorded automatically.

The data were used to test and develop dynamic carbon cycle models. We used the land-surface model BASGRA (Höglind *et al.*, 2016) developed for grasslands. The model was used inside a platform called Predictive Ecosystem Analyzer (PEcAn, LeBauer *et al.*, 2013) which enables analysing the role of individual parameters in the overall performance and stability of the model. In addition, the Yasso model (Tuomi *et al.*, 2011) describing the decomposition of soil organic matter was further developed to describe the dynamics in agricultural soils in more detail including nitrogen cycling.

These models will later form the core of the verifying methodology that describes the stocks (vegetation, soil) and fluxes (photosynthesis, plant and microbial respiration, plant growth, litter production, harvest, leaching) of carbon in agricultural fields according to the Guidelines and Good Practice Guidance of the Intergovernmental Panel on Climate Change (IPCC). Other relevant climate impacts resulting from CH_4, N_2O and albedo will also be accounted for in the developed methodology.

Results and discussion

Considering net CO_2 exchange (uptake of ca. 70-220 g C m^{-2}), the yield (outflow of *ca.* 100-170 g C m^{-2}) and the organic fertilisation events (input of ca. 90 g C m^{-2}), the annual carbon balance was negative at the southern site, i.e. the site acted as an overall sink of carbon (ca. 70-150 g m^{-2}). In 2019, the growing season temperatures were lower and precipitation higher and, according to preliminary analysis, the sink was notably greater than in the hot and dry 2018. The remote sensing data highlighted a great difference in the leaf area index between 2018 and 2019. The seasonal CO_2 fluxes were greatly dependent on weather conditions and management practices; for example, a change in cutting height was clearly visible in the net ecosystem exchange. Preliminary analysis with BASGRA indicated that the main cause for the differences in the leaf area and thus also in net exchange arises from changes in weather, whereas the changes in the management had smaller effects. However, currently BASGRA seems to be too sensitive for high temperatures as the model performance was weak during the hottest periods in 2018.

Analogue analysis for the northern peat site will be presented after one full year of measurements. Nevertheless, the night-time CO_2 emissions were higher at the peat site compared with the mineral site, indicating faster decomposition of soil organic matter. Nevertheless, the overall carbon balance in the northern site also seemed to be negative during the growing season of 2019.

The seasonal dynamics of the leaf area index derived from Sentinel-2 equalled the one measured in the field. NDVI covaried with photosynthetic capacity derived from eddy covariance measurements. Thus, it seems that momentary rate of grass photosynthesis could be easily and accurately predicted using vegetation indices and weather as drivers.

Conclusions

Both sites acted as carbon sinks and weather was initially behind many observed changes in carbon sequestration. Photosynthetic potential of grasses seems to follow the changes in green area, whereas predicting the changes in soil carbon dynamics is still underway.

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References

Heimsch L. (2019) Carbon balance of agricultural grasslands based on direct micrometeorological flux measurement method. Master's thesis. Aalto University, Finland. 57 p. Available at: http://urn.fi/URN:NBN:fi:aalto-201908254937.

Höglind M., Van Oijen M., Cameron D. and Persson T. (2016) Process-based simulation of growth and overwintering of grassland using the BASGRA model. *Ecological Modelling* 335, 1-15.

LeBauer D.S., Wang D., Richter K.T., Davidson C.C. and Dietze M.C. (2013). Facilitating feedbacks between field measurements and ecosystem models. *Ecological Monographs* 83, 133-154.

Paustian K., Lehmann J., Ogle D., Robertson G. and Smith P. (2016). Climate-smart soils. *Nature* 532, 49-57.

Rawls W.J., Pachepsky Y.A., Ritchie J.C., Sobecki T.M. and Bloodworth H. (2003) Effect of soil organic carbon on soil water retention. *Geoderma* 116, 61-76.

Tuomi, M., Laiho, R., Repo, A. and Liski, J. (2011) Wood decomposition model for boreal forests. *Ecological Modelling* 222, 709-718.

Vekuri H., Lohila A., Kulmala L., Tuovinen J.-P., Rainne J., Mäkelä T., Hatakka J., Joki-Tokola E., Liski J. and Laurila T. (2019) Greenhouse gas flux measurements on an agricultural grassland on peat soil. In: Laurila T., Lintunen A. and Kulmala M. (eds.) *Proceedings of The Center of Excellence in Atmospheric Science. Report series in aerosol science, No 226.* Finnish Association for Aerosol Research, Finland. pp. 713-715.

Dairy cows back to arable regions? Grazing leys for eco-efficient milk production systems

Loges R., Mues S., Kluß C., Malisch C., Loza C., Poyda A., Reinsch T. and Taube F.
Group of Grass and Forage Science/Organic Agriculture, Kiel University, Kiel, Germany

Abstract

Recent intensification in European agricultural production has been accompanied by serious environmental trade-offs, thus questioning the sustainability of current specialized production systems for both all-arable cash crops and animal products. Under the temperate conditions of North-West Europe, ruminant-based integrated crop-livestock systems are considered as a strategy towards ecological intensification. The object of the interdisciplinary project: 'Eco-efficient pasture-based milk production', established in 2016, is to provide relevant ecosystem services linked to dairy systems: high quantity and quality of agricultural commodities, low nutrient surpluses, a low carbon footprint and contributions to agro-biodiversity. Four-year data are presented based on a 98 spring-calving Jerseys/crossbred dairy herd on a former all-arable farm as an alternative to traditional specialized systems. Measurements include forage yield and quality, nitrogen fluxes, as well as milk production and quality. The results illustrate the capability of a rotational ley grazing system to provide both a high milk performance per ha combined with low environmental footprints and additionally offer significant yield benefits for the arable crops in the crop rotation.

Keywords: rotational grazing, grass clover, eco-efficiency, integrated crop-livestock systems

Introduction

The recent intensification in European agriculture has raised environmental and sustainability questions, many of which are related to the increasing simplification of agricultural systems. Mixed farming is considered as a strategy to enhance sustainability (Ryschawy *et al.*, 2012). Under the maritime conditions of Northern Germany, ruminant-based mixed farming systems are discussed as an alternative to specialized systems. With respect to ruminant nutrition, pasture is considered a cheap and environmentally friendly forage source (Dillon *et al.*, 2008; Rotz *et al.*, 2009). This is the background for the interdisciplinary project: 'Eco-efficient pasture-based milk production' that started in 2016 at Kiel University's research farm Lindhof in Northern Germany. The project focuses on a whole-farm approach to provide high amounts of energy and protein-rich forages from biodiverse pastures, while simultaneously reducing nutrient surpluses and the carbon footprint. We thus analyse the potential of pasture-based milk production on grass-clover leys to strengthen sustainability of an organic arable crop rotation by comparing the main agronomic and environmental performance indicators of the experimental farm to those of the average performance of almost 1000 dairy farms of the North German state of Schleswig-Holstein (S-H) as reported by the Landwirtschaftskammer S-H (2019).

Materials and methods

At the Lindhof research farm (N 54°27, E 9°57; mean air temperature 8.7 °C; mean annual precipitation 785 mm) a pasture-based dairy herd of 98 spring-calving Jerseys was introduced in 2016 to make use of 7 ha permanent grassland and 55 ha organic grass-clover leys grown in rotation with arable crops. Grass-clover swards at Lindhof are used as two-year leys and are established by under-sowing winter spelt in the preceding year with a seed mixture consisting of 20 kg ha^{-1} *Lolium perenne*, 2 kg ha^{-1} *Trifolium repens* and 6 kg ha^{-1} *Trifolium pratense*. Grasslands and leys in walking-distance to the milking parlour were grazed at least 8 times per year with one cut for baled silage in between. Swards outside of that range were cut 4 times per year for silage. No additional nitrogen was applied to the grass-clover, and all organic manures produced during winter were used for fertilising 56 ha organic cash crops. Forage yield was determined using a rising plate meter and hand sampling, forage quality was determined using NIRS spectroscopy. Nitrate leaching to groundwater was determined continuously with ceramic suction cups during the winters 2016/17 to 2018/19 and analysed for NO_3 concentrations. The volume of drainage water was calculated by a general climatic water balance model.

Results and discussion

Table 1 relates the performance of forage and milk production achieved at the experimental farm Lindhof to those of the currently dominant milk production systems of Schleswig-Holstein which is characterized by all year indoor-feeding and the use of Holstein-Friesians. Despite of a 37% lower cow live weight and 70% less concentrate feeding, the grazing-based Jersey cows at Lindhof reached 80% of the average annual milk yield of consulted dairy farms at 6,907 kg energy corrected milk (ECM). Using intensive rotational grazing in combination with reduced concentrate feeding at Lindhof lead to 37% higher milk yields produced from forage compared to the S-H average. Compared to the typical forage production based on silage maize and grass silage the intensive grazing system at Lindhof lead to 38% reduced production costs per unit metabolizable energy and 36% lower production costs per kg of ECM. Due to the symbiotic N_2-fixation in the grass-clover swards, Lindhof is independent of external N-fertiliser input, while the average dairy-farm bought in 123 kg ha^{-1} of mineral N fertiliser. On average the 944 considered specialized dairy farms of S-H showed a critical high N-balance per ha forage area with a surplus of 168 kg N.

High rates of grass-clover N_2 fixation in combination with relatively low N-exports via milk and meat are the background for the high surplus of 88 kg N per ha forage area at Lindhof. Exports of manure equivalent to 60 kg N per ha to the 56 ha organic cereal crops improve cereal yields by 15% compared to unfertilised fields. This combination of intensive dairy production and cash crop production leads to a whole-farm N balance of 18 kg N per ha, which is below the threshold values for Germany and still constitutes unavoidable N losses. Figure 1 shows the nitrate-N leaching losses over winter for differently managed grasslands and leys at Lindhof in a range of 4 to 9 kg nitrate-N ha^{-1} as the average over three

Table 1. Production parameters, economic results and nitrogen balance (2017/18) of the experimental farm Lindhof compared to the average of 944 dairy farms consulted by the chamber of agriculture of Schleswig-Holstein.[1]

Parameter	Unit	1. average of consulted dairy farms (n=944)	2. Lind-hof	2. relative to 1.
Annual milk yield	kg ECM cow^{-1}	8,601	6,907	(80%)
Milk solids (fat + protein)	kg cow^{-1} year^{-1}	661	539	(82%)
Concentrates fed	kg cow^{-1} year^{-1}	2,538	770	(30%)
Live weight[2]	kg cow^{-1}	680	430	(63%)
Performance per kg cow live weight	kg ECM kg^{-1}	12.6	16.1	(127%)
Milk from forage	kg ECM cow^{-1}	3,195	4,386	(137%)
Milk yield per unit forage area[3]	kg ECM ha^{-1}	13,345	11,009	(82%)
Production costs per unit ME	ct MJ^{-1}	1.71	1.06	(62%)
Total feeding costs	ct kg^{-1} ECM	22.22	14.31	(64%)
Forage costs	ct kg^{-1} ECM	13.22	9.15	(69%)
Concentrate costs	ct kg^{-1} ECM	9.00	5.16 [5]	(57%)
Forage area mineral N input	kg N ha^{-1}	123	0	
Forage area N balance[4]	kg N ha^{-1}	168	88	(52%)

[1] ECM = energy corrected milk; ME = metabolizable energy.

[2] Estimated value for black and red Holsteins.

[3] Including imported feed.

[4] Farm-gate nitrogen balance of farm area dedicated to dairy production.

[5] Produced organically.

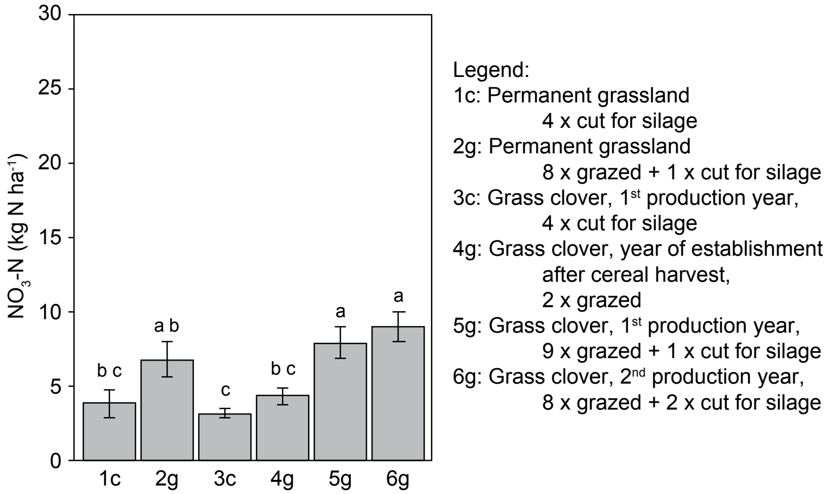

Figure 1. Over-winter NO_3-N leaching losses (kg N ha^{-1}) to groundwater across differently managed grasslands and leys at Lindhof (average over 3 leaching periods 2016/17 to 2018/19). Different lower-case letters indicate significant differences at $P<0.05$.

leaching periods. As expected, grazing experienced higher NO_3-N losses compared to cutting. Within grazed leys, leaching losses increased with the age of the swards. On average there were no significant differences between permanent grasslands and leys. The relatively low nitrogen losses of grazed swards at Lindhof can be explained with (1) lower sward clover contents on grazed than on cut swards and (2) removal of N via at least 1 silage cut taken during summer also from grazed fields. Averaged across the total forage production area, nitrate concentrations in leakage to the groundwater remained at 12 mg NO_3 l^{-1}, far below the EU-threshold value of 50 mg l^{-1} (not shown in the diagram).

Conclusions

The high milk yields at very low costs and almost no nitrate losses show the capability of a rotational ley grazing system to be economically competitive at reduced environmental burdens. The findings underline the strength of ruminant-based crop-livestock systems as a tool towards ecological intensification under the temperate conditions of Northern Germany.

References

Dillon P., Hennessy T., Shalloo L., Thorne F. and Horan B. (2008) Future outlook for the Irish dairy industry: a study of international competitiveness, influence of international trade reform and requirement for change. *International Journal of Dairy Technology* 61, 16-29.

Landwirtschaftskammer Schleswig-Holstein (2019) Ergebnisse der Vollkostenauswertung der Rinderspezialberatungsringe in Schleswig-Holstein, Auswertungsjahr 2017/2018 (in German/ Full cost evaluation of dairy production at Schleswig-Holstein of season 2017/2018).

Rotz C.A., Soder K.J., Skinner R.H., Dell C.J., Kleinan P.J., Schmidt J.P. and Bryant R.B. (2009) Grazing can reduce the environmental impact of dairy production. *Forage and Grazinglands* 7. https://doi.org/10.1094/FG-2009-0916-01-RS

Ryschawy J., Choisis N., Choisis J.P., Joannon A. and Gibon A. (2012) Mixed crop-livestock systems: an economic and environmental-friendly way of farming? *Animal* 6:10, 1722-1730.

Carbon storage in long- and short-term grasslands in Norway

Sturite I.[1], Bárcena T.G.[2], Moni C.[2], Øpstad S.[1] and Riley H.[3]
[1]*Norwegian Institute of Bioeconomy Research (NIBIO), Division of Food Production and Society, Department of Grassland and Livestock, 1431 Ås, Norway;* [2]*Norwegian Institute of Bioeconomy Research (NIBIO), Division of Environment and Natural Resources, Department of Biogeochemistry and Soil Quality, 1431 Ås, Norway;* [3]*Norwegian Institute of Bioeconomy Research (NIBIO), Division of Food Production and Society, Department of grain and forage seed agronomy, 1431 Ås, Norway*

Abstract

Perennial versus short term (<3 years) grass vegetation cover is likely to have considerable differences in root density and thus carbon (C) inputs to soil. Carbon inputs are important to maintain soil organic carbon (SOC) and may even increase it. In Norway and Scandinavia, the SOC content in soil is often higher than in other parts of Europe, due to the cold climate and high precipitation (i.e. slower turnover rates for soil organic matter) and a dominance of animal production systems with a large amount of grassland. Here we aimed to evaluate differences in SOC content, down to 60 cm depth, of a long-term grassland (without ploughing for decades) and a short-term grassland (frequently renewed by ploughing) under contrasting climate, soil and management conditions. Quantification of SOC was carried out on three long-term experimental sites on an extended latitude gradient in West and North Norway. The samples were taken from 4 depth increments (0-5, 5-20, 20-40 and 40-60 cm) in treatments that have not been ploughed for at least 43 years, and in treatments that were ploughed every third year until 2011. Preliminary results suggest that there is no significant difference in SOC storage down to 60 cm between long-term and short-term grasslands.

Keywords: ploughing frequency, soil organic carbon, sward

Introduction

Soils play a major role in the global C cycle and long-term grasslands can store significant amounts of C in soil over time (Soussana *et al.*, 2004). This is due to enhanced root C input which has been found to be 2.3 times higher than that from above-ground residues (Kätterer *et al.*, 2012). However, C accumulation in grassland soils may reach equilibrium over time (Smith, 2014). Likewise, some studies have concluded that changes in soil organic carbon (SOC) content of grasslands in recent decades are marginal (Hopkins *et al.*, 2009). A complex interaction of factors influences the SOC storage capacity in grasslands, including for example management practices, soil properties and (seasonal) climate. In addition, in Norway and Sweden, the dominance of animal production systems provides significant C inputs to grassland in the form of manure (Röing *et al.*, 2005; Riley and Bakkegård, 2006). The effect of management (including renewal strategies with respect to the age of the grassland) on SOC, remains poorly understood, especially in Norway. The objective of our study was to measure SOC content down to 60 cm depth in long- and short-term grasslands under contrasting climate, soil and management conditions. In this study the long-term grassland represents fields without ploughing for >40 years, while short-term grasslands have been ploughed and reseeded every 3rd year. We hypothesised that long-term grasslands might store more C than short-term grasslands, due to less disturbance by tillage and related C losses.

Materials and methods

Quantification of C was carried out on 3 experimental sites with long- and short-term grassland, hereafter denoted as V1 and E3, respectively, located on a latitude gradient (more details for site characteristics and management Table 1).

Table 1. Experimental sites characteristics and management practices.

	Særheim	Fureneset	Svanhovd
General information			
Coordinate	58°47'N 5°41'E	61°18'N 5°4'E	69°27'N 30°3'E
Grassland experiments established in	1968	1974	1968
Soil type	Sandy loam of moraine origin	Sandy loam of moraine origin	Peat soil on marine silt loam sediment
Mean C content in top 5 cm (%)	7.1±2.0	9.0±2.4	21.6±8.8
C/N	14.7	17.2	15.1
Grass seed mixture per treatments			
Treatment V1 Permanent grassland without ploughing >40 years	Timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.), smooth grass (*Poa pratensis* L.)		
Treatment E3 Ploughed and reseeded every 3rd year	Perennial ryegrass (*Lolium perenne*)	Perennial ryegrass (*Lolium perenne*)	Timothy (*Phleum pratense* L.)
Fertilisation			
Type	Mineral N and cattle slurry in combination with mineral fertiliser		
Number of applications year^{-1}	2 to 3	2 to 3	1 to 2
Application rate	Site specific	Site specific	Site specific
Plant biomass harvest	2 to 3 times	2 to 3 times	1 to 2 times

The experiments were arranged in a strip-split-block design with the longevity of grassland as vertical treatments (6.0×29.4 m), and fertiliser rate as subplots (3.0×9.8 m), all replicated three times.

For soil analyses, of each treatment and for each site, soil pits were dug and the soil profile described down to 1 m depth. From each soil pit, two undisturbed soil profile faces were sampled with metal augers down to 60 cm. The distance between each sampled profile face never exceeded 1 m. SOC stocks were calculated based on C concentration and bulk density assessments. The samples were taken with a soil corer (~9 cm diameter) from 4 fixed depth increments (0-5; 5-20; 20-40 and 40-60 cm depth) in V1 and E3 in summers 2017/2018. A linear mixed effect model with block as a random effect was used to evaluate the effects of ploughing frequency on the total SOC stocks at each site.

Preliminary results and discussion

On average for both treatments, the SOC stocks evaluated from the top 60 cm of the soil depth amounted to 177±37 Mg C ha^{-1} at Særheim, 246±41 Mg C ha^{-1} at Fureneset, and 309±87 Mg C ha^{-1} at Svanhovd, suggesting an increase with latitude. Relative to the long-term V1 treatment, the average SOC stock in the short-term E3 treatment was lower at Fureneset, similar at Særheim and higher at Svanhovd (Figure 1A). However, these differences were not statistically significant (linear mixed effect model, $P>0.05$). This is in contrast with previous studies of Soussana *et al.* (2004) in France, which suggested that converting short-term to long-term grassland increased SOC accumulation. The reason that we were unable to observe any clear difference between unploughed (V1) and regularly ploughed (E3) grassland on the SOC stock at 0-60 cm is most likely due to the extremely variable thickness of the organic horizons, both between and within treatments. Because this variability is independent of the treatment/block distribution, it may strongly mask the effect of the ploughing frequency treatment. To avoid this confounding effect, we looked also at the SOC stock in the 0-20 cm depth increment, which never included any transitions between organic and mineral horizons. The evaluation of SOC from the top 20 cm of the soil amounted to 112±15 Mg C ha^{-1} at Særheim, 128±17 Mg C ha^{-1} at Fureneset, and 192±45 Mg C ha^{-1} at Svanhovd. Relative to the long term V1 treatment, the average carbon stock in the short term E3 treatment was

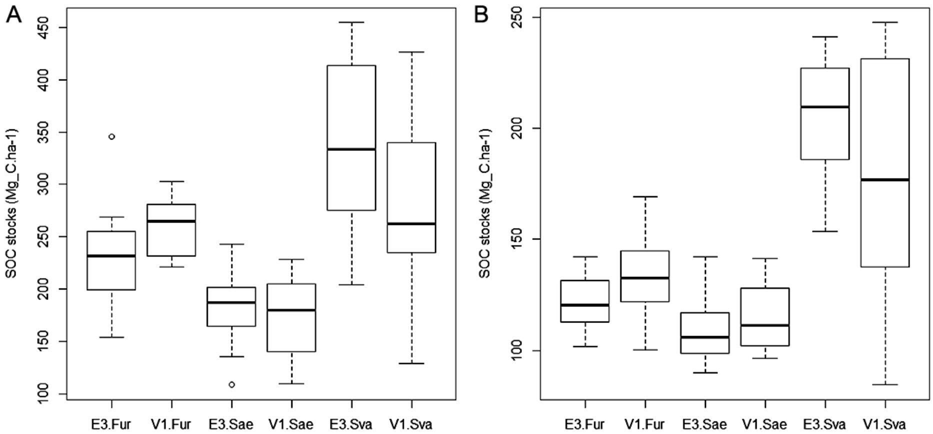

Figure 1. Soil organic carbon stock (in Mg C per hectare) down to 60 cm depth (A) and to 20 cm depth (B) for short-term grassland (E3) and long-term grassland (V1) at Fureneset (Fur), Særheim (Sae) and Svanhovd (Sva).

lower at Fureneset and at Saerheim, but higher at Svanhovd (Figure 1B). Again, however, none of these differences were statistically significant.

Another possible explanation for the reason that we were still unable to find any clear difference between unploughed and regularly ploughed grasslands may be an effect of previous land use history. In the period between 1968 and 1990, additional experimental treatments were present in the trials, which included grazing with sheep and cattle. From 1991 onwards, these grazing treatments were excluded and treatments with the use of cattle manure in combination with mineral fertiliser were included. Furthermore, very high variability of the SOC stock of the top 20 cm at Svanhovd suggests a strong lateral variability of the carbon content that is not related to the variation in the horizon depth.

Conclusions

Our experimental setting did not allow us to characterize any variation of SOC stock in relation to the ploughing frequency. Our original hypothesis is therefore rejected and possible explanations for the results could be the large heterogeneity found in these soils, as well as, the influence of previous treatments in SOC. We will continue the studies and investigate the data further.

References

Hopkins D.W., Waite I.S., McNicol J.W., Poulton P.R., Macdonald A.J. and O'Donnell A.G. (2009) Soil organic carbon contents in long-term experimental grassland plots in the UK (Palace Leas and Park Grass) have not changed consistently in recent decades. *Global Change Biology* 15, 1739-1754.

Kätterer T., Bolinder M.A., Berglund K. and Kirchmann H. (2012) Strategies for carbon sequestration in agricultural soils in northern Europe. *Acta Agriculturae Scandinavica Section A*. 62, 181-198.

Riley H. and Bakkegard M. (2006) Declines of soil organic matter content under arable cropping in southeast Norway. *Acta Agriculturae Scandinavica* 56, 217-223.

Röing K., Andren O. and Mattsson L. (2005) Long-term management effects on plant N uptake and topsoil carbon levels in Swedish long-term field experiments: cereals and ley, crop residue treatment and fertilizer application. *Acta Agriculturae Scandinavica Sect. B, Soil and Plant Sci*. 55, 16-22

Smith P. (2014) Do grasslands act as a perpetual sink for carbon? *Global Change Biology* 20, 2708-2711.

Soussana J.F., Loiseau P., Vuichard N., Ceschia E., Balesdent J., Chevallier T. and Arrouays D. (2004) Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use and Management* 20, 219-230.

Using GWP* (an alternative application of the Global Warming Potential) to report temporal trade-offs in greenhouse gas footprints of alternative Finnish cattle diets

Lynch J.[1], Järvenranta K.[2] and Pierrehumbert R.[1]
[1]*Atmospheric Oceanic and Planetary Physics, University of Oxford, OX2 3PU, United Kingdom;* [2]*Luke, Halolantie 31 A, 71750 Maaninka, Finland*

Abstract

Agricultural emissions reductions can play an important role in meeting global climate targets. As agricultural emissions include significant amounts of methane (CH_4) and nitrous oxide (N_2O), not just carbon dioxide (CO_2), alternative emission mitigation options are typically assessed by comparing total 'CO_2-equivalent (CO_2e) carbon footprints' (where non-CO_2 gases are reported as a CO_2e quantity by scaling by their 100-year Global Warming Potential). However, this approach can obscure important time-dependent differences between gases. Here, we show how an alternative application of Global Warming Potentials, GWP*, can report CO_2-warming-equivalent emissions that include these temporal complexities. We use the Finnish 'FootprintBeef' model as a case-study, comparing business-as-usual (BAU) production of Finnish beef to two alternative scenarios where production shifts to lower footprint grass-fed or grain-fed systems. The grain-fed alternative system achieves a reduction in all three greenhouse gases, and so provides a straightforward climate benefit. However, the grass-fed alternative achieves a slightly lower footprint through reductions to N_2O and CO_2 emissions at the expense of a small increase in CH_4 emissions. Reporting these emissions over time using GWP* indicates that despite the lower CO_2-equivalent footprint, this scenario increases warming relative to BAU for 75 years after changing production, due to the initial impacts of the increased methane, before being beneficial for the longer-term.

Keywords: greenhouse gas emissions, GWP*, methane, CO_2 equivalent, cattle diet

Introduction

Cattle production is a relatively emissions-intensive activity: livestock make a significant contribution to anthropogenic global warming, and reducing livestock emissions could provide an important contribution to climate change mitigation. The potential benefits of different emission mitigations are generally assessed using carbon dioxide equivalent (CO_2e) 'carbon footprints' which aggregate multiple gases using an emission weighting metric, with the 100-year Global Warming Potential (GWP100) the most commonly used metric. However, typical metrics, including the GWP100, must collapse both the radiative strength and lifetime of different greenhouse gases to a single value, which necessitates value judgements regarding the timescale over which impacts are assessed (Myhre *et al.*, 2013).

Gas-specific temporal trade-offs can be especially significant for ruminant production assessments, as ruminant emissions footprints are typically dominated by the short-lived gas methane (CH_4), with smaller nitrous oxide (N_2O) and CO_2 components. A change in animal diet or production system may achieve a reduction to total CO_2e footprint through smaller emissions of some gases, but larger emissions of others, entailing a temporal trade-off that is not revealed through a simple footprint comparison. In this paper, we explore this by using emission footprints for Finnish beef production. We demonstrate the utility of a novel application of the Global Warming Potential termed GWP* (Cain *et al.*, 2019) to assess temporal trade-offs.

Materials and methods

We use Finnish beef production footprints from the Luke 'FootprintBeef' model (H. Heusala *et al.*, unpublished data). The model includes both the dairy and suckler herds, and all animal types within both production systems. The model generates emission estimates of CO_2, CH_4 and N_2O for representative current diets and optimised grass-fed and grain-fed alternative scenarios for both herds. We scale up footprints for each system to give total annual emissions of each gas for each production scenario (BAU, optimised grass-fed and optimised grain-fed) assuming fixed beef output of 84 million kg, with the output from each herd also assumed unchanged at 68% from the dairy herd and 32% from the beef herd (Table 1).

To illustrate the impacts of a shift to either production type, we assume that current and recent annual emissions are BAU, changing linearly to one of the two alternative diets between 2020 and 2030, then remaining in the new diet from hence. CO_2-warming-equivalent (CO_2-we) emissions are calculated for each year using GWP* (Cain *et al.*, 2019), where methane is:

$$E_{\mathrm{CO_2\text{-}we}} = \mathrm{GWP}_{100} \times \left[75 \times \frac{\Delta E_{CH_4}}{20} + 0.25 \times E_{CH_4}\right]$$

in which GWP_{100} is the standard 100-year Global Warming Potential for methane, ΔE_{CH_4} the change in rate of methane emissions over the preceding 20 years, and E_{CH_4} the current annual methane emission rate. In short, this means that a change in rate of methane emissions is described as a large CO_2-we release or removal for the first 20 years, but then a relatively small CO_2-we if emissions are sustained at the same rate beyond this initial period. N_2O emissions are described as CO_2-we by multiplying directly by GWP100, as in conventional application, and CO_2 emissions added directly. As cumulative CO_2-we has a linear correspondence with temperature change (Cain *et al.*, 2019), we use the difference in cumulative CO_2-we over time between BAU and the two new dietary scenarios to indicate the global warming implications over time resulting from a change to either alternative.

Results and discussion

While the optimised grain diet, which has reduced emissions of all three greenhouse gases, results in a straightforward climate benefit, for the grass-fed diet the initial increase in methane means that it provides an increase, rather than a reduction, in total CO_2-warming-equivalent emissions until 2105 (Figure 1). I.e. the increased warming from increasing methane emissions under the optimised grass-fed diet means there is no overall benefit seen from this lower GWP100 CO_2e footprint for 75 years. Although the impacts are marginal here, they demonstrate dynamic trade-offs that may be important across other agricultural assessments, with potentially significant implications for near-term climate targets.

This behaviour could also be inferred by comparing metrics using different time-horizons. Using the 20-year Global Warming Potential (Myhre *et al.*, 2013) reports higher annual CO_2e emissions for the grass-fed diet than BAU (8,263 vs 8,229 Mt CO_2e, respectively). However, using GWP* has the advantage of

Table 1. Annual emissions of CO_2, CH_4 and N_2O, and total GWP100 CO_2-equivalents using conversion factors from the IPCC 5th Assessment Report (AR5, Myhre *et al.*. 2013), for Business As Usual (BAU; current production footprints), optimised grass-fed and optimised grain-fed Finnish dairy and suckler herds producing 84 million kg beef.

	CO_2 (kt)	CH_4 (kt)	N_2O (kt)	CO_2e (kt, AR5 GWP100)
BAU	791	75.7	4.08	3,992
Optimised grass-fed	788	76.4	4.00	3,988
Optimised grain-fed	762	74.6	4.01	3,913

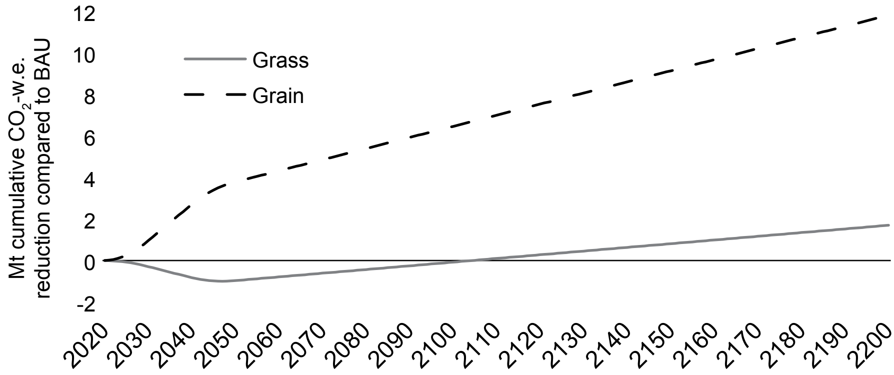

Figure 1. Reduction in cumulative CO_2-warming-equivalent emissions (using GWP*) from switching from business as usual Finnish beef production to optimised grass- or grain-fed diets over 10 years from 2020 to 2030, then remaining under the new diets.

illustrating the temporal dynamics over whatever timeframe is of interest using a simple equation, without requiring multiple individual metrics.

These results do not necessarily mean the grass-fed diet is suboptimal. If production continues over the long-term, the optimised grass-fed scenario still has a lesser climate impact than BAU, and the reversibility of methane-induced warming may mean the grass-fed diet is a better option depending on future developments (e.g. further means of reducing emissions). Comparisons with the optimised grain-fed diet would also need to consider factors beyond the climate impact, such as nutritional quality, animal health, and multifunctional outputs of either system. Further work is also required to confirm the emissions themselves, including the potential for improved soil carbon storage in grass-fed systems.

Conclusions

GWP* provides a simple way to explore climate impacts, including gas-specific temporal trade-offs that may be important when appraising alternative agricultural mitigation options.

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References

Cain M., Lynch J., Allen M.R., Fuglestvedt J.S., Frame D.J. and Macey A.H. (2019) Improved calculation of warming-equivalent emissions for short-lived climate pollutants. *npj Climate and Atmospheric Science* 2, 29.

Myhre G. *et al.* (2013) Anthropogenic and Natural Radiative Forcing. In: Stocker, T.F. *et al.* (eds.) *Climate change 2013: the physical science basis. Contribution of Working Group 1 to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, Cambridge, UK and New York, USA.

Incorporating plant diversity into biogeochemical models to better infer ecosystem services

Korhonen P.[1], Van Oijen M.[2], Barcza Z.[3,4,5], Confalonieri R.[6], Klumpp K.[7], Kröel-Dulay G.[8], Lellei-Kovács E.[8], Louarn G.[7], Louault F.[7], Martin R.[7], Moulin T.[9], Movedi E.[6], Picon-Cochard C.[7], Rolinski S.[10], Wirth S.[10], Seddaiu G.[11], Viovy N.[12] and Bellocchi G.[7]

[1]Natural Resources Institute Finland, Halolantie 31A, 71750 Kuopio, Finland; [2]CEH-Edinburgh, Bush Estate, Penicuik EH26 0QB, United Kingdom; [3]Department of Meteorology, Eötvös Loránd University, Pázmány P. s. 1/A, 1117 Budapest, Hungary; [4]Excellence Center, Faculty of Science, Eötvös Loránd University, Brunszvik u. 2., 2462 Martonvásár, Hungary; [5]Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Czech Republic; [6]Università degli Studi di Milano, Cassandra lab, via Celoria 2, 20133 Milan, Italy; [7]UCA, INRAE, VetAgro Sup, UREP, 63000 Clermont-Ferrand, France; [8]Institute of Ecology and Botany, MTA Centre for Ecological Research, Alkotmány u. 2-4, 2163 Vácrátót, Hungary; [9]Agroscope, Agroecology and Environment, Reckenholzstrasse 191, 8046 Zurich, Switzerland; [10]Potsdam Institute for Climate Impact Research, Member of the Leibniz Association, P.O. Box 601203, 14412 Potsdam, Germany; [11]Department of Agricultural Sciences, Desertification Research Centre, University of Sassari, Viale Italia, 39, 07100 Sassari, Italy; [12]Laboratoire des Sciences du Climat et de l'Environnement, LSCE/IPSL, CEA-CNRS-UVSQ, Université Paris-Saclay, 91191 Gif-sur-Yvette, France

Abstract

Multi-species grasslands are a reservoir of biodiversity and provide multiple ecosystem services, including fodder production and carbon sequestration. The provision of these services depends on the control exerted on plant diversity by the interplay of biotic and abiotic factors (e.g. grazing or mowing intensity). Biogeochemistry models incorporate a mechanistic view of the functioning of grasslands and provide a sound basis for studying the underlying processes. Although major advancements in modelling have been made recently, the simulation of biogeochemical cycles mostly remains disjointed from plant species dynamics, with considerable uncertainty in the quality of predictions. In contrast, ecological models account for plant diversity with approaches adopted from biological demography, without sufficiently linking the dynamics of plant species to the biogeochemical processes occurring at the community level (which is essential to assess resilience against, for instance, drought or nutrient limitation). The MODIPRAS project explored and highlighted the role of plant diversity in the regulation of ecosystem functions. An extensive literature and model survey were carried out with an emphasis on technically advanced models reconciling biogeochemistry and biodiversity, and models readily applicable to managed grasslands in Europe. The survey sets out state-of-the-art of biogeochemical and ecological modelling.

Keywords: multispecies grassland, biogeochemistry, ecosystem, diversity

Introduction

Due to the large coverage of temperate agricultural area by grasslands (Gilmanov *et al.*, 2007), it is crucial to understand the ecosystem services (ES) they provide. In particular, temperate grasslands are carbon sinks and a small net source of greenhouse gases (GHGs), thus contributing to climate regulation (e.g. Chang *et al.*, 2015). Biogeochemistry models (BGMs) are often used to simulate land-surface exchanges of carbon and nitrogen fluxes, and the dynamics of grassland growth and productivity. Despite recent major advancements in model development (Rolinski *et al.*, 2018; Movedi *et al.*, 2019), the assessment of biodiversity-mediated ES are limited because current BGMs are mostly lacking functions describing the temporal and spatial development of the plant diversity characterising grassland communities. In contrast, ecological models describing ecosystem functioning via population dynamics are lacking a detailed description of the fluxes needed to quantify many ES (e.g. Koffel *et al.*, 2018).

We carried out a literature and model survey with the aim to (1) review the current understanding of the dynamics of plant diversity in grasslands, and the impact of plant diversity on ES, (2) review state-of-the-art models for grassland biogeochemistry, and for the dynamics of plant diversity, (3) discuss how BGMs can be modified to simulate both the dynamics of plant diversity itself and the impacts of plant diversity on ES, and (4) propose ways for future model development.

Materials and methods

The focus of the literature survey was both on managed and unmanaged grasslands in temperate climates. We considered mainly regulating (e.g. carbon sequestration, GHG-emission reductions) and provisioning (e.g. productivity) ES that have been intensively studied during the recent years. The included drivers behind the changes in plant diversity were management effects (e.g. intensity, frequency and timing of defoliation, fertilisation and choice of plant species on sown grasslands), climatic effects (temperature and precipitation) and to lesser extent effects of pests and diseases. We mainly considered studies at the field-scale, and modelling studies having either an explanatory or a predictive approach to the observed phenomena.

Results and discussion

The literature based on empirical studies provided several examples of how environmental change impacts grassland plant diversity and how, in turn, plant diversity affects other ES provided by grassland ecosystems on field and landscape scales. However, high uncertainties remain in the scale and direction of impacts due to differences among studies in contrasting environmental conditions and different grassland systems. For instance, the response of a grassland's species diversity to changes in temperature and precipitation is highly dependent on the grassland productivity (Grime *et al.*, 2008) and geographical location. Meta-analyses should avoid conflating naturally evolved and experimentally manipulated plant diversity dynamics and a Bayesian hierarchical approach could be used to account for interactions (Gurevitch *et al.*, 2018).

For biodiversity modelling we were able to recognize differences in model structures, mathematical formulations, data-use and spatial scale that all hamper integration of BGMs and ecological models. For instance, the spatial scale in BGMs is commonly the field scale whereas it is the region or landscape scale in biodiversity models. To modify BGMs to simulate the dynamics and impacts of plant diversity, three possible approaches were distinguished: (1) representing plant diversity as a constant or dynamic metric, (2) representing multiple species or plant functional types without competition and (3) representing multiple competing species or plant functional types. Case (1) is sometimes applied in ecological models for biodiversity and represented as a scalar constant or variable (Doležal *et al.*, 2019). In BGMs, global flux-modifiers can sometimes be quantified as functions of biodiversity, but mostly functional diversity, instead of species richness, is the minimum requirement. An example of case (2) is CoSMo-approach (Confalonieri, 2014), which has been used successfully to modify an existing BGM to take plant diversity into account (Movedi *et al.*, 2019) by computing suitability of existing and alternative species to different environmental and management conditions. Case (3) is closest to the mechanistic philosophy underlying BGMs and includes explicit simulation of competition for resources (light, water and nutrients). Examples include models such as DynaGraM (Moulin *et al.*, 2018) for multi-species simulations. The most advanced methods for mechanistic competition modelling are 3D functional-structural models (Evers *et al.*, 2018; Faverjon *et al.*, 2019).

Conclusions

The importance of representing biodiversity in BGMs depends on the aims of model application and on the state of the simulated grassland (e.g. nutrient- and water-limited vs potential growth). However, importance of understanding and including effects and dynamics of plant diversity is emphasised when modelling resilience of grasslands against disturbances.

The future trends needed for model improvement are diverse. These include: (1) parallel development of different model types, (2) collecting benchmarking data and carrying out model comparison against those data, (3) increasing data availability (also with new-generation data collection systems, e.g. remote sensing), (4) increasing the explicit representation of spatial heterogeneity in BGMs, (5) increasing the role of hybrid modelling (meaning an extension of process-based models statistically to provide values for output variables that are not in the mechanistic part of the model), and (6) utilising machine-learning approaches.

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References

Chang J., Ciais P., Viovy N., Vuichard N., Sultan B. and Soussana, J.F. (2015) The greenhouse gas balance of European grasslands. *Global Change Biology* 21(10), 3748-3761.

Confalonieri R. (2014) CoSMo: a simple approach for reproducing plant community dynamics using a single instance of generic crop simulators. *Ecological Modelling 286*, 1-10.

Doležal J., Lanta V., Mudrák O. and Lepš J. (2019) Seasonality promotes grassland diversity: interactions with mowing, fertilization and removal of dominant species. *Journal of Ecology* 107(1), 203-215.

Evers J.B., Van Der Werf W., Stomph T.J., Bastiaans L. and Anten N.P. (2018) Understanding and optimizing species mixtures using functional-structural plant modelling. *Journal of Experimental Botany* 70(9), 2381-2388.

Faverjon L., Escobar-Gutiérrez A., Litrico I., Julier B. and Louarn G. (2018) A generic individual-based model can predict yield, nitrogen content, and species abundance in experimental grassland communities. *Journal of Experimental Botany* 70(9), 2491-2504.

Gilmanov T.G., Soussana J.F., Aires L., Allard V., Ammann C., Balzarolo M., Barcza Z., ... and Wohlfahrt G. (2007) Partitioning European grassland net ecosystem CO2 exchange into gross primary productivity and ecosystem respiration using light response function analysis. *Agriculture, Ecosystems & Environment* 121(1-2), 93-120.

Grime J.P., Fridley J.D., Askew A.P., Thompson K., Hodgson J.G. and Bennett C.R. (2008) Long-term resistance to simulated climate change in an infertile grassland. *Proceedings of the National Academy of Sciences* 105(29), 10028-10032.

Gurevitch J., Koricheva J., Nakagawa S. and Stewart G. (2018) Meta-analysis and the science of research synthesis. *Nature* 555 (7695), 175.

Koffel T., Boudsocq S., Loeuille N. and Daufresne T. (2018) Facilitation vs. competition driven succession: the key role of resource ratio. *Ecology Letters* 21(7), 1010-1021.

Moulin T., Perasso A. and Gillet F. (2018) Modelling vegetation dynamics in managed grasslands: responses to drivers depend on species richness. *Ecological Modelling* 374, 22-36.

Movedi E., Bellocchi G., Argenti G., Paleari L., Vesely F., Staglianò N., Dibari C. and Confalonieri R. (2019) Development of generic crop models for simulation of multi-species plant communities in mown grasslands. *Ecological Modelling* 401, 111-128.

Rolinski S., Müller C., Heinke J., Weindl I., Biewald A., Bodirsky B.L., Bondeau A., Boons-Prins E., Bouwman A., Leffelaar P., Te Roller J., Schapoff S. and Thonicke K. (2018) Modeling vegetation and carbon dynamics of managed grasslands at the global scale with LPJmL 3.6. *Geoscientific Model Development* 11, 429-451.

A management-based typology for European permanent grasslands

Tonn B.[1], Bausson C.[2], Ten Berge H.[3], Buchmann N.[4], Bufe C.[3], Eggers S.[5], Fernández-Rebollo P.[6], Forster-Brown C.[7], Hiron M.[5], Klaus V.H.[4], Korevaar H.[3], Lellei-Kovács E.[8], Lombardi G.[9], Markovic B.[10], Ravetto Enri S.[9], Schils R.L.M.[3], Stypinski P.[11] and Newell Price P.[12]

[1]University of Göttingen, Department of Crop Sciences, Germany; [2]Chambre régionale d'agriculture de Normandie, France; [3]Wageningen Plant Research, Agrosystems Research, the Netherlands; [4]ETH Zürich, Department of Environmental Systems Science, Switzerland; [5]Swedish University of Agricultural Sciences, Department of Ecology, Sweden; [6]University of Córdoba, Department of Forestry, Spain; [7]ADAS Medenvale, United Kingdom; [8]MTA Centre for Ecological Research, Institute of Ecology and Botany, Hungary; [9]University of Turin, Department of Agricultural Forest and Food Sciences, Italy; [10]University of Montenegro, Department of Livestock Science, Montenegro; [11]Warsaw University of Life Science, Faculty of Agriculture and Biology, Poland; [12]ADAS Gleadthorpe, United Kingdom

Abstract

European permanent grasslands (PG) vary widely in their delivery of agricultural outputs and other ecosystem services and hence in their challenges and opportunities for sustainable grassland management. To facilitate communication and knowledge transfer, improve inventories, ease mapping and provide a framework for future data collection across the whole range of European PG, we have developed a two-level grassland typology that focuses on PG management (defoliation, fertilisation, renewal) and its determinants (productivity potential, presence of woody plants, additional site attributes affecting management). The typology consists of eight first-level and 18 subordinate second-level classes, based on management intensity, productivity potential, presence of woody plants and grassland renewal intervals. It is applicable both at field and regional scales and is cross-referenced with existing classification schemes such as the EUNIS and Natura 2000 habitats classes. We present the typology and its main classification criteria, and discuss options for its future implementation.

Keywords: management intensity, permanent grassland, typology

Introduction

The great diversity of permanent grasslands (PG) across Europe can be an obstacle for effective knowledge transfer and policy making. Identifying types of PG across countries that are similar in terms of ecosystem delivery, challenges and opportunities for sustainable management would greatly enhance communication between stakeholders and provide more meaningful inventories of European PG and their contributions to agricultural production and public goods. Recognizing this, the European Grassland Federation has proposed a classification of grassland types to be used in agricultural statistics (Peeters *et al.*, 2014). In it, PG are classified based on their management intensity into 'semi-natural' PG, 'improved' PG and PG 'no longer used for production', with semi-natural PG further subdivided into pastures and traditional hay meadows. Building on this, we propose a two-level PG typology that extends the classification to eight first-level and 18 subordinate second-level classes (Figure 1), complemented by a list of additional attributes. We here present the classification criteria used and provide an outlook on further implementation within the EU H2020 project 'Developing SUstainable PERmanent Grassland systems and policies' (SUPER-*G*).

Classification criteria

Management intensity – 'low', 'intermediate' or 'high' – forms a central criterion of the proposed typology. We quantify this based on two indicators, namely defoliation intensity and fertilisation intensity.

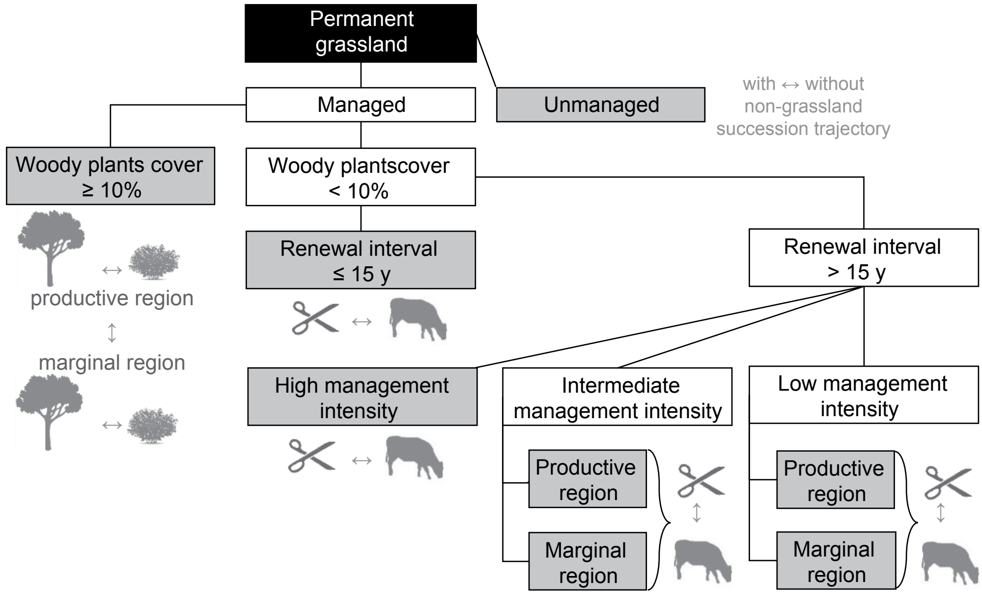

Figure 1. Overview of the proposed permanent grassland typology. First-level classes of the typology are in grey boxes, subordinate second-level classes are indicated either by text or by two different sets of symbols (tree- vs shrub-dominated; predominantly cut vs predominantly grazed).

Defoliation intensity integrates stocking rate of grazing animals (livestock-unit grazing-days per ha and year) and cutting frequency (number of mowing or mulching operations per year). Fertilisation intensity is quantified through the total agricultural nitrogen input from mineral and organic fertilisers as well as excreta of grazing animals on the site. Preliminary thresholds for each indicator have been set based on a multi-national stakeholder survey and will be further validated within the SUPER-*G* project. Management intensities for selected combinations of the indicators are shown in Figure 2.

Our concept of management intensity is input- rather than outcome-based. It does not, in itself, consider the site-specific PG productivity potential, which modifies the effect of a given management intensity and determines the maximum feasible intensity level. To take account of this important aspect, PG types result from the combination of management intensity and two classes of pedo-climatic productivity potential, called 'productive region' and 'marginal region' (Figure 1). In all these cases, subordinate second-level classes differentiate between predominantly mown and predominantly grazed PG.

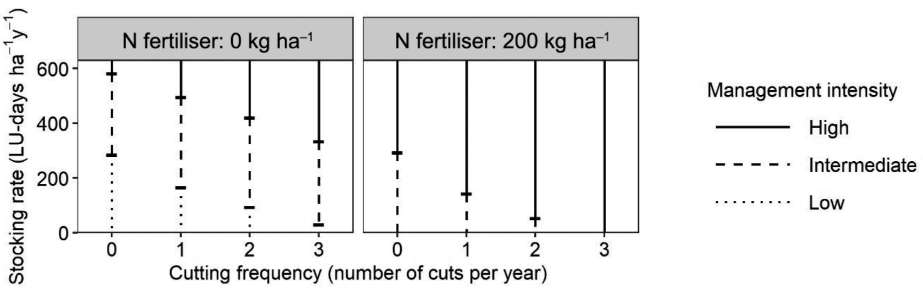

Figure 2. Illustration of management intensity classes resulting from selected combinations of cutting frequency, nitrogen fertiliser application and stocking rates of grazing animals.

As defined in the SUPER-*G* project, PG includes any land dominated by grasses or herbaceous forage that can be grazed/mown and has not been included in an arable crop rotation for five years. It thus encompasses habitats that also include shrubs or trees, grasslands that are not currently agriculturally used, and frequently renewed grasslands. Each of these three special cases forms a separate PG type (Figure 1). The first type consists of open habitats with a woody plant cover of 10% or more, as long as herbaceous plants make up 50% of the vegetation cover. This type is subdivided into four second-level classes, depending on pedo-climatic productivity potential and on predominance of either trees or shrubs among the woody plants. The second type includes PG with no management for at least three successive years. It is further differentiated into PG that will remain as grassland even without management ('natural grassland') and grassland that will enter a succession trajectory towards non-grassland unless management is resumed ('abandoned grassland'). Finally, grassland that is frequently resown after chemically or mechanically removing the existing sward, with a renewal interval of 15 years or less, forms a third type distinguishing it from older PG swards.

The PG types are complemented by further attributes that are important for management and the delivery of ecosystem services and that cut across the types. These include limitations to productivity by short vegetation period, extended summer drought or acidic soils; limitations to management by steep slopes, stony or shallow soil, and other characteristics strongly modifying ecosystem service delivery.

Implementation

The final typology will be made available as an atlas including an online classification tool that can link to PG type portraits and management options. To maximize transferability, the PG typology will be cross-referenced with the habitat types of the European Nature Information System (EUNIS, 2019). Maps of the PG types and attributes will be produced using available spatial data, especially the distribution of EUNIS habitats, and expert-based decision rules. Currently, insufficient spatial information about grassland management is the greatest obstacle for accurate mapping of PG types, but developments in remote sensing and big data analysis may alleviate this problem in the near future (Estel *et al.*, 2018). With its classes largely defined by management intensity, the typology aims to cover the full range of grassland uses in European farming systems and to provide an accessible knowledge base for conditions, challenges and opportunities linked to PG management across Europe.

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References

Estel S., Mader S., Levers C., Verburg P.H., Baumann MK. and Kuemmerle T. (2018) Combining satellite data and agricultural statistics to map grassland management intensity in Europe. *Environmental Research Letters* 13, 0704020.

EUNIS (2019) *EUNIS Habitat Classification*. Available at: http://www.eea.europa.eu/data-and-maps/data/eunis-habitat-classification.

Peeters A., Beaufoy G., Canals R.M., De Vliegher A., Huyghe C., Isselstein J., Jones G., Kessler W., Kirilov A., Mosquera-Losada M.R., Nilsdotter-Linde N., Parente G., Peyraud J.-L., Pickert J., Plantureux S., Porqueddu C., Rataj D., Stypinski P., Tonn B., van den Pool–van Dasselaar A., Vintu V. and Wilkins R.J. (2014) Grassland term definitions and classifications adapted to the diversity of European grassland-based systems. *Grassland Science in Europe* 19, 743-750.

Extensive grassland management promotes greater above- and belowground community richness in two contrasting agroclimatic regions in Switzerland

Fox A.[1,2], Widmer F.[2], Muller R.[1], Barreiro A.[3], Dimitrova Mårtensson L-M.[3], Silva L.[4], Vieira Â.F.[4], Musyoki M.[5], Zimmermann J.[5], Rasche F.[5] and Lüscher A.[1]
[1]Forage Production and Grassland Systems, Agroscope, Reckenholzstrasse 191, 8046 Zurich, Switzerland; [2]Molecular Ecology, Agroscope, Reckenholzstrasse 191, 8046 Zurich, Switzerland; [3]Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Alnarp, Sweden; [4]InBIO Laboratório Associado, Universidade dos Açores, Ponta Delgada, Azores, Portugal; [5]Institute of Agricultural Sciences in the tropics, University of Hohenheim, Stuttgart, Germany

Abstract

This study investigated whether extensive management of permanent grasslands promoted greater aboveground (plant) and belowground (fungal) biodiversity. Three grassland management types, i.e. high intensity (INT), low intensity (LI) and extensive (EXT) were sampled in two contrasting agroclimatic regions in Switzerland, the Lowlands and the Alps. The latter two grassland management types are supported through the 'Ecological Compensation Areas' (ECAs) payment scheme. The number of distinct plant species were counted at each site and soil samples were taken, from which fungal operational taxonomic units (OTUs) were constructed. In both regions, EXT had significantly higher plant species and fungal OTU richness (P<0.01) compared to INT. Additionally, the correlation between plant and fungal community richness was stronger in the Lowlands (R=0.59, P<0.001) than in the Alps (R=0.45, P=0.006). These results underline the success of the ECA payment scheme at protecting above- and belowground biodiversity in Swiss grassland ecosystems.

Keywords: species richness, biodiversity, plants, fungi, permanent grassland

Introduction

Since 1993, Switzerland has implemented a scheme of 'Ecological Compensation Areas' (ECAs) aimed at promoting and protecting biodiversity in agronomic systems. Representing approximately 90,000 hectares, extensive (EXT) grasslands are the dominant ECA type (BLW, 2006). In the Alps, climatic and topographical limitations have restricted the agricultural intensification of grasslands and therefore these sites have largely maintained their compositionally complex plant communities (Peter *et al.,* 2008). There are no such limitations to agricultural intensification in the Lowlands, therefore the biodiversity promotion potential of EXT grasslands may be greater in this region. While their effectiveness at promoting aboveground biodiversity is known (Kampmann *et al.,* 2012), whether EXT grasslands promote belowground biodiversity demands attention. The present study addressed this and investigated their role in promoting both plant and fungal community richness (as well as the structural relationship between these) in the Lowlands and Alps, to see if ECAs are comparably effective in both regions.

Materials and methods

Three different management types were sampled in two agroclimatically contrasting regions in Switzerland, i.e. the Lowlands and the Alps. Management types represented were high intensity (INT) (conventional fertilisation rate, early and frequent utilisations), low intensity (LI, is a Swiss ECA category with strongly reduced fertilisation rate and infrequent utilisations) and EXT (is a Swiss ECA category with no fertilisation and infrequent utilisations). There were 72 plots in total (12 plots per management category in each region), with the sampling area comprising a standardised study unit with four subplots of 4 m^2 each. The number of different plant species present was determined on each subplot. Plant species richness per plot was calculated as the total number of individual species present between the subplots. Four soil cores were also taken per subplot, and the resultant 16 cores were then combined and homogenized as a representative soil sample of the plot. Soil DNA was extracted from each sample, with the fungal internal transcribed spacer region (ITS2) being PCR amplified and an amplicon-based Illumina Miseq sequence analysis conducted (Frey *et al.*, 2016). The richness of the resultant fungal operational taxonomic units (OTUs, analogous to a fungal species) was then calculated (Jost, 2006). Statistical differences in both richness values between INT and the other management types was determined by analysis of variance. Pearson correlation was used to determine the relationship between plant species richness and fungal OTU richness.

Results and discussion

Compared to INT, there was a highly significant increase in fungal OTU richness in both LI and EXT in the Lowlands (P<0.001, Figure 1A). While there was also a significant increase in fungal OTU richness in EXT in the Alps (P<0.01), there was no significant increase in LI (P>0.05, Figure 1A). Such findings are in agreement with Barreiro *et al.* (2019) who found an increase in the biomass of saprophytic fungi in EXT grasslands in both of these regions (using the same sites used in this study), indicating that such grassland types represent favourable habitats for soil fungi. There was also a highly significant increase in plant species richness in EXT over INT in both the Lowlands and the Alps (P<0.001, Figure 1B). The increase associated with LI was stronger in the Lowlands (t value=5.38, P<0.001) than in the Alps (t value=2.94, P<0.01, Figure 1B).

In both regions, there was a significant correlation between plant species richness and fungal OTU richness, with the effect being stronger in the Lowlands (R=0.59, P<0.001; Figure 2A) than in the Alps (R=0.45, P=0.006; Figure 2B). This correlation would imply a relationship between the above- and belowground biodiversity (De Deyn, Quirk and Bardgett, 2011), highlighting additional advantages of implementing grassland management extensification strategies though ECA schemes. The more pronounced increases in plant and fungal community richness in both LI and EXT in the Lowlands would indicate that the biodiversity promotion potential is greater in this region. This is likely a consequence of the plant community richness of Lowland INT grasslands being so low, even being significantly lower than INT in the Alps (P=0.012, Figure 1B). To encourage increased implementation of EXT grasslands in the Swiss Lowlands, more targeted economic incentives for farmers in this region could be a potential strategy.

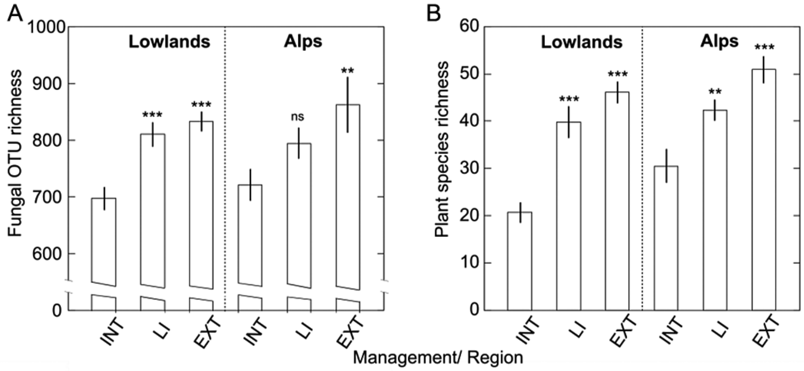

Figure 1. (A) Fungal OTU richness and (B) plant species richness in the three grassland management types; high intensity (INT), low intensity (LI) and extensive (EXT) in the two differing agroclimatic regions in Switzerland (Lowlands and the Alps). Asterisks denote a significant difference to INT. *** P<0.001, ** P<0.01, ns P>0.05.

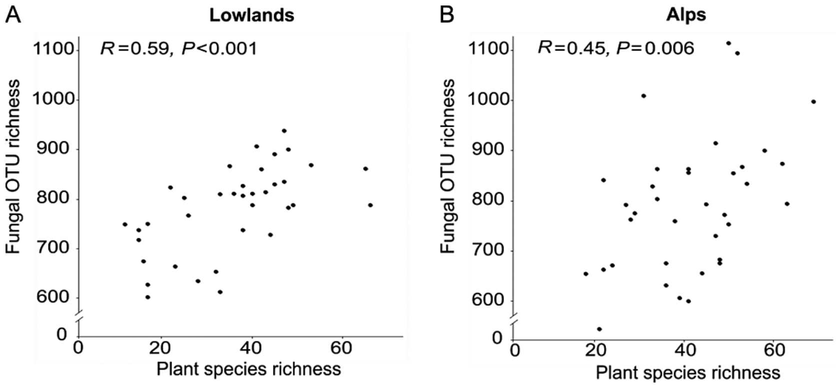

Figure 2. Correlation between fungal operational taxonomic units (OUT) richness and plant species richness in the two agroclimatic regions of Switzerland. (A) Lowlands and (B) Alps. Displayed is the Pearson *R* and its significance.

Conclusions

EXT grasslands supported by the Swiss ECA payment scheme significantly increased both plant and fungal community richness. This study highlights the success of this scheme for protecting above- and belowground biodiversity in Swiss grassland ecosystems. This is particularly apparent in the Swiss Lowlands, where grasslands tend to be more intensively managed.

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References

Barreiro A., Fox A., Lüscher A., Widmer F., Vieira Â., Parelho C., Silva L., Melo J., Cruz C., Musyoki M., *et al.* (2019) Soil microbial biomass and community structure in differently managed grasslands along a European gradient. *Grassland Science in Europe* 24, 464.

BLW (2006) Agrarbericht. Swiss Federal Office for Agriculture, Berne.

De Deyn G.B., Quirk H. and Bardgett B. (2011) Plant species richness, identity and productivity influence key groups of microbes in grassland soils of contrasting fertility. *Biology Letters* 7, 75-78.

Frey B., Rime T., Phillips M., Stierli B., Hajdas I., Widmer F. and Hartmann M. (2016) Microbial diversity in European alpine permafrost and active layers. *FEMS Microbiology Ecology* 92, fiw018.

Jost L. (2006) Entropy and diversity. *Oikos* 113, 363-375.

Kampmann D., Lüscher A., Konold W. and Herzog F. (2012) Agri-environment scheme protects diversity of mountain grassland species. *Land Use Policy* 29, 569-576.

Peter M., Edwards P.J., Jeanneret P., Kampmann D. and Lüscher A. (2008) Changes over three decades in the floristic composition of fertile permanent grasslands in the Swiss Alps. *Agriculture, Ecosystems and Environment* 125, 204-212.

Pollination function is positively influenced by floral traits functional diversity in semi-natural grasslands

Goulnik J.[1], Plantureux S.[1], Théry M.[2], Baude M.[3], Delattre M.[4], Van Reeth C.[5], Villerd J.[1] and Michelot-Antalik A.[1]

[1]*Université de Lorraine, Inrae, LAE, 54000 Nancy, France;* [2]*UMR 7179 CNRS-MNHN, Mécanismes Adaptatifs et Evolution, Brunoy, France;* [3]*Université d'Orléans, EA 1207 LBLGC, 45067 Orléans, France;* [4]*UMR MIA-Paris, AgroParisTech, INRA-Université Paris-Saclay, Paris, France;* [5]*Centre de Recherches sur les Ecosystèmes d'Altitude (CREA Mont-Blanc), 67 lacets du Belvédère, 74400 Chamonix Mont-Blanc, France*

Abstract

Semi-natural grasslands are well studied regarding factors affecting their vegetative traits diversity, but not with regard to floral traits. Our objective was to determine if floral trait diversity of semi-natural grasslands influences pollination function. We selected 16 French grasslands with relatively homogeneous landscape features. Functional diversity indices were calculated from measurements of four floral traits: flower area, flowering height, nectar tube depth and nectar sugar production, on 47 plant species. A total of 2,823 pollinators visiting these species were caught during a flowering season. We found a positive relation between floral trait functional diversity and pollination function as estimated by the interaction frequency (i.e. number of pollinator visits in a given time) at the community scale. More specifically, we found a positive effect of nectar sugar production per flower unit diversity on interaction frequency. These results confirm that diversity of floral traits, and particularly nectar production, can promote niche partitioning of pollinators and ecosystem functioning.

Keywords: plant-pollinator interactions, nectar, niche partitioning, functional trait

Introduction

In a context of a global pollinator decline, semi-natural grasslands (hereafter, 'grasslands) are suitable habitats for pollinators by providing pollen and nectar resources. Floral traits are a main component of plant-pollinator interaction patterns. Along with phenology, they include cues or signals (e.g. flower colour), exploitation barriers (e.g. nectar tube depth), and rewards (e.g. sugar production). Environmental factors can influence the functional diversity (FD) values of floral traits with important cascading effects on the pollination function (Lavorel *et al.*, 2013), often approximated by the number or frequency of interactions. The relation between floral trait FD and interaction frequency between plants and pollinators have been seldom studied and there is no consensus in the literature. Our study aimed at determining the influences of floral trait community FD on pollination function using an experimental network of 16 semi-natural grasslands.

Materials and methods

We selected 16 grasslands in a 12 km^2 circle (48°73N, 7°05E, 250 m a.s.l.) in Moselle, France, with relatively homogeneous landscape features, using a Geographic Information System (QGIS) and land-cover data from BD TOPO® (IGN) 2014. These grasslands are managed by mowing. From May-August 2017, a single observer (J.G.) sampled pollinators that were clearly interacting with sexual organs of flowers, with a sweep net on one 400 m^2 transect (100 m long × 4 m wide) per grassland during a 15 min walk, without counting the time needed to process captured pollinators, yielding a total of 70 sampling events. At each sampling event, flower cover for each currently flowering species was estimated as the mean percentage of cover (within 1%) from seven 1 m^2 quadrats distributed regularly along the entire transect. We measured the height of the top floral unit (FU) (i.e. aggregation of flowers accessible by a pollinator without flying) and FU area for 3-330 individuals (mean=71) per species for 47 of the 50 flowering plant species found. Nectar tube depth (i.e. distance between the nectaries and the entrance of a flower where only mouthparts can penetrate (Stang *et al.*, 2006)) were measured for 10 individuals per species of a single flower per FU stored in 70° alcohol. Nectar sugar production per FU in 24 h was estimated following Baude *et al.* (2016) for 5 individuals per plant species.

For FD indices, Rao's quadratic entropy indices (FD_Q) were calculated for all floral traits collected and for each floral trait separately using the package FD. We then created null models using the name-shuffling approach to disentangle FD from taxonomic diversity and we used the obtained standardised effect sizes as FD indices in subsequent analyses.

We used general linear mixed models with a negative binomial distribution to test the influence of total FD and FD of each trait on interaction frequency with grassland identity (grassland ID) and sampling session as random terms. For all models, we considered a correlation coefficient (|r|) of 0.5 to be the threshold for collinearity and thus for variable selection (Dormann *et al.*, 2013). We selected models using Akaike's Information Criterion (AICc). We tested parameter significance using Wald-tests with the R package lme4. We calculated R^2m which informs about the deviance explained by fixed terms only for final models using the R package MuMIn.

Results and discussion

In total 2,823 pollinators were caught on flowers, comprising 44.3% Hymenoptera, 40.1% Diptera, 11.0% Lepidoptera and 4.3% Coleoptera. Eight plant species represented more than 75% of total plant-pollinator interactions: *Centaurea jacea* (17.1% of total interactions), *Daucus carota* (15.0%), *Trifolium repens* (13.2%), *Silaum silaus* (8.2%), *Ranunculus acris* (7.4%), *Lotus corniculatus* (6.7%), *Jacobea aquatica* (6.1%) and *Trifolium pratense* (5.1%).

Interaction frequency was positively related to FD total (P=0.004, Figure 1A) and FD sugar of nectar (P<0.001, Figure 1B). R^2m of the models which also integrated the total flower cover as explanatory variable, were respectively 0.41 and 0.48. This result contributes to the positive biodiversity-ecosystem functioning framework and differs from those of Fornoff *et al.* (2017) and Uyttenbroeck *et al.* (2017) who displayed a negative influence of floral trait FD on interaction frequency. This difference can be attributed to the pollinator richness considered in the previous studies (67 and 68 species), versus more than 200 species in our study. Our result underlined the importance of floral trait diversity in the context of pollinator decline, which could favour pollinator abundance independently of plant species richness.

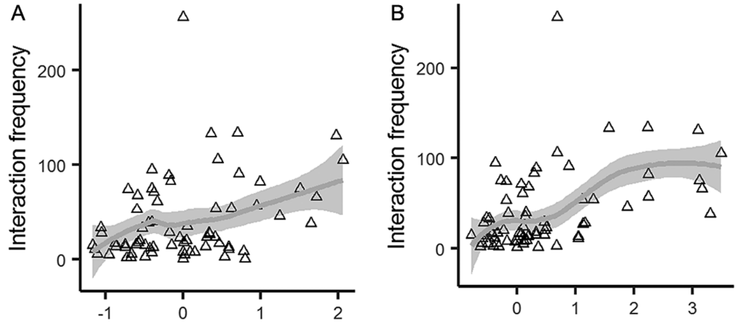

Figure 1. Predicted fit of general linear mixed-effects models of the relation between interaction frequencies and (A) FD of total floral trait, (B) FD of nectar sugar. Grey shading around the line: 95% confidence intervals.

This positive relation could be due to an increase of niche partitioning explained by an increase of pollinator diversity combined with an increase in floral trait FD (e.g. Junker *et al.*, 2015). Moreover, when we modelled interaction frequency using a combination of all floral trait FD, we found a positive relation only with diversity of sugar production per FU. This suggests that an increase in reward production diversity was a main factor for niche partitioning of pollinators, probably according to their metabolic needs (Vaudo *et al.*, 2015). However, differences between metabolic needs of pollinators still need to be considered to validate this hypothesis.

Conclusions

Our results underlined the importance of considering flower functional diversity, in addition to species diversity, for maintaining pollinator abundance and pollination function in agroecosystems. Future work is needed to understand the mechanisms under these relations and to improve grassland management according to these observations.

Acknowledgements

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References:

Baude M., Kunin W.E., Boatman N.D., Conyers S., Davies N., Gillespie M.A.K., Morton R.D., Smart S.M. and Memmott J. (2016) Historical nectar assessment reveals the fall and rise of floral resources in Britain. *Nature* 530, 85-88.

Dormann C.F., Elith J., Bacher S., Buchmann C., Carl, G. Carré G., Marquéz J.R.G., Gruber B., Lafourcade B., Leitão P.J., Münkemüller T., McClean C., Osborne P.E., Reineking B., Schröder B., Skidmore A.K., Zurell D. and Lautenbach S. (2013) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography* 36, 27-46.

Fornoff F., Klei, A.-M., Harti, F., Benad, G., Venjako, C., Schaefe, H.M. and Ebelin, A. (2017) Functional flower traits and their diversity drive pollinator visitation. *Oikos* 7, 1020-1030.

Junker R.R., Blüthgen N. and Keller A. (2015) Functional and phylogenetic diversity of plant communities differently affect the structure of flower-visitor interactions and reveal convergences in floral traits. *Evolution Ecology*. 29, 437-450.

Lavorel S., Storkey J., Bardgett R.D., de Bello F., Berg M.P., Le Roux X., Moretti M., Mulder C., Pakeman R.J., Díaz S. and Harrington R. (2013) A novel framework for linking functional diversity of plants with other trophic levels for the quantification of ecosystem services. *Journal of Vegetation Science*. 24, 942-948.

Stang M., Klinkhamer P.G.L. and Van der Meijden E. (2006) Size constraints and flower abundance determine the number of interactions in a plant-flower visitor web. *Oikos* 112, 111-121.

Uyttenbroeck R., Piqueray J., Hatt S., Mahy G. and Monty A. (2017) Increasing plant functional diversity is not the key for supporting pollinators in wildflower strips. *Agriculture Ecosystems and Environment*. 249, 144-155.

Vaudo A.D., Tooker J.F., Grozinger C.M. and Patch H.M. (2015) Bee nutrition and floral resource restoration. *Current Opinion in Insect Science* 10, 133-141.

Considerable floristic biodiversity potential in roughs on golf-courses

Grant K.[1], Boehling N.[2] and Elsaesser M.[1]
[1]Agricultural Centre Baden-Wuerttemberg (LAZBW), Atzenberger Weg 99, 88326 Aulendorf, Germany; [2]Flora-X, Römersteinstr. 12, 73230 Kirchheim unter Teck, Germany

Abstract

The decline of biodiversity in grasslands is a major topic in Germany. Even in Baden-Wuerttemberg, where governmental programmes maintain the highest percentages of species rich meadows in Germany, many of these grasslands are losing a high percentage of plant species. The main causes for this are intensification of grassland management and construction activities. In these circumstances the roughs of golf courses. with around 47,000 ha in Germany, have potential for extraordinary floristic biodiversity. In order to show the potential of roughs for biodiversity, we investigated 15 courses from 88 in Baden-Wuerttemberg. On each course, plant species composition was examined at 10 plots in roughs. We showed that, depending on the ecological attributes of the landscapes, up to 100 and more herbaceous plant species, including several endangered species, could be found on well managed golf courses. The following simple management requirements should be followed: one late cut or up to two cuts per year, no mulching and very low fertilisation.

Keywords: floristic diversity, golf course, roughs, extensive grassland management, hay meadow, indicator species

Introduction

Species-rich grassland has a high ecological value but its floristic and faunistic diversity has been declining continually due to intensive management for better forage yield and quality (Manning *et al.,* 2015). Furthermore, there is an ongoing loss of grassland due to construction activities for buildings and roads. Besides the grasslands used primarily for agriculture there are considerable grassy areas throughout Germany that have potential for high biodiversity, namely golf courses and parks. In general, the public view of golf courses is not very good in terms of floristic diversity. The perception focuses usually on the fairways and greens consisting of a few lawn species which are kept very short by intensive management. However, about half the area of each golf course is comprised of 'roughs', areas with higher grass vegetation, fallow land, and other landscape elements (e.g. sand, ponds, streams, rocks, trees, hedges). Depending on the geologic and climatic region, as well as the greenkeeping of the golf course, the vegetation of these roughs can be quite diverse and of high ecological value. In Baden-Wuerttemberg (southwestern Germany), golf roughs comprise about 6,000 ha. A vegetation survey of a part of this area (15 of 88 golf courses) was done by the Agricultural Centre Baden-Wuerttemberg (LAZBW) in 2019 to investigate the potential of roughs for biodiversity and thereby disprove the critical attitude of the public against golf. Furthermore, management strategies were developed for each golf course studied to further increase its floristic diversity.

Materials and methods

Vegetation surveys were done between 14 May and 5 July 2019 on 15 golf courses in Baden-Wuerttemberg (Table 1). On each golf course, 10 plots of 25 m^2 were examined in roughs focussing on plant species identity and abundance using the Braun-Blanquet method. The additional parameters were: total cover, vegetation height, vegetation density and observed disturbances. Species composition of all 150 plots was assigned to six habitat types: Lowland hay meadow (n=6), Mountain hay meadow (n=104), Humid

Table 1. Details of 15 golf courses including number of all detected (total and endangered) vascular plant species.

Golf course no.	Name	Landscape of BW	Av. height above sea level (m)	Species richness	Number of endangered species[1]
1	Niederreutin	Obere Gäue	495	66	6
2	Freudenstadt	Schwarzwald-Randplatten	655	91	5
3	Gröbernhof	Mittlerer Schwarzwald	225	60	4
4	Bad Waldsee	Oberschwäbisches Hügelland	620	92	14
5	Kirchheim u.T.	Mittleres Albvorland	320	63	2
6	Owingen	Bodenseebecken	560	60	5
7	Sonnenbühl	Mittlere Kuppenalb	768	79	10
8	Rickenbach	Hochschwarzwald	770	94	19
9	Liebenstein	Neckarbecken	265	87	4
10	Monrepos	Neckarbecken	260	81	4
11	Schaichhof	Schönbuch	510	73	3
12	Langenstein	Hegau	485	87	6
13	St.Leon-Rot	Nördliches Oberrheintiefland	107	105	13
14	Mönsheim	Neckarbecken	445	71	6
15	Illerrieden	Unteres Illertal	620	91	14

[1] Including vulnerable species.

meadow (n=15), Wetland/Marsh (n=5), Semi-natural dry grassland in two forms: nutrient-poor (n=9) and dry/semidry (n=7), and undefined habitat type (n=6).

Species abundance data were recoded from the field coding for statistical analyses using a replacement code (Table 2).

An unconstrained ordination was applied to illustrate the (dis)similarity between species compositions of each plot. We used non-metrical multidimensional scaling (NMDS) according to the procedure recommended by Minchin (1987) using the function metaMDS of package 'vegan 2.5-4' for the R statistics system. Dissimilarity index used by NMDS was Bray-Curtis. In order to test for differences in species composition between habitat types, we conducted analyses of similarities (ANOSIM). In the case of significant differences, pair-wise comparisons were performed between all levels of the factor. Additionally, the data set was analysed for indicator species using package 'labdsv version 1.8-0'.

Results and discussion

The vegetation survey showed a wide diversity of vascular plant species. In total 349 species were found. On average, roughs had 23 species with a range between 7 and 40. The survey only gives a small but good representation of the roughs within each golf course, and for the golf courses of Baden-Wuerttemberg in general. Therefore, more species and habitat types may very likely be found if more detailed examinations were made. Indicator species analyses revealed *Arrhenatherum elatius* and *Galium album* as the main species in grassland roughs when total vegetation cover was above 80%. In roughs with less total cover *Festuca rubra* was the most frequent species, indicating less nitrogen and more dry conditions.

Table 2. Replacement code for transferring Braun-Blanquet field code into mean vegetation cover.

Field code BB	r	+	1	2m	2a	2b	3	4	5
Code replacement (average cover %)	0.05	0.55	2.5	3.5	10	20	37.5	62.5	87.5

Most roughs belonged to Lowland hay meadow which also explains the finding *A. elatius* as an indicator species. NMDS (Figure 1) and ANOSIM revealed that the assumed group of Mountain hay meadow roughs were not significantly different from the Lowland hay meadow group (P=0.99). However, species of wetland/marsh habitats, mainly found along the edges of ponds, rivers or water bodies, and semi-natural dry grassland habitats were clearly different from the mesophile grassland species. One remarkable feature was the edge of nutrient-poor dry grassland interlacing with the lowland hay meadows as a hotspot for several orchid and endangered species (Figure 1, e.g. *Orchis ustulata, Dactylorhiza majalis, Muscari botryoides, Campanula glomerata*) clearly indicating the importance of extensive management. Ideally, and if possible economically, this should be one late cut per year, but no more than two cuts, with no mulching and very low fertilisation.

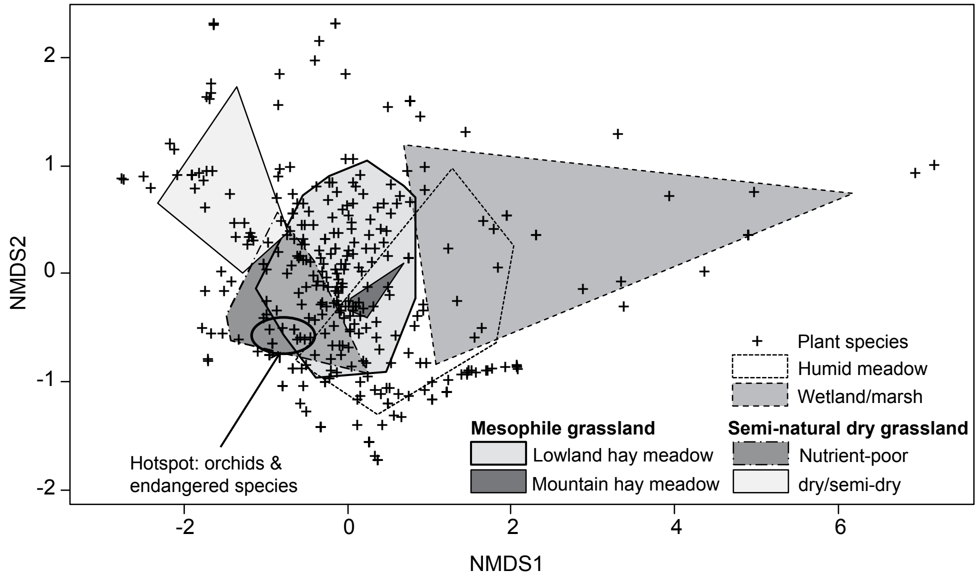

Figure 1. Non-metrical multidimensional scaling ordination (NMDS) of species (+) surveyed in 150 plots in golf course roughs; Outlines of the positions of the assigned habitat types in ordination space (shortest distance) are shown. A hotspot of endangered species is marked with an ellipse.

Conclusions

The study showed golf roughs to be quite diverse and mainly of high ecological value. If greenkeeping follows extensive management, the golf courses can further increase their importance for species diversity and offer valuable habitats for endangered species.

Acknowledgements

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References

Manning P. *et al.* (2015) Grassland management intensification weakens the associations among the diversities of multiple plant and animal taxa. *Ecology* 96, 1492-1501.

Minchin P.R. (1987) An evaluation of the relative robustness of techniques for ecological ordination. *Vegetatio* 69, 89-107.

Impacts of forb abundance on plant nutrition indexes along the growing season in intensively managed permanent grasslands

Perotti E.[1], Huguenin-Elie O.[2], Meisser M.[3], Dubois S.[4], Probo M.[1] and Mariotte P.[1]
[1]*Grazing Systems, Agroscope, 1260 Nyon, Switzerland;* [2]*Forage Production and Grassland Systems, Agroscope, 8046 Zurich, Switzerland;* [3]*Mandaterre, 1400 Yverdon-les-Bains, Switzerland;* [4]*Feed Chemistry Group, Agroscope, 1725 Posieux, Switzerland*

Abstract

Plant nutrition indexes are important tools to estimate forage nutritional status and adjust fertilisation to plants requirements. However, these indexes have initially been developed for grasses and may be ill-suited to grasslands with relatively high abundance of forbs. To test the validity of nutrition indexes (NI) for forb-rich grasslands, we set up an experiment involving nine intensively managed permanent grasslands across Switzerland with contrasted plant composition and soil fertility. The three first harvests of the year 2018 were sorted into grasses, forbs and legumes. Forb abundance in plots ranged from 2 to 74% while legumes represented less than 5% on average. We measured nitrogen (N), phosphorus (P) and potassium (K) content of bulk forage and grasses, and calculated the respective nutrition indexes (NNI, PNI, KNI) separately for both. The PNI and KNI calculated for bulk forage and grasses were slightly different and differences varied among harvests. In addition, we found a strong discrepancy between grasses and forage NNI (up to 70% difference), which was independent of the harvest but influenced by forb abundance. Overall, our findings suggest an increasing underestimation of N fertiliser needs of grasses with an increasing abundance of forbs when using bulk NNI.

Keywords: botanical composition, fertilisation, plant nutrition indexes, permanent grasslands, herbs, forbs

Introduction

Plant nutrition indexes are helpful tools to estimate forage nutritional status and adjust fertilisation to plants requirements. Nitrogen (N), phosphorus (P) and potassium (K) nutrition indexes (NI), respectively NNI, PNI and KNI, have initially been developed for grasses (Duru and Thélier-Huché, 1997; Lemaire and Salette, 1984; Salette and Huché, 1991) but are now widely used for different types of grasslands. While a correction of these indexes has been developed to account for the abundance of legumes within the sward (Jouany *et al.,* 2005; Cruz *et al.,* 2006), the abundance of forbs has not been taken into consideration up to now. As a consequence, these indexes may not be adapted to grasslands with relatively high abundance of forbs. To test the validity of the nutrition indexes for forb-rich grasslands, we set up an experiment using nine intensively managed permanent grasslands across Switzerland with contrasted plant composition, forb abundance and soil fertility.

Materials and methods

Dominant grass species were *Alopecurus pratensis*, *Agrostis capillaris, Lolium* × *hybridum* and *Lolium perenne*, while *Taraxacum officinale* and *Achillea millefolium* were the dominant forbs. Three replicated plots (1.05 m × 5 m) were established at each site and managed according to intensive cutting practices (i.e. forage harvest approximatively every 6 weeks). We considered the first three harvests of 2018, which together yielded more than 75% of annual yield. Forage harvests were split in two subsamples: one representing forage bulk and one separated into grass, forb and legume biomasses. We measured N, P and K content separately for bulk forage and for the grass biomass only, and calculated the nutrition indexes (NNI, PNI, KNI) of the grasslands based on the N, P and K content and dry matter (DM) of either the bulk forage or the grass biomass according to Lemaire *et al.* (1989) and Duru and Thélier-Huché (1997):

$NNI = 100 \times \%N / (4.8\ DM^{-0.32})$
$PNI = 100 \times \%P / (0.15 + 0.065 \times \%N)$
$KNI = 100 \times \%K / (1.6 + 0.525 \times \%N)$

Indexes calculated for bulk forage were corrected for legume abundance (Cruz *et al.,* 2006; Jouany *et al.,* 2005) following the equations:

$NNI_c = NNI - (0.7 \times \%legume)$
$PNI_c = PNI + (0.5 \times \%legume)$
$KNI_c = KNI + (0.5 \times \%legume)$

Nutrient contents in plant biomass are expressed in percentage, DM in Mg ha^{-1} and legume abundance in percentage of the total community biomass. In order to determine the percentage error between nutrition indexes calculated on bulk forage compared to grasses only, we calculated the response ratio (RR) as follows:

$RR_{index} = (Index_{Bulk} - Index_{Grass}) / Index_{Grass}$

We ran regressions between nutrition indexes, calculated for the bulk and grass fractions across different sites, and harvests to highlight any discrepancy between both measurements. The effect of harvest (1st, 2nd, 3rd) on RR_{index} across sites was tested using linear mixed effect models specifying 'plot' nested into 'site' to take into account of repeated measures (harvest) within the same plot and in different sites. To investigate the importance of forbs on the discrepancy between indexes measured for bulk forage and grasses, we ran regressions between RR_{index} and abundance of forbs in plots.

Results and discussion

Forb abundance in plots ranged from 2 to 74%, while legumes represented less than 5% on average. The nine grasslands and the three harvests used in this study yielded a wide range of values for all three nutrition indexes. Our results showed that indexes calculated from the forage bulk and grass biomasses were relatively similar for the phosphorus (PNI) and potassium (KNI) nutrition indexes (Figure 1B,C) with an average percentage error (RR) of 3 and 10%, respectively. Higher percentage error was found during the first and second harvests for KNI (about 10% error) and during the third harvest for PNI (about 8% error), but were not related to forb abundance. By contrast, we found strong discrepancy between nitrogen nutrition index (NNI) calculated from bulk and grass biomass (Figure 1A) with an average percentage error of 20%. Contrarily to PNI and KNI, the percentage error of NNI was

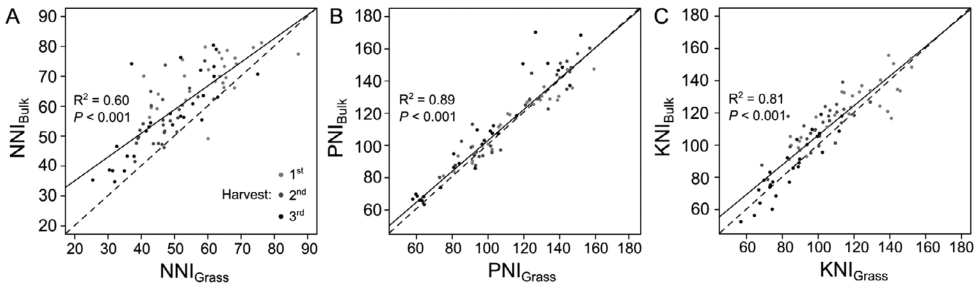

Figure 1. Relationships between plant nutrition indexes calculated on the bulk and grass fractions, (A) Nitrogen nutrition index [NNI], (B) Phosphorus nutrition index [PNI], (C) Potassium nutrition index [KNI] across sites and harvests. Dashed lines indicate cases where bulk and grasses indexes have the same value.

independent on the harvest along the vegetation growing season. This important difference between NNI_{Bulk} and NNI_{Grass} was strongly associated with forb abundance (Figure 2). Indeed, our results showed that discrepancy between NNI_{Bulk} and NNI_{Grass} remained relatively low (10%) up to 20% forb abundance within the community and then quickly increased to reach 70% discrepancy in plots with forb abundance above 60%. These results suggest that forbs may have very different N needs compared to grasses, which strongly impact NNI calculated on bulk forage. It is important to note that vegetation of the nine grasslands included in the study was rarely limited by P and K (i.e. mean PNI and KNI >80; Soil P [Olsen] varying between 14 and 85 mg kg^{-1} across sites), but according to recommendations was insufficiently supplied with N (i.e. mean NNI <80). Limitations in N, but not in P and K, might have affected the observed results and percentage error in PNI_{Bulk} and KNI_{Bulk} might be higher in P- and K-limited grasslands.

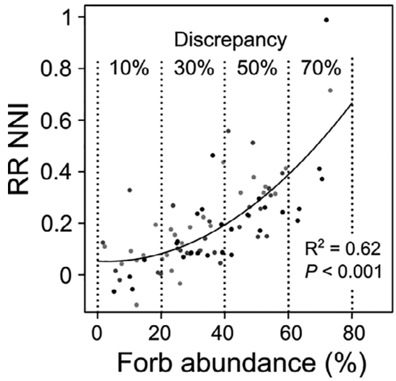

Figure 2. Relationship between the response ratio (RR) of nitrogen nutrition index (NNI) and forb abundance in plots. Discrepancy corresponds to average RR NNI calculated for each class of forb abundance (0-20, 20-40, 40-60, 60-80%).

Conclusions

Overall, our findings show that using plant nutrition indexes calculated on bulk forage might induce important bias in determining vegetation needs for nitrogen, above all in grasslands with forb abundance higher than 20%. Further research is thus needed to better understand nutrient needs in forb-rich permanent grasslands and adapt nutrient supply accordingly.

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References

Cruz P., Jouany C., Theau J.P., Petibon P., Lecloux E. and Duru M (2006) L'utilisation de l'indice de nutrition azotée en prairies naturelles avec présence de légumineuses. *Fourrages* 187, 369-376.

Duru M. and Thélier-Huché L. (1997) N and P-K status of herbage: use for diagnostic on grasslands. In: Diagnostic procedures for crop N management. *Les colloques de l'INRA* 82, 125-138.

Jouany C., Cruz P., Theau J.P., Petibon P., Foucras J. and Duru M. (2005). Diagnostic du statut de nutrition phosphatée et potassique des prairies naturelles en présence de légumineuses. *Fourrages* 184, 547-555.

Lemaire G. and Salette J. (1984) Relation entre dynamique de croissance et dynamique de prélèvement d'azote pour un peuplement de graminées fourragères. I. Etude de l'effet du milieu, II. Etude de la variabilité entre génotypes. *Agronomie* 4, 423-430.

Salette J. and Huché L. (1991) Diagnostic de l'état de nutrition minérale d'une prairie par analyse de végétal: principes, mise en œuvre, exemples. *Fourrages* 125, 3-18.

Derogation for increased cattle manure application on grassland in Flanders: effects on crop yield, N export and nitrate-N residues

Vanden Nest T.[1], Odeurs W.[2], Vandervelpen D.[2], Elsen A.[2], De Vliegher A.[1], Ruysschaert G.[1], D'Hose T.[1], Bries J.[2] and Vandendriessche H.[2]

[1]Flanders Research Institute for Agriculture, Fisheries and Food, Merelbeke, Belgium; [2]Soil Service of Belgium, Heverlee, Belgium

Abstract

In September 2015 the European Commission granted the derogation requested by Belgium for the region of Flanders pursuant to Council Directive 91/676/EEC. The derogation implies that under certain conditions a higher amount of livestock manure can be applied. A monitoring network should reveal the impact of derogation on the nitrogen and phosphorus losses from the soil and water quality. In this paper the results of the monitoring network on grassland on sandy and sandy loam soils (2016-2018) are discussed. No significant effects of the derogation were detected on crop yield and N export in grassland. The average nitrate-N residues in autumn 2016 and 2017 were on average higher on derogation farms. However, the nitrate-N residues were low enough to represent no environmental hazard. In general nitrate-N residues were higher in 2018 compared with 2016 and 2017, largely due to extremely dry summer conditions and sward damage. In 2018 there was no effect of the derogation on nitrate-N residues in grassland.

Keywords: Nitrates Directive, derogation, crop yield, N export, nitrate-N residues

Introduction

Following the Nitrates Directive 91/676/EEC with the aim of protecting ground and surface waters, the Government of Flanders (northern region of Belgium) implemented several manure action programmes starting in the 1990s, imposing severe restrictions on mineral and organic fertiliser use. In September 2015 – at the start of The Fifth manure action programme (2015-2018) – the European Commission granted the derogation requested by Belgium (Decision 2015/1499) for the third time. The derogation implies that under certain conditions, livestock manure in higher quantities than the general application standard of 170 kg N ha^{-1} can be applied. Specifically, for grassland, 250 kg N_{total} ha^{-1} may be applied from cattle manure. However, the total application standard remains 300 and 235 kg $N_{available}$ ha^{-1} for mown and grazed grassland on sandy soils, respectively, and 310 and 245 kg $N_{available}$ ha^{-1} for mown and grazed grassland on other soil types. $N_{available}$ of cattle slurry and farmyard manure is calculated as 60 and 30% of N_{total}. The objective of this research is to set up and follow a monitoring network of farms in order to provide data on the impact of derogation. The monitoring network provides data on production factors, such as fertilisation and yield as well as monitoring data on nitrates and phosphorus in soil and water. In this paper we evaluate the effect of derogation on grassland in sandy and sandy loam soils in 2016, 2017 and 2018.

Materials and methods

In 2016 we created a network of 160 farms to monitor the effect of derogation on potential nutrient losses. All farms are located on sandy and sandy loam soils, since derogation is mainly requested on farms located on these soils. Derogation can be requested for several crops; however, for this paper we focus only on monoculture grass pastures (72 farms). On each farm, three pastures were selected for monitoring. Half of them are derogation farms, half are not. The farms are evenly spread over sandy and sandy loam soils. On each farm general information was gathered about the type of farm, livestock and acreage. From each pasture information was gathered about the soil chemical conditions, the fertilisation, cutting dates, estimated yield and number of grazing livestock and grazing days if applicable for 2016, 2017 and 2018. The farmer's management choices were not influenced by the researchers. On each pasture, soil samples were taken in all three years during the period from 1 October to 15 November, to determine the nitrate-N residue in the soil profile (0-90 cm). Soil sampling and analysis were done by accredited samplers and in an accredited soil lab, respectively. Some pastures were excluded from further research, for example because soil sampling to a depth of 90 cm was not possible (e.g. too dry). In 2017 and 2018, twenty pastures were selected for crop yield determination: 10 parcels on derogation farms and 10 on non-derogation farms. On each pasture and just before each cut, 3 representative strips of 9 m^2 were harvested to determine the yield. From each strip a grass sample was dried at 70 °C for 48 hours to determine the dry matter content. Samples were ground using a plant mill and analysed in a total N analyser for N content. The dry matter yield and N export were calculated. Several parcels were excluded due to missing data from one or more cuts. Differences in yield, N export and nitrate-N residue were statistically analysed.

Results and discussion

Although grassland is generally managed more intensively on derogation farms than non-derogation farms, no statistical differences were detected in crop yield and N export in 2017 and 2018 (Table 1). We determined no crop yields in 2016. We assume this to be the result of exceptional weather conditions. Spring and early summer of 2017 were exceptionally dry, and 2018 extremely dry. This had an effect on the number of cuts and the crop yield of all grasslands throughout Flanders. In the whole of Flanders crop yields were generally 25% lower than normal in 2017 and 2018. In 2016 and 2017 the nitrate-N residues were significantly higher in grassland on sandy loam soils from derogation farms than on non-derogation farms (Table 2). In both years the same trend was detected for sandy soils, but the difference was not significant. In 2018 no differences in derogation versus non-derogation was observed. Probably this is linked to the weather conditions. Due to extremely dry conditions in summer 2018, many swards were severely damaged. Dead grass thus started mineralizing in the autumn when precipitation increased. Except for derogation farms in sandy loam soils in 2018, the nitrate-N residues were below 90 kg NO_3^--N ha^{-1}, which was the legal threshold value in the manure action programme 2015-2018. This is the average of many parcels, however; some of the parcels did exceed the threshold.

Table 1. Mean dry matter yield and N export of the first cut and in total from a selection of grassland parcels in the monitoring network with crop yield determination for every cut.

Year		Derogation?	n[1]	Dry matter (DM) yield		N-export	
				Mg DM ha^{-1}	*P*-value	kg N ha^{-1}	*P*-value
2017	1st cut	Yes	10	4.16	0.94	110	0.82
		No	10	4.04		104	
	total	Yes	5	10.67	0.46	308	0.92
		No	5	11.85		289	
2018	1st cut	Yes	9	3.10	0.20	97	0.96
		No	9	3.89		97	
	total	Yes	9	9.16	0.86	298	0.27
		No	9	9.48		253	

[1] n = number of parcels in the analysis.

Table 2. Nitrate-N residue on all grassland parcels of the monitoring network.[1]

Year	Soil	Derogation?	n	Nitrate-N residue, kg NO_3^--N ha^{-1} (0-90 cm)		*P*-value
				Median	Mean	
2016	S	Yes	52	37	51	0.06
		No	53	23	36	
	SL	Yes	53	28	41	0.03
		No	54	18	27	
2017	S	Yes	49	61	69	0.07
		No	50	33	52	
	SL	Yes	50	40	55	0.02
		No	50	22	35	
2018	S	Yes	52	50	65	0.93
		No	52	64	79	
	SL	Yes	49	61	97	0.21
		No	51	43	64	

[1] n = number of parcels in the analysis; S = sandy soil; SL = sandy loam soil.

Conclusions

In 2017 and 2018, we observed no effects of the derogation on crop yield and N export. We assume that this was because of exceptionally dry conditions in both years. Although nitrate-N residues were increased for derogation farms compared with the non-derogation farms, in most cases nitrate residues were well below the acceptable level.

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CO_2 and N_2O balance of a legume-based grassland in eastern Finland

Shurpali N.J.[1], Li Y.[1], Korhonen P.[2] and Virkajärvi P.[2]
[1]Biogeochemistry research group, Department of Environmental and Biological Sciences, University of Eastern Finland, Yliopistoranta 1 D, P.O. Box 1627, 70211 Kuopio Finland; [2]Milk Production group, Production Systems unit, Natural Resources Institute Finland, Halolantie 31A, 71750 Kuopio Finland

Abstract

Legume-based grasslands have many advantages. Several earlier studies have shown that perennial legumes help in soil carbon (C) sequestration and mitigation of nitrous oxide (N_2O) emissions. Legume-based grasslands are an important part of the regional economy in Finland as they provide high protein fodder for the cattle and thus are highly relevant to the meat and dairy industries. In view of the changing climate, we need to ensure that the fodder grass production is environmentally sustainable. Therefore, we are measuring carbon dioxide (CO_2) and N_2O balance using the eddy covariance technique at a site near the Luke Kuopio (Maaninka) research station in eastern Finland. We present in this paper seasonal balance of CO_2 from a grassland site growing timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.) and discuss the climate impact on vegetation dynamics in the legume grassland ecosystem. These data are important in evaluating the impact on the environment of producing a unit kg of meat or a litre of milk.

Keywords: environmental sustainability, climate change, food security, eddy covariance technique, GHG exchange

Introduction

The introduction of legumes in cropping systems reduces the dependence on nitrogen (N) fertilisers and could mitigate the carbon (C) footprint of cropping systems (Saliendra *et al.*, 2018). Legumes are seen as competitive crops because of their economically sustainable advantages for farming and nutritional benefits for humans and livestock alike. However, their role in sustainable agricultural production under boreal environments is not well understood yet. There is a dearth of regionally based, ecosystem-scale field experimental data on agriculture in Finland. Such data are crucial in improving model predictions of current and future climate feedbacks from agricultural management practices. Here, we measured net exchange of carbon dioxide (CO_2) and nitrous oxide (N_2O) from a legume-grass mixture cultivated on a loamy soil in eastern Finland with the aim to evaluate the sustainability of legume grassland management with respect to soil organic carbon (SOC) sequestration and climate change mitigation.

Materials and methods

The experimental site (a 6.3 ha agricultural field of 280×220 m) is located in Maaninka (63°09'49'N, 27°14'3'E, 89 m a.s.l.) in eastern Finland. Annual long-term mean air temperature in the region is 3.2 °C and annual precipitation 612 mm (reference period 1981-2010; Pirinen *et al.,* 2012). The soil is classified as a Haplic Cambisol/Regosol (Hypereutric, Siltic) (IUSS Working Group WRB, 2007). The main experimental field was established with a mixture of timothy (*Phleum pratense* L. cv. Nuutti; seed rate 15 kg ha^{-1}) and red clover (*Trifolium pratense* L. cv. Ilte 5 kg ha^{-1}) in May 2015 with barley as a cover crop during the first year. The field was oversown in 2017 with red clover (cv. Ilte) with a seed rate of 3 kg ha^{-1}. The eddy covariance measurements began in May 2017. The field was divided into two parts based on the frequency of wind directions with the eddy covariance (EC) tower located in the middle of the field. The treatments were (1) MinN, fertilised with mineral N, and (2) OrgN, fertilised with digestate residue from a farm scale anaerobic biogas plant (input cow slurry and timothy-red clover silage) located at the Luke Kuopio (Maaninka) research station. The MinN part received (in ha^{-1} $year^{-1}$) 106 kg N, 28 kg P and 50 kg K, whereas OrgN received 98 kg total N of which 53 kg was soluble N, 13 kg P and 83 kg K. The yield was harvested twice per growing season (late June to early July and second cut in late August). The EC system consists of a sonic anemometer (RS-50, Gill Solent Ltd., UK) for measuring turbulent wind components, an infrared gas analyser (LI7000 – Li-Cor Inc., Lincoln, NE, USA) and a laser spectrometer (Aerodyne, Inc., Billerica, USA) at 2.5 m height for detecting high frequency CO_2, water vapour and N_2O concentrations. The same EC system measured the fluxes from both MinN and OrgN parts depending on the prevailing wind direction (about 65% of the total data were from OrgN side and 35% from MinN part of the study area). Likewise, a weather station located at the site monitored continuously several micrometeorological variables such as air temperature and humidity, atmospheric pressure, wind speed and direction, radiation balance components, photosynthetically active radiation (PAR), profiles of soil temperature and moisture, etc. Details of EC data processing procedures are described in our earlier publications (Rannik *et al.*, 2015; Shurpali *et al.*, 2016).

Results and discussion

The 2017 and 2018 growing seasons under investigation here presented stark differences in the short-term and seasonal weather conditions. The 2017 season was cooler with a May – September mean temperature of 11.6 °C, whereas for the similar period in 2018 the seasonal average temperature was 15.1 °C. These large seasonal differences resulted from exceptionally warm temperatures during the 2018 spring. The period from snowmelt in the spring to early June marks the most crucial time for the development of soil thermal and hydrological conditions and vegetation growth in the Nordic region. Favourable climatic conditions (optimal temperatures, well-distributed rain events and high available soil moisture and nutrients) during this part of the season ensure vigorous crop growth and high productivity for the entire season. Climatic stress during this time is generally considered detrimental to the overall seasonal crop performance. The seasonal and year-to-year variability in net CO_2 balance in our study resulted primarily from these differences in plant productivity at the start of the growing season, which sets the stage for the year's growth. There were clear differences in the net amount of CO_2 fixed under MinN and OrgN treatments during the two seasons. The cumulative net amount of CO_2 fixed by the legume-grass mixture in 2017 from the start of the measurements until the end of September (May 19 – Sep 30) was 10.1 Mg ha^{-1} in MinN and 8.3 Mg ha^{-1} in OrgN. However, during a similar period in the warmer 2018 season, the MinN part fixed 19.8 Mg ha^{-1} and the OrgN part fixed 12.8 Mg ha^{-1} of CO_2 (Table 1).

The dry matter (DM) yield in 2017 was 5.9 Mg ha^{-1} for MinN and 4.9 Mg ha^{-1} for OrgN, while in 2018 it was 6.3 and 6.8 Mg ha^{-1}, respectively, with a 7 to 39% higher yield advantage from the warmer year. Most likely some part of applied OrgN was leached due to wetter conditions during the digestate spreading in 2017. In 2017, the DM yield from the second cut was 20% higher than from the first cut. The trend was reversed in 2018. The yield from the first cut in 2018 was 135% higher than that from the second cut. The seasonal N_2O balance indicated that the OrgN had negligible N_2O emissions and MinN treatment was a sink in 2017. Both treatments were a net sink for N_2O in 2018 with OrgN being a smaller sink than the MinN treatment. The enhanced sequestration of atmospheric CO_2 under high temperatures has been possible owing to higher photosynthetic capacity of the vegetation during the early spring in 2018 (MinN GPP during 2018 season was higher by 35%, while that of OrgN site was higher by 57% compared with the corresponding 2017 seasonal GPP values). Considering the yield advantage and exceptionally high rates of atmospheric CO_2 uptake during the 2018 season, it is intuitive to conclude that the climatic conditions that existed during the 2017 season, especially during the early, crucial crop development phase, were suboptimal (Table 1).

Table 1. Seasonal net CO_2 balance for the legume grassland during the 19 May – 30 Sept. period in 2017 and 2018.[1]

Year	Treatment	NEE Mg CO_2 ha^{-1}	N_2O[2] Mg CO_2e ha^{-1}	Yield[3] Mg CO_2 ha^{-1}	Organic fertiliser C[4] Mg CO_2e ha^{-1}	Seasonal balance Mg CO_2e ha^{-1}
2017	Min N	-10.1	-0.6	10.4	0.0	-0.3
	Org N	-8.3	0	8.6	-1.6	-1.3
2018	Min N	-19.8	-0.7	10.2	0.0	-10.3
	Org N	-12.8	-0.3	11.0	-1.8	-3.9

[1] All units are in Mg CO_2-equivalents ha^{-1}. The net balance is estimated as the sum of net ecosystem CO_2 exchange (NEE), net N_2O exchange, crop dry matter yield and C input in organic fertiliser (digestate). Negative values represent net CO_2 uptake by the ecosystem and positive values imply CO_2 losses from the system.

[2] Expressed in Mg CO_2-equivalents ha^{-1} with a global warming potential (GWP) value of 265 for a 100-year time horizon.

[3] 43.9% C content in dry matter.

[4] Organic fertiliser contained 37.8±1.9% C in dry matter.

Conclusions

Based on the net carbon balance estimated as the sum of seasonal NEE, N_2O flux and crop yield, the MinN site was a weak sink in 2017 and a stronger sink in 2018, while OrgN site was a sink for atmospheric CO_2 in both years (Table 1). The OrgN site was a bigger sink during the warmer year 2018. While the OrgN site had high GPP during 2018, the total respiration from this site was also high. Therefore, the mineral site sequestered more CO_2 than the organic site in 2018. A negative value for the net carbon balance after accounting for the loss of carbon (biomass removal in harvest) suggests that the ecosystem retained a part of the sequestered CO_2 in the soil. Based on the net CO_2 and N_2O exchange, climate and phenological development patterns observed at our study site, the grassland vegetation in boreal region appeared to have a strong SOC sequestration potential in a climatically extreme 2018. The results from this study are especially relevant to the Paris Climate Agreement that places a strong emphasis on adapting to climate change through innovative solutions to increasing soil organic carbon.

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References

Pirinen P., Simola H., Aalto J., Kaukoranta J., Karlsson P. and Ruuhela R. (2012) *Climatological statistics in Finland 1981-2010*. Finnish Meteorological Institute, Helsinki, Finland.

Rannik Ü, Haapanala S., Shurpali N.J., Mammarella I., Lind S., Hyvönen N., Peltola O., Zahniser M., Martikainen P.J. and Vesala T. (2015) Intercomparison of fast response commercial gas analysers for nitrous oxide flux measurements under field conditions. *Biogeosciences* 12, 415-32.

Saliendra N.Z., Liebig M.A. and Kronberg S.L. (2018) Carbon use efficiency of hayed alfalfa and grass pastures in a semiarid environment. *Ecosphere* 9, e02147.

Shurpali N.J., Rannik Ü., Jokinen S., Lind S., Biasi C., Mammarella I., Peltola O., Pihlatie M., Hyvönen N., Räty M., Haapanala S., Zahniser M., Virkajärvi P., Vesala T. and Martikainen, P.J. (2016) Neglecting diurnal variations leads to uncertainties in terrestrial nitrous oxide emissions. *Scientific Reports* 6, 25739.

The effect of spring melt conditions on phosphorus losses in surface runoff from grassland fertilised with mineral P or slurry

Järvenranta K. and Virkajärvi P.
Natural Resources Institute Finland, Halolantie 31 A, 71750 Maaninka, Finland

Abstract

Phosphorus (P) losses after slurry or fertiliser application cause eutrophication in surface waters. There are few comparisons between different application techniques and timing, as the experimental set up is demanding with multiple factors. We measured surface runoff from 11 different fertiliser and slurry treatments simultaneously in a surface runoff simulator (SIMU) located in an adjustable climatic chamber. Treatments were 15 and 30 kg P ha^{-1} in mineral fertiliser P or slurry P spread by broadcasting, trailing hose and injection in summer or in autumn. In addition, two different spring conditions (snowy spring and rainy spring) were applied for all 11 treatments. The experiment was repeated in the two following years. There were no differences in phosphorus losses in surface runoff between the years (Total P 0.039 g m^{-2} and dissolved P 0.030 g m^{-2}). The amount of phosphorus loss was lower during a snowy winter than in rainy winter (0.034 g m^{-2} vs 0.045 g m^{-2}), but the proportion of dissolved P was higher (83 vs 68%). In both years, slurry caused more P losses than mineral P, and autumn spreading more losses than summer spreading. Highest risk for large P losses occurred when soil was wet during slurry spreading in autumn.

Keywords: slurry, phosphorus, surface runoff, fertiliser, grass

Introduction

Phosphorus (P) losses after slurry or fertiliser application cause eutrophication in surface waters. Comparisons between different application techniques and timing are seldom made, as the experimental set up is demanding with multiple factors such as type and timing of fertilisation, weather conditions prior and post application affecting both soil and vegetation ability to bind phosphorus, and also the weather circumstances during winter and spring. Slurry has caused an increased risk for P losses in surface runoff compared with mineral fertiliser especially when used in large amounts, late in the autumn or by broadcasting method (Puustinen *et al.,* 2019). Changing climate with heavy winter rains may also increase P loads from fields (Fisher and Knutti, 2015).

The aim of this study was to produce coefficients for P runoff for 11 different fertiliser and slurry treatments in snowy or rainy spring circumstances for modelling purpose. Runoff experiments were performed in a surface runoff simulator (SIMU) combined with adjustable climatic chamber.

Materials and methods

The study was carried out at the Natural Resources Institute Finland (Luke), Kuopio (63°14' N, 27°31' E). The soils of the experimental area were moderately coarse-textured sandy soil in the plough layer with pH 6.3-6.5 and soil P_{aac} concentration 13.1±0.8 mg l^{-1} (mean ± standard deviation). The mean annual air temperature is 3.1 °C and the mean annual rainfall 612 mm (FMI open data). Soil samples (0 to 7 cm) were taken from each plot. The soil test P concentrations expressed as ammonium acetate-extractable P (P_{aac}) was determined by Eurofins Ltd. (Vuorinen and Mäkitie, 1955). The experiments were accomplished during 2017-2018 on a grass mixture with timothy (*Phleum pratense* L.) -meadow fescue (*Festuca pratensis* Huds.). The first fertilisation was given equally to all plots as mineral fertiliser in May and the first silage cut in June. The first cut was not included in the experiment. The experimental treatments were: 0, soil test based P-fertilisation, 15 and 30 kg P ha^{-1} either in mineral fertiliser or slurry P spread by broadcasting, trailing hose or injection. The treatments were applied after first silage cut or in the autumn. In addition, the treatments were two different spring conditions (snow cover and irrigation), and were applied for all 11 treatments.

The soil mats (one per each plot; 0.3×0.9 m, 6-7 cm thick) were cut from the field in November in both years, stored in -2 °C prior to the runoff experiment. Each runoff period lasted for 7 days in the SIMU chamber (Saarijärvi and Virkajärvi, 20, with temperature pattern adjusted at night time -3 and at daytime +15 °C. To generate surface runoff, each soil mat was either covered with snow or irrigated with 90 mm water divided between three consecutive days at the beginning of each runoff period. Runoff water was collected and analysed for total P (SFS 3026) and dissolved P (SFS 3025) among other analyses. The experiment was repeated in the two following years. Experimental design was a randomised complete block design with four replicates. P runoff coefficients were calculated relative to 30 kg P ha^{-1} mineral fertilisation given in summer and are presented as treatment medians. Data were analysed by ANOVA. Model included P fertilisation, spring circumstances (snow or irrigation) and their interaction as fixed factors and replicate as random factor with Mixed-procedure SAS 9.4.

Results and discussion

There was no difference in phosphorus losses in surface runoff between the years (Total P 0.039 g m^{-2} and dissolved P 0.030 g m^{-2}). The proportion of dissolved P was higher during the first year (80 vs 72%).

The amount of phosphorus loss was lower during the snowy spring compared with rainy spring (0.034 g m^{-2} vs 0.045 g m^{-2}), but the proportion of dissolved P was higher (83 vs 68%). Even though the overall amount of P loss was higher for rainy circumstances for all treatments compared to snow melt, the differences between treatments for P runoff coefficients were larger for snow melt conditions (Figure 1A). As expected, 15 kg P ha^{-1} caused less P runoff than 30 kg slurry P ha^{-1}. However, it was surprising that there was no difference between the trailing hose and injection methods, even though the P loss was clearly lower with injection when 30 kg slurry P ha^{-1} was applied. The P load from slurry was higher than from mineral fertiliser especially in the snowy spring whereas the difference was less obvious in the rainy spring. The effect is especially pronounced for broadcast and trailing hose slurry applications in summer.

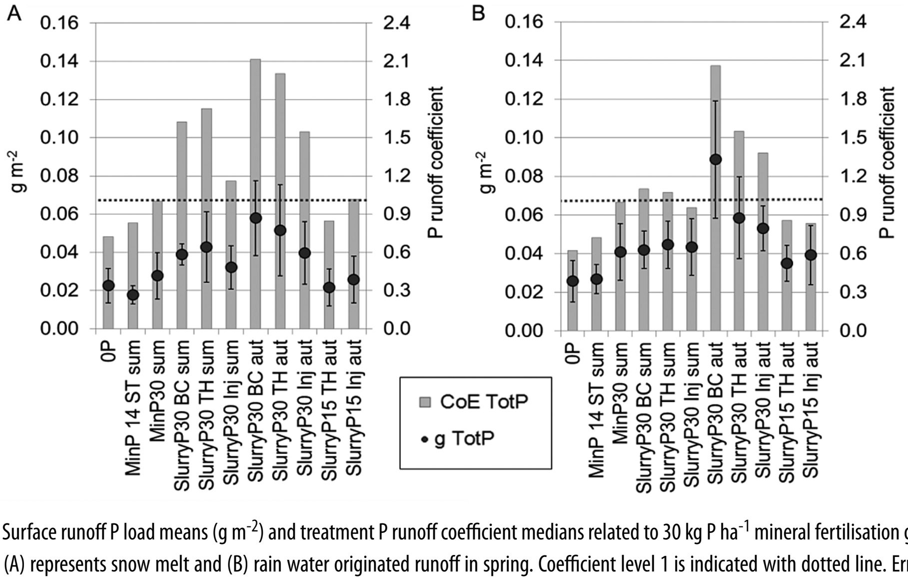

Figure 1. Surface runoff P load means (g m^{-2}) and treatment P runoff coefficient medians related to 30 kg P ha^{-1} mineral fertilisation given in summer. (A) represents snow melt and (B) rain water originated runoff in spring. Coefficient level 1 is indicated with dotted line. Error bars represent std. Min = mineral fertiliser, ST = based on soil test recommendation, BC = broadcast, TH = trailing hose, Inj = injection, sum = summer and aut = autumn.

Combination of bare soil (irrigation) and freeze/thaw cycles increased P losses compared to snow cover melting on soil with equal freeze/thaw cycles. Snow cover probably protects soil surface from sub-zero conditions that extract soil and microbiome P and small soil particles from the surface (Freppaz *et al.,* 2007; Uusi-Kämppä *et al.,* 2012).

The SIMU method with intact surface soil mats is unique and efficient way to make a direct comparison between a large number of treatments, here there were 22 in total. Runoff coefficients achieved this way seem to be a potential aid for modelling purposes. However, environmental circumstances during the growing season have a marked effect on P losses that reflects on coefficients.

Conclusions

Slurry caused more P losses than mineral P, and autumn spreading more than summer spreading. The highest risk for large P losses occurred when soil was wet during spreading in autumn. A rainy spring increased P losses compared with snowy spring conditions. Runoff coefficients achieved in controlled circumstances (SIMU) are a potential tool for modelling purposes.

References

Fisher E.M. and Knutti R. (2015) Anthropogenic contribution to global occurrence of heavy-precipitation and high-temperature extremes. *Nature Climate Change* 5, 560–564.

Freppaz M., Williams B.L., Edwards A.C., Scalenghe R. and Zanini E. (2007) Simulating soil freeze/thaw cycles typical of winter alpine conditions: Implications for N and P availability. *Applied Soil Ecology* 35, 247-255. https://doi.org/10.1016/j.apsoil.2006.03.012

Puustinen M., Tattari S., Väisänen S., Virkajärvi P., Räty M., Järvenranta K., Koskiaho J., Röman E., Sammalkorpi I., Uusitalo R., Lemola R., Uusi-Kämppä J., Lepistö A., Hjerppe T., Riihimäki J. and Ruuhijärvi J. (2019) Ravinteiden kierrätys alkutuotannossa ja sen vaikutukset vesien tilaan. KiertoVesi-hankkeen loppuraportti. *Suomen ympäristökeskuksen raportteja* 22/2019. 142 p. (In Finnish)

Saarijärvi K. and Virkajärvi P. (2010) Surface Runoff Simulator (SIMU) hastens the research on phosphorus losses from grassland. *Grassland Science in Europe* 15, 78-80.

Uusi-Kämppä J., Turtola E., Närvänen A., Jauhiainen L. and Uusitalo R. (2012) Phosphorus mitigation during springtime runoff by amendments applied to grassed soil. *Journal of Environmental Quality* 41, 420-426. https://doi.org/10.2134/jeq2010.0441

Vuorinen J. and Mäkitie O. (1955) The method of soil testing in use in Finland. *Agrogeological Publications.* 63 pp. 1-44.

Improving phosphorus use efficiency on extensive grassland farms without compromising productivity

Higgins S., Nicholl G., Vero S., Bailey J.S. and Doody D.
Agri-Food and Biosciences Institute, Newforge Lane, Belfast, United Kingdom

Abstract

Improving phosphorus (P) use efficiency on farms is essential in order to minimize the contribution that agriculture makes to P export to waterbodies within catchments. Lowering P fertiliser use on extensive grassland farms without compromising on productivity, is being studied at three farms in Ireland. These grasslands receive nitrogen inputs of on average less than 60 kg N ha^{-1} yr^{-1} of chemical N fertiliser and manure loadings of less than 120 kg N ha^{-1} yr^{-1}. Sixty experimental plots (each plot 16 m^2) have been established at three grassland sites where soil P content is 10-20 mg l^{-1} Olsen P and soil pH is sub-optimal (average pH 5.7). Plots receive 60 kg N ha^{-1} yr^{-1} along with either low, medium or high P fertiliser inputs over a three-year period, each rate with and without the addition of lime. Grass dry matter yields, nutrient uptake, nutrient use efficiency and grass quality are being monitored. Data for the first year of application demonstrate that revised P recommendations for extensive grassland were sufficient for production. Overall nutrient balance however is essential, with one of the experimental sites experiencing potassium deficiency in both the soil and grass that was severely hampering production. There were no significant beneficial effects of lime application on yields or nutrient uptake in Year 1.

Keywords: phosphorus, extensive grassland, water quality, grass yield

Introduction

Many waterbodies are failing to achieve the targets of the Water Framework Directive (WFD) due to phosphorus export from agricultural land (Boardman *et al.*, 2018). Extensive farms comprise a large percentage of the farming enterprises in Ireland and are likely to be making a significant contribution to nutrient enrichment of freshwater systems. In a recent soil survey in Northern Ireland (NI), 50% of fields on dairy farms and almost 40% of fields in beef and sheep enterprises were found to be above the recommended agronomic optimum for soil Olsen P (16-25 mg l^{-1}). Extensively managed grassland in Northern Ireland is classified as grassland which receives less than 60 kg chemical N ha $year^{-1}$ and less than 120 kg manure N loading per annum (NAP, 2019). Generally, although not exclusively, this grassland supports beef/sheep enterprises with grazed pasture and 1-2 silage cuts per year. The Nitrates Action Programme (NAP) and Phosphorus Regulations in Northern Ireland were established in order to meet the requirements of the EU Nitrates Directive (91/676/EEC) and are revised every four years. One of the main objectives is to promote efficient management of animal manures and manufactured fertilisers in order to reduce pollution and improve water quality. In a recent revision it was proposed that grassland managed 'extensively' with relatively low nitrogen inputs, should have lower P requirements and therefore a lower target soil P level (16-20 mg l^{-1}) than grassland managed 'intensively' with high N inputs driving high levels of grass production and P removal. These new fertiliser guidelines are being evaluated as a component of a wider five-year project implementing a series of catchment restoration actions, supported by the European Union's INTERREG VA Programme and managed by the Special EU Programmes Body (SEUPB). One of the hypotheses being evaluated, and presented here, is that a soil Olsen P level of 16-20 mg l^{-1} is sufficient to meet crop requirements on extensively managed grassland farms in Northern Ireland.

Materials and methods

Experimental field plots were established in 2019 on three extensive grassland farms in the Blackwater river catchment, a cross-border catchment straddling the border region of Northern Ireland and the Republic of Ireland. Sixty 16 m^2 experimental plots were established in one field per each of the three farms. Two of the fields were managed by grazing animals and one of the fields was managed for silage in a two-cut system. Experimental plots on the grazed field were fenced off during the trial to prevent animal access, and two grass harvests were taken in a simulated cutting regime. The soil nutrient status and field management of each site are presented in Table 1. Site 3 contained an overall mean farm application of less than 60 kg N $ha^{-1}yr^{-1}$, but as would be common practice, a larger application of 75 kg N ha^{-1} was applied for first cut silage. The plots on the two grazed fields (Sites 1 and 2) received 60 kg N ha^{-1} split between two applications along with either a low, medium or high rate of P fertiliser, as shown in Table 2. P Rate 1 was the new revised P fertiliser recommendations for extensive grassland under grazing management. P Rate 2 was the P fertiliser rate recommended for extensive grassland under silage production on an extensive farm (based on the current soil P status per field) and P Rate 3 was the P recommendation for a silage field managed within a typical intensive dairy system. Potassium (K) was applied per field as recommended per soil K status and management type. Lime was applied to half the plots at each site, with the other half receiving no lime. Rate of lime applied was 2.5 Mg ha^{-1} at Site 1 and Site 2 and 2 Mg lime ha^{-1} at Site 3. Fertiliser was applied in late May/early June 2019. Experimental plots were cut 6-8 weeks following the fertiliser application using a Haldrup harvester, immediately followed by a second fertiliser application and second harvest 6-8 weeks later. No organic manure was applied to the sites in 2019. Grass dry matter (DM) yield was measured on each plot along with grass nutrient status, nutrient use efficiency and grass quality (protein, water soluble carbohydrate, acid detergent fibre). An Analysis of Variance was carried out to ascertain the significance of P application rate and lime on each of the measured variables.

Table 1. Soil nutrient status and management of three selected field sites.

	Site 1	Site 2	Site 3
Soil P mg l^{-1}	13	17.8	18.3
Soil K mg l^{-1}	120	275	75
Soil pH	5.69	5.74	5.86
Inorganic N fertiliser use kg N ha^{-1}	60	60	75 1^{st} cut silage; 75 for 2^{nd} cut
	Whole season rate	Whole season rate	Per cut application
Management	Grazing	Grazing	Silage

Table 2. Revised P fertiliser recommendations for extensive grassland farms in Northern Ireland.

Maximum P fertiliser application limits, kg P per ha	Soil P, mg l^{-1}				
	0-9	10-15	16-20	21-25	26-45
Grazed grass (whole season) extensive farm	22	15.4	8.8	0	0
First cut silage extensive farm	30.8	24.2	17.6	0	0
First cut silage intensive dairy farm	44	30.8	17.6	17.6	8.8

Results and discussion

In Year 1 the revised P recommendations for extensive farms were shown to be sufficient for production. Rate of P fertiliser applied had no significant ($P<0.01$) effect on grass DM yields within the two grazed fields (Site 1 and Site 2). Under the silage regime (Site 3), mean total grass DM yield over 2 cuts was 6.55 Mg DM ha^{-1}, which was lower than the total yield of the two grazed fields (Site 1: 7.23 Mg DM ha^{-1} and Site 2: 6.87 Mg DM ha^{-1}) despite Site 3 receiving a greater rate of N fertiliser. Site 3 was found to have severe potassium deficiency in both the soil (75 mg K l^{-1}) and in the grass silage. 60% of the experimental plots at Site 3 contained negative DRIS (Diagnosis and Recommendation Integrated System) indices for K. DRIS-*ryegrass* is a mathematical model which interprets the results of grass tissue analysis to give a series of indices for N, P, K and S. Negative DRIS N, P, K or S indices are indicative of deficiencies in these nutrients serious enough to limit sward production (Bailey *et al.*, 1997a). An index of -1 indicates that at least 10% of the maximum DM yield has been lost (Bailey *et al.*, 1997b), and indices between -10 and -30 suggest that anything up to 40% of the possible DM yield has been lost. At Site 3, 31% of plots contained a DRIS K Index of between -10 and -30. Only 1% of plots displayed negative DRIS P indices, at either cut, and these were control plots receiving no P fertiliser, indicating that grass P content was sufficient and not limiting production. Even at the lowest P application rate (8.8 kg P per ha), a negative P index was not displayed at any of the 3 sites, including Site 1 where starting soil Olsen P content was lowest (13 mg l^{-1}). Lime application did not have a significant beneficial effect on any of the parameters measured in Year 1, even at Site 1 where soil pH was pH 5.69 and is where lime would likely have most benefit. There are a further two years of data to be collected from all three field sites, during which time lime may display greater effectiveness.

Conclusions

Sufficient quantities of all nutrients and an adequate soil pH (pH 6.0-6.2) are required to optimise grass production. On extensive grassland farms, the revised P recommendations can potentially maintain production to meet specific farm requirements and will be evaluated further over the coming two years.

Acknowledgements

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References

Bailey J.S., Beattie J.A.M. and Kilpatrick, D.J. (1997a) The diagnosis and recommendation integrated system (DRIS) for diagnosing the nutrient status of grassland swards: I Model Development. *Plant and Soil* 197, 127-135.

Bailey J.S., Cushnahan A. and Beattie J.A.M. (1997b) The diagnosis and recommendation integrated system (DRIS) for diagnosing the nutrient status of grassland swards: II Model calibration and validation. *Plant and Soil* 197, 137-147.

Boardman J., Vandaele K., Evans R. and Foster I.D.L. (2018) Offsite impacts of soil erosion and runoff: why connectivity is more important than erosion rates. *Soil Use and Management* 2019, 1-12.

Productivity and persistency of multicomponent swards with different legume contents, using two nitrogen fertilisation rates

Adamovics A. and Gutmane I.
Latvia University of Life Sciences and Technologies, Liela iela 2, 3001 Jelgava, Latvia

Abstract

Field trials were carried out with the aim to investigate the forage yield and quality during four production years of grass and legume-grass swards, fertilised with two nitrogen rates (N0 and N120). Mixtures were grouped into three types: composed only of grasses (G); white clover (*Trifolium repens*) and grass (Tr+G); white clover, red clover (*Trifolium pratense*) and grass (Tr+Tp+G). Significant differences in dry matter (DM) yield were found between successive production years and mixture types (MT). More stable productivity was demonstrated by grass-only swards, with a yield decrease between the first and fourth year of 2.59 Mg ha^{-1} (or 32%). Grass MT had the highest DM yield in the fourth production year, in comparison with the legume-containing mixtures. For all legume-containing MT, there was more yield decrease by year 4 (by 11.43 Mg ha^{-1} or 71% for Tr+Tp+G mixtures). N rate increase from 0 to 120 kg ha^{-1} contributed to a significantly increased DM yield and crude protein (CP) for all MT. The positive influence of N fertilisation on DM yield and CP was more pronounced on grass-only swards. Legume-containing mixtures achieved a higher CP content in comparison with grass-only swards at all fertilisation rates in all production years.

Keywords: grass-legume mixture, persistency, nitrogen fertilisation, crude protein

Introduction

In Latvia's grassland farming, the use of multicomponent mixtures is a traditional practice, because mixture swards can secure better persistence and more stable productivity with high-quality forage (Adamovics and Gutmane, 2016). Botanical composition of grassland is very important for sward persistence, not just for forage yield and quality. Insufficient winter survival and a fast decline of productivity over successive years are often observed for *Lolium* species in monoculture swards in the Baltic region (Aavola, 2005; Lemežiene *et al.*, 2004). The objective of our study was to investigate forage yield and quality during four production years of multispecies swards containing different contents of legume-and-*Lolium*-grasses.

Materials and methods

Field trials were conducted at three experimental sites in Latvia. At each of the three sites, the same mixtures were sown in June 2014 – without a cover crop, in three replications, and with a 10-m^2 plot size. The following grass combinations were used in mixtures: *Lolium perenne*, ×*Festulolium loliaceum* in equal parts (L); *Phleum pratense, Festuca pratensis, Lolium perenne Poa pratensis, Festuca rubra* in equal parts (Mix). The legume-containing swards were composed of *T. repens* 20% and grasses 80%: Tr+G(L) and Tr+G(Mix); *T. repens* 10%, *T. pratense* 20% and grasses 70%: Tr+Tp+G(L) and Tr+Tp+G(Mix). Only-grass swards were composed of *Dactylis glomerata* 50% and *P. pratensis* and *F. rubra* in equal parts: G(Dc); grasses Mix 100%: G(Mix). The following fertilisation treatments were used for all mixture types: P78, and K90, and two N-fertilisation levels – N0 and N120 (60+60) kg ha^{-1}. Swards were cut four times during the vegetation season. The CP content of plants was determined by modified Kjeldahl. The data were statistically analysed using two-way analysis of variance, and difference among means was detected by LSD at the P<0.05 probability level (Excel for Windows, 2003).

Results and discussion

The legume-containing mixtures provided significantly higher dry matter (DM) yields in the first and second production year (Table 1). In the third year of sward use, there were no significant differences detected in yield between legume-containing and grass-only mixtures. In the fourth production year, grass-only mixtures showed the highest DM yield in comparison with legume-containing mixtures. There were significant differences between the G(Dc) and Tr+Tp+G(L), G(Mix) and both Tr+G swards. The mixtures with higher contents of *Lolium* provided a significantly higher productivity in comparison with Tr+G(Mix) and Tr+Tp+G(Mix) only in the first and second production years.

Legume-containing mixtures achieved a higher CP content in comparison with grass-only swards in all years of sward use. The *D. glomerata*-containing mixture had the lowest CP content in all production years. The positive influence of *Lolium* sp. on CP content was not stated. A successive CP content decrease with sward aging was not observed. The significant CP differences between the years of sward use can be explained not only by higher legume contents in the second and third production year, but also by different meteorological conditions: dry summer in the first and fourth production year.

The N rate increase from 0 to 120 kg ha^{-1} contributed to a significant DM yield increase for all mixture types in all production years. The highest DM yield increase was found for grass-only swards (an increase of 3.47 Mg ha^{-1} on average for four production years). For all legume-containing mixture types, the increase in DM yield was lower, but still significant (Table 2). Comparing legume-containing mixtures, the highest DM increase (on average for four production years) was found for the Tr+Tp+G(Mix) and Tr+G(Mix) swards (by 1.33 and 1.29 Mg ha^{-1}, or 15 and 16% respectively). For swards Tr+Tp+G(L) and Tr+G(L) with the highest *Lolium* content, the positive effect of increased N fertiliser rates on DM yield increase (by 0.71 and 1.08 Mg ha^{-1}, or 7 and 12% respectively) was expressed to a lesser extent.

The increase in N fertiliser rate from 0 to 120 kg ha^{-1} contributed to a significant CP content increase in the DM yield of all mixtures. Comparing legume-containing mixtures, the highest CP increase (on average for four production years) was found for the Tr+Tp+G(Mix) and Tr+G(Mix) swards (by 26.4 and 18.4 g kg^{-1}, or 18 and 12% respectively). For swards Tr+Tp+G(L) and Tr+G(L) with the highest *Lolium* content, the positive effect of increased N fertiliser rates on CP content increase (by 12.8 and 14.7 g kg^{-1}, or 8 and 10% respectively) was expressed to a lesser extent.

Table 1. Dry matter yield, Mg ha^{-1}, and crude protein content in dry matter yield, g kg^{-1}, during four production years.[1]

Mixture type (MT)	Dry matter yield, Mg ha^{-1} Year of sward use (Y)				Crude protein content, g kg^{-1} Year of sward use (Y)			
	First	Second	Third	Fourth	First	Second	Third	Fourth
G(Dc)	8.54	8.84	6.79	5.39	117.3	111.2	128.8	103.7
G(Mix)	7.73	7.15	6.42	5.70	129.0	119.9	150.3	124.4
Tr+G(L)	15.44	10.94	6.58	4.84	138.1	180.9	175.6	142.7
Tr+G(Mix)	14.00	9.75	6.41	4.50	158.5	169.1	183.5	128.4
Tr+Tp+G(L)	16.68	12.54	6.97	4.26	143.1	179.9	179.8	136.9
Tr+Tp+G(Mix)	15.35	11.29	6.41	4.91	158.8	176.5	172.8	144.8
Mean	12.95	10.09	6.60	4.93	140.8	156.3	165.1	130.1
$LSD_{0.05}$	MT=1.02; Y=0.83; MT/Y=2.04				MT=14.10; Y=11.51; MT/Y = ns			

[1] LSD = least significant difference; ns = not significant.

Table 2. Effect of N rate on the average dry matter yield, Mg ha^{-1}, and crude protein content in dry matter yield, g kg^{-1}.[1]

Mixture type (MT)	Dry matter yield, Mg ha^{-1} N rate, kg ha^{-1} (N)		Crude protein content, g kg^{-1} N rate, kg ha^{-1} (N)	
	N0	M120	N0	M120
G(Dc)	5.60	9.17	106.6	123.9
G(Mix)	5.07	8.43	117.9	143.8
Tr+G(L)	8.91	9.99	152.0	166.7
Tr+G(Mix)	8.02	9.31	150.7	169.1
Tr+Tp+G(L)	9.76	10.47	153.5	166.3
Tr+Tp+G(Mix)	8.82	10.15	150.0	176.4
Mean	7.70	9.59	138.4	157.7
$LSD_{0.05}$	MT=1.04; N=0.60; MT/N=1.47		MT=18.74; N=10.82; MT/N=ns	

[1] LSD = least significant difference; ns = not significant.

Conclusions

A significant successive DM yield decrease was found among the sward production years, with the highest yield in the first harvest year. The mixtures with the highest contents of *Lolium* provided a significantly higher productivity only in the first and second production years. There was no positive significant influence of *Lolium* sp. on CP content.

Increasing the N fertiliser rate from 0 to 120 kg ha^{-1} contributed to a significant increase in DM yield and CP content for all mixtures. The highest DM yield increase was found for grass-only swards.

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References

Aavola R. (2005) The yield potential of Estonian perennial ryegrass (*Lolium perenne*) cultivars at different mineral fertilisation levels and cutting frequencies. *Grassland Science in Europe* 10, 449-453.

Adamovics A. and Gutmane I. (2016) Productivity and quality of multicomponent grass swards on three soil types. *Grassland Science in Europe* 21, 329-331.

Lemežiene N., Kanapeckas J., Tarakanovas P. and Nekrošas S. (2004) Analysis of dry matter yield structure of forage grasses. *Plant Soil Environment* 50, 277-282.

The trade-off between enteric and manure methane emissions from lactating dairy cows

Bayat A.R., Rasi S., Seppänen A.M., Ahvenjärvi S. and Tapio I.
Production Systems, Natural Resources Institute Finland (Luke), 31600 Jokioinen, Finland

Abstract

To evaluate the trade-offs between enteric and manure CH_4 emissions, four Nordic-Red cows were fed diets containing 65:35 or 35:65 grass-silage to concentrate ratio and 0 or 50 g kg^{-1} rapeseed oil on a dry matter (DM) basis in a 4×4 Latin square with 21-day periods. Feed intake increased by feeding more concentrates and tended to decrease with oil supplementation. Digestibility of DM and nutrients were not influenced by dietary factors with the exception of neutral detergent fibre digestibility reduced by feeding more concentrates. Daily CH_4 emission was not affected by concentrate ratio but was reduced by oil supplementation especially in the high-compared with low-grass silage diet (20.0 vs 8.9%). Methane yield (g kg^{-1} DM intake), however, was reduced by both greater concentrate (6.2%) and oil (9.7%). Faecal biochemical CH_4 potential (g CH_4 cow^{-1}) increased numerically (9%) or significantly (15%) by greater concentrate and oil supplementation, respectively. In contrast, when faecal CH_4 production was measured *in vitro* over 75 d with adding water but not inoculant to mimic the manure storage condition, greater concentrate ratio reduced (89.4%) while oil tended to reduce (69.5%) CH_4 production indicating lack of trade-off between enteric and manure CH_4 emissions by dietary mitigation.

Keywords: grass silage, enteric methane, manure methane, trade-off, dairy cow

Introduction

Increasing the global demand for food over the next decades and simultaneously decreasing greenhouse gas emissions from the ruminant sector remains challenging. There are several nutritional strategies available to reduce enteric methane (CH_4) emissions from ruminants (Hristov *et al.*, 2013) but it is not known how these strategies will affect potential CH_4 emissions from manure via changes in excretion of nutrients or manipulation of microbial populations. Therefore, this study was conducted to evaluate the possible trade-offs between mitigation of enteric- and manure CH_4 emissions.

Materials and methods

The experiment was conducted in Jokioinen, Finland in spring 2019. Four multiparous lactating Nordic-Red cows producing 38.8±1.9 kg d^{-1} milk in their 101±15.9 days in milk were randomly assigned to diets containing 65:35 or 35:65 grass silage to concentrate ratio and 0 or 50 g kg^{-1} rapeseed oil on a dry matter (DM) basis in a 4×4 Latin square with four 21-day periods (15 days adaptation). Diet comprised grass silage, rolled barley, oats, molassed sugar beet pulp, rapeseed meal and vitamin-mineral premix fed as a total mixed ration. Rapeseed oil replaced concentrate ingredients. Rumen samples were taken by stomach tubing 4 hours post morning feeding on the last day of study. Enteric CH_4 was measured over 3 days using respiration chambers when total urine and faeces were collected simultaneously. Milk yield and composition was measured over 4 days using infrared analysis (MilkoScan 133B, Foss Electric, Hillerød, Denmark). Energy corrected milk was calculated based on the yields of fat, CP, and lactose (Sjaunja *et al.,* 1990). Chemical analysis of feed and excreta was conducted using routine analytical methods. Biochemical CH_4 potential (BMP) was measured under mesophilic (37 °C) conditions using automated testing equipment (Bioprocess Control Ltd, Sweden) over 4 weeks. The bottles were mechanically mixed (84 rpm) for one min h^{-1}. Tests were done in 500 ml bottles, using 400 ml liquid volume with duplicate test bottles. Sample to inoculum OM ratio of 1:1 was used. The inoculum was obtained from a farm-scale biogas plant treating cattle slurry. All bottles were buffered with $NaHCO_3$ (3 g l^{-1}) and flushed with N_2

to obtain anaerobic conditions. The gas was collected in gas bags and analysed for CH_4 and CO_2 using gas chromatography. For static *in vitro* CH_4 measurements (75 days) the same equipment was used as in BMP assay while no inoculant and mixing was used under temperature of 25 °C and water was added with ratio of urine from each cow. Experimental data were analysed using ANOVA for a factorial arrangement of treatments using Mixed procedure of SAS (version 9.4) with a model including fixed effects of period, concentrate ratio, oil, and their interaction, and random effect of cow. Least square means are reported. The effects are declared significant at $P\leq0.05$ or a trend at $0.05<P<0.10$ and Least Significant Difference (LSD) test was used to differentiate the least square means at $P\leq0.05$.

Results and discussion

Feed intake increased (P=0.015) by feeding more concentrate and tended to be lower (P=0.09) with oil supplementation (Table 1). Ruminal pH, total volatile fatty acid and ammonia concentrations were not significantly affected ($P\geq0.16$) by the treatments (data not presented). Rumen molar acetate to propionate ratio was lower ($P\leq0.03$) with both greater dietary concentrate ratio and oil supplementation but the oil effect tended to be stronger in high forage diet (P=0.09 for interaction). Digestibility of DM and nutrients was not influenced ($P\geq0.11$) by dietary factors with the exception of NDF digestibility, which was reduced (P=0.01) by feeding more concentrates (Table 2). Feeding more concentrates increased milk yield and oil increased milk yield only in the high-concentrate diet (P=0.01 for the interaction). However, energy corrected milk (ECM) yield was not influenced by oil supplementation while it was increased (P<0.01) with greater concentrate level. Daily CH_4 emission was not affected by concentrate ratio but was reduced by oil supplementation especially in high-grass silage diet (20.0% compared with 8.9% for low grass silage diet; P=0.015 for interaction). Methane yield (g kg^{-1} DM intake), however, was lower (P<0.01) with both greater concentrate level (6.2%) and oil supplementation (9.7%).

Faecal BMP (g CH_4 cow^{-1}), measured *in vitro* over four weeks, increased numerically (9%; P=0.12) or significantly (15%; P=0.03) by greater concentrate ratio and oil supplementation, respectively. In contrast, when CH_4 production was measured *in vitro* under static conditions over 75 days with adding

Table 1. Effect of forage to concentrate ratio and dietary rapeseed oil supplement on nutrient intake, milk production and apparent total tract digestibility in lactating dairy cows.[1]

	Treatment[2]				SEM	*P*-value[3]		
	L	LRO	H	HRO		FC	RO	FC×RO
Intake, kg d^{-1}								
DM	24.7[a]	22.3[b]	25.8[a]	25.6[a]	0.83	0.015	0.091	0.13
NDF	12.4[a]	10.9[b]	10.4[b]	10.0[b]	0.43	0.005	<0.001	0.020
EE	0.92[c]	1.80[b]	0.97[c]	2.13[a]	0.060	0.015	0.091	0.13
Milk yield, kg d^{-1}	31.2[c]	30.5[c]	36.2[b]	40.6[a]	1.47	<0.001	0.038	0.011
ECM, kg d^{-1}	33.3[b]	32.9[b]	39.4[a]	39.4[a]	1.95	<0.001	0.82	0.82
Fat, g d^{-1}	1,400[b]	1,403[b]	1,644[a]	1,490[ab]	95.1	0.028	0.24	0.22
Protein, g d^{-1}	1,124	1,085	1,372	1,460	70.2	<0.001	0.26	0.020
Apparent total tract digestibility, g kg^{-1}								
OM	676	658	685	679	8.1	0.11	0.17	0.48
NDF	639[a]	612[ab]	585[b]	575[b]	12.9	0.011	0.19	0.52
EE	620	598	631	635	17.0	0.19	0.61	0.45

[1] Values with different superscripts in a row differ significantly; DM = dry matter; EE = ether extract; ECM = energy corrected milk; SEM = standard error of the mean; OM = organic matter; NDF = neutral detergent fibre.

[2] Refers to the diets based on low (0.35) or high (0.65) concentrate ratio supplemented with 0 (L and H, respectively) or 50 (LRO and HRO, respectively) g kg^{-1} DM of rapeseed oil.

[3] FC = effect of forage to concentrate ratio in the diet; RO = effect of rapeseed oil supplement; FC×RO = interaction of FC and RO.

Table 2. Effect of forage to concentrate ratio and dietary rapeseed oil supplement on enteric methane (CH_4) emission and faecal *in vitro* CH_4 production in lactating dairy cows.[1]

	Treatment[2]				SEM	*P*-value[3]		
	L	LRO	H	HRO		FC	RO	FC×RO
Enteric CH_4								
g d^{-1}	535^{a}	428^{c}	516^{a}	470^{b}	27.7	0.25	<0.001	0.015
g kg^{-1} OMI[3]	23.3^{a}	20.7^{bc}	21.5^{b}	19.6^{c}	0.87	0.005	<0.001	0.31
g kg^{-1} DOM[4]	34.4^{a}	31.4^{b}	31.3^{b}	28.8^{c}	1.24	0.004	0.005	0.68
g kg^{-1} ECM	16.0^{a}	13.0^{b}	13.2^{b}	12.0^{b}	0.64	0.006	0.003	0.087
Biochemical CH_4 potential								
ml g^{-1} OM	219^{b}	267^{a}	238^{ab}	266^{a}	9.2	0.36	0.005	0.30
g d^{-1}	1,164^{b}	1,359^{ab}	1,293^{ab}	1,467^{a}	75.3	0.12	0.029	0.87
Faecal static CH_4 production								
ml g^{-1} OM	26.4^{a}	10.8^{ab}	3.2^{b}	0.3^{b}	5.83	0.028	0.16	0.32
g d^{-1}	143^{a}	48^{ab}	19^{b}	1.6^{b}	28.4	0.022	0.089	0.21

[1] Values with different superscripts in a row differ significantly; OMI = organic matter intake; DOM = digestible organic matter; ECM = energy corrected milk; OM organic matter.
[2] Refers to the diets based on low (0.35) or high (0.65) concentrate ratio supplemented with 0 (L and H, respectively) or 50 (LRO and HRO, respectively) g kg^{-1} dry matter of rapeseed oil.
[3] FC = effect of forage to concentrate ratio in the diet; RO = effect of rapeseed oil supplement; FC×RO = interaction of FC and RO.

water but not inoculant to mimic more the actual storage condition, greater concentrate ratio reduced (P=0.02) and oil tended (P=0.09) to reduce CH_4 production indicating a lack of trade-off between enteric and manure CH_4 emissions. Thus, there was a major difference between quantity of BMP and static CH_4 production from the same faecal sample. In addition, there were two main discrete phases of gas production in the static assay affected by both dietary concentrate ratio and oil supplement (graph not presented). The first phase of gas production occurred during d 1 to 11 where high concentrate diets had clearly faster CH_4 production rate. This phase probably reflects the fermentation of soluble fractions. The second phase of gas production occurred between days 30 to 75 and 56 to 75 for low and high concentrate diets, respectively. This phase reflects the fermentation of slowly degradable fractions. Diets supplemented with oil had a longer delay (about 10 days) in starting the second phase of CH_4 production and had a clearly lower rate of CH_4 production compared with unsupplemented diets. Microbial population of incubated faecal samples over time will be analysed to follow up and link CH_4 production to different microbes.

Conclusions

Enteric CH_4 yield as well as static faecal *in vitro* CH_4 production which mimics natural manure CH_4 emissions were lowered by both feeding more concentrate and oil. No trade-off between enteric and manure CH_4 emissions were observed due to dietary strategies tested.

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References

Hristov A.N., Oh J., Firkins J.L., Dijkstra J., Kebreab E., Waghorn G., … and Tricarico J.M. (2013) Mitigation of methane and nitrous oxide emissions from animal operations: a review of enteric methane mitigation options. *Journal of Animal Science* 91, 5045-5069.

Sjaunja L.O., Baevre L., Junkkarinen L., Pedersen J. and Setälä J. (1990) A Nordic proposal for an energy corrected milk (ECM) formula. *Proc. 27th ICAR session*, Paris, France. pp. 156.

Impact of tillage methods and sowing rates on yield and weed suppression in multi-species swards

Beaumont D.A.[1], Storkey J.[2], Eales G.[3], Jones H.E.[3], LeCocq K.[1], Saunders K.S.[1], Stagg B.[3] and Lee M.R.F.[1,4]

[1]Rothamsted Research, North Wyke, Okehampton, Devon EX20 2SB, United Kingdom; [2]Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom; [3]Duchy College, Stoke Climsland, Callington, Cornwall PL17 8PB, United Kingdom; [4]University of Bristol, Bristol Veterinary School, Langford, Somerset BS40 5DU, United Kingdom

Abstract

Recent experimental studies have demonstrated increased plant diversity in agricultural pastures can benefit a variety of ecosystem functions including increased biomass production and weed suppression. With rising global demand for more sustainable ruminant production systems, multi-species swards are growing in popularity as an alternative to high-input ryegrass monocultures. Minimum tillage reduces disturbance to soil structure compared with conventional ploughing, however, weed infestation can be potentially higher under non-inverted, shallow cultivation as seeds remain near the soil surface. A multi-factorial field experiment was established to assess the impact of three tillage methods and four seed mixes, with increasing diversity, sown at two different rates on sward productivity and weed ingress. We found tillage depth, increased plant diversity or sowing rate had limited beneficial effect on dry matter yield in the first growing year. However, increased species diversity and ploughing reduced weed pressure.

Keywords: seed mixes, cultivation, sown and unsown species, establishment, productivity

Introduction

Many studies have shown a positive relationship in grasslands between plants species richness and a variety of ecosystem functions. Increasing the complexity of forage seed mixes can improve weed suppression (Suter *et al.*, 2017) and enhance above ground biomass (Küchenmeister *et al.*, 2012) through positive species interactions and species niche complementarity in resource capture. Pasture weeds can lower forage yield by competing for water, sunlight and nutrients and can also reduce forage quality (DiTomaso, 2000). As the application of herbicides is not an option to control weeds in multi-species leys it is imperative to select tillage methods and sowing rates to minimize weed ingress, thus reducing a decline in sward productivity and consequently the frequency and associated economic and environmental costs of re-seeding. Cultivation techniques which minimise soil disturbance are recommended for reducing establishment costs, increasing soil nutrient stocks, improving soil structure and increasing beneficial soil fauna compared with conventional ploughing. However, there are some concerns regarding increased weed densities and shifts in weed species communities with minimal tillage practices (Derksen *et al.*, 2002). The objective of this research was to investigate the impact of three tillage practices and four seed mixes with increasing diversity sown at two different rates on above ground biomass and relative weed abundance in the first growing year.

Methods and materials

A randomised split-plot design field experiment with three replicates was established in autumn 2018 at Rothamsted Research North Wyke, Devon, UK on a permanent, species-poor grassland on clay soil. Prior to cultivation the field was sprayed with glyphosate (2.16 kg ha^{-1}). Experimental plots were cultivated using three contrasting tillage depths: shallow, intermediate and deep by (1) power harrow (PH) to a depth of 5-7 cm; (2) disc harrow (DH) to a depth of 10-15 cm; (3) conventional ploughing (P) to a depth of 20-25 cm. Plots of 24 m^2 (8×3 m) were sown with four seed mixes of increasing plant diversity, a binary mix of *Lolium perenne* and *Trifolium repens* (PC) and mixes that comprised three functional groups (grass, legume and non-leguminous forbs), six species (A), twelve species (B), eighteen species (HD) each sown at a conventional rate of 34.59 kg ha^{-1} (CS) and a high rate of 51.89 kg ha^{-1} (HS). Plots were cut three times, early, mid and late during the 2019 growing season, at a 5 cm height with a Haldrup harvester. Application rates of inorganic fertiliser to each cultivation treatment were based on soil concentrations found in soil samples collected during autumn 2018. In 2019 all three cultivation types received 13 kg ha^{-1} of P and 48 kg ha^{-1} of S, however, potassium rates differed with 158, 100 and 174 kg ha^{-1} of K applied to the PH, DH and P plots respectively. Botanical percentage cover was assessed visually in two 0.25 m^2 quadrats per plot before each harvest. There were not resources available for destructive sampling and separation of species at each harvest, but this was done once on two 0.25×0.5 m quadrats per plot, cut to ground level immediately following the May cover assessments to assess the accuracy of the visual estimates. Above-ground dry matter yield (DMY) and sown and unsown plant abundance were analysed using general analysis of variance models with GenStat, release 19.1 (VSN International 2018).

Results and discussion

No significant treatment effects for average annual DMY were found; however, there were some temporal differences. At the first harvest in May (overall mean 2.60 Mg ha^{-1}) there were no significant differences between the treatments, but by July the ploughed plots had significantly more biomass than either DH or PH plots (P=0.039). The average values were 2.32, 1.81 and 1.68 Mg ha^{-1} for P, DH and PH, respectively. There were significant differences between seed mixes in July with the highest biomass recorded in seed mix A (P=0.006). Highest productivity was recorded on the ploughed plots sown at the higher rate with a 6-species mix (2.70 Mg ha^{-1}), however, sowing rate had no significant effect in July or at any other timepoint. After the second cut the ploughed plots lost the advantage yielding the least in October (average 2.74, 3.24 and 3.27 Mg ha^{-1} for P, DH and PH respectively) although cultivation treatment was not significant. Seed mix had no significant effect at the last harvest.

To assess the effect of the treatments on sward composition across the season, % cover data were analysed. Although not as accurate as relative dry weights, all visual assessments were done by a single expert to minimize observer error and a Pearson's correlation between the proportion of sown species in the destructive sample taken in May and the corresponding visual estimate was highly significant (r=0.58, P<0.001). While there were no significant differences in total biomass in May between the treatments, the seed mixes differed in the relative cover of sown (P=0.023) compared with unsown species (P=0.019) recorded prior to the first cut (Figure 1a). In May the binary mix (PC) had the lowest percentage cover of sown species and highest weed pressure; the best performing mix in terms of weed suppression was A (6 species). These seed-mix trends were similar for the PC mix prior to the second (sown species P<0.001, weed species P=0.003) and third (sown species P=0.003, weed species P<0.001) cuts although the HD

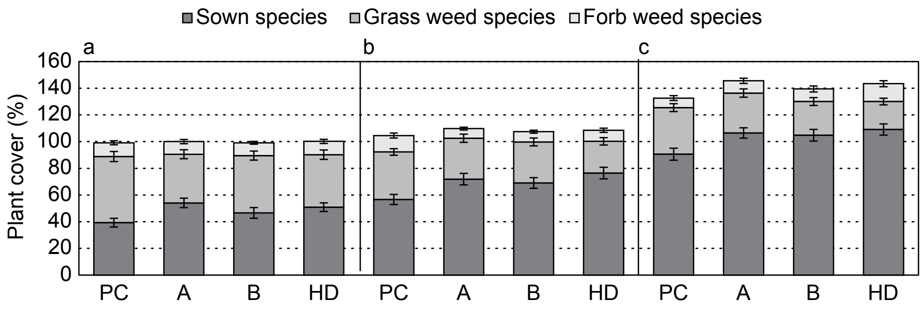

Figure 1. Average percentage cover of sown, weed grass and weed forb species in seed mixes, binary (PC), 6 species (A), 12 species (B) and 18 species (HD) at: (a) May, (b) July (c) October 2019.

(18 species) mix had the highest sown species and lowest weed cover and overall weed cover decreased with time (Figure 1). Cultivation was also a significant treatment in July (sown species P=0.015, weed species P=0.026) and almost significant in September for sown species (P=0.052) with the highest sown species establishment and lowest weed ingress recorded in the ploughed plots (July average weed species cover: P=23.6%, DH=53%, PH=43.2%, September average weed species cover: P=19.4%, DH=47.7%, PH=45.34%). The higher sowing rate had no significant impact on sown or unsown species relative abundance during the growing season. At all three sampling timepoints grass species dominated the weed community (Figure 1).

Conclusions

Tillage depth or increased plant diversity had no overall impact on annual yield in the first growing season. However, abundance of sown species was higher and weeds generally lower under ploughing, indicating that deeper cultivation is beneficial for establishment of desired species. Complex seed mixes reduced weed invasion compared with a binary mix which may have implications on forage quality and longevity of leys. Our results suggest there is no benefit of sowing at a higher rate in terms of yield or weed suppression.

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References

Derksen D.A., Anderson R.L., Blackshaw R.E. and Maxwell B.D. (2002) Weed dynamics and management strategies for cropping systems in the northern Great Plains. *Agronomy Journal* 94, 174-185

DiTomaso J.M. (2000) Invasive weeds in rangelands: species, impacts, and management. *Weed Science* 48, 255-265.

Küchenmeister F., Küchenmeister K., Wrage N., Kayser M. and Isselstein J. (2012) Yield and yield stability in mixtures of productive grassland species. Does species number or functional group combination matter? *Grassland Science* 58, 94-100.

Suter M., Hofer D. and Lüscher A. (2017) Weed suppression enhanced by increasing functional trait dispersion and resource capture in forage ley mixtures. *Agricultural Ecosystems & Environment* 240, 329-339.

Climate change vulnerability of Alpine pastures: first results of the project PASTORALP

Bellocchi G.[1], Argenti G.[2], Bassignana M.[3], Bindi M.[2], Brilli L.[4], Costafreda Aumedes S.[2], Filippa G.[5], Martin R.[1], Moriondo M.[4], Napoléone C.[6], Staglianò N.[2], Targetti S.[6] and Dibari C.[2]

[1]INRAE, UREP, 5 chemin de Beaulieu, 63000 Clermont-Ferrand, France; [2]DAGRI, University of Florence, Piazzale delle Cascine 18, 50144 Florence, Italy; [3]IAR, Rég. La Rochère 1/A, 11100 Aosta, Italy; [4]CNR, IBIMET, via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy; [5]ARPA-VdA, Climate Change Unit, Loc. La Maladière 48, 11020 Saint-Christophe (AO), Italy; [6]INRA, ECODEV, 228 route de l'aérodrome, 84914 Avignon, France

Abstract

Over the last 120 years, temperatures in the Alps have increased by ~1.6 °C, almost twice the global average, and summer precipitation has decreased by ~30%, resulting in temperature extremes and aridity. Pastoralism is an integral part of Alpine life, which contributes to the maintenance of a rich grassland biodiversity and supports local economy and rural vitality. In the face of the recent changes induced by global warming, the EU-LIFE project PASTORALP (2017-2022) develops strategies to reduce the vulnerability of pastures in two Western Alpine parks: Écrins National Park (France) and Gran Paradiso National Park (Italy). Climate simulations anticipate increasing temperatures in both parks, with an increased risk of drought periods, and a lengthening of the growing season of about two months in the near future, coupled with a reduction in the snow depth. This results in decreased fodder stocks and a shift in species composition towards lower forage quality. Earlier melting of snowpack is also limiting access to water availability for the grazing livestock. These are all risk factors for Alpine pastoral production systems. Local officials, park staff and agricultural actors are engaged into a participative process for the implementation of better management of forage resources.

Keywords: grazing management, protected areas, land use, vulnerability assessment

Introduction

Pastoralism is an integral part of alpine life, where it supports mountain landscapes and local economy (including tourism), and the maintenance of a rich grassland biodiversity. However, alpine pastoralism manifests its fragility in the face of the changes induced by recent global warming. Climate changes and their impacts are visible in the Alpine region, which has experienced a temperature increase of almost 2 °C over the last century, along with an important reduction of precipitation in the summer season (Gobiet *et al.*, 2014). These changes likely alter grassland productivity and quality (Dibari *et al.*, 2016), harm cold-tolerant high-altitude grassland communities (Gottfried *et al.*, 2012) and lead to a decline of the areas suitable for some vegetation types (Dibari *et al.*, 2013). Moreover, land abandonment and rural depopulation phenomena determine relevant changes in mountain ecosystems and depletion of plant species richness (Orlandi *et al.*, 2016). Appropriate management can preserve grassland biodiversity, maintain ecosystem services and counteract climate change impacts (Felber *et al.*, 2016; Nori and Gemini, 2011). However, in many Alpine regions specific measures to manage pastures coping with climate change are still not implemented, despite the adoption of *ad hoc* policies (e.g. European Agricultural Policy, Reg. EU 1305/2013). With focus on two parks, representative of both sides of the French-Italian border, the LIFE project PASTORALP ('Pastures vulnerability and adaptation strategies to climate change impacts in the Alps'; start October 2017) combines biophysical and socio-economic features to assess and reduce the vulnerability of Alpine pastures.

We present some results obtained in the Écrins National Park (French Southern Alps) by combining observations and model-based simulations with stakeholder participation. The study area includes ca. 70,000 ha of summer pastures (covering ~30% of the park area), which are grazed by about 115,000 sheep (75% of total stocking rate), 5,800 cows and >1000 of goats and horses. Transhumance (which is declining across Europe) is still relevant in the study region, with ~1/3 of total sheep stocking rate in summer pastures being involved in transhumance (Brien, 2018).

Materials and methods

In 2018 and 2019, a participatory-based process involved ~20 stakeholders (farmers, technicians and delegates of local institutions), mostly from the partnership-based action-research scheme Alpages Sentinelles (Dobremez *et al.*, 2013), through meetings, interviews and discussions, which paralleled data collection and GIS analyses (participative process illustrated in Figure 1). In parallel, outputs from the Aladin RCM simulations (http://www.umr-cnrm.fr/gmgec-old/site_engl/aladin/aladin_en.html) were obtained with daily downscaled weather data (0.25° spatial resolution) on species-rich grasslands (dominated by *Agrostis rupestris*, *Trifolium alpinum*, *Agrostis alpina*, *Plantago alpina*, *Leontodon hispidus*, *Festuca nigrescens*, *Agrostis capillaris*, *Plantago atrata* and *Alchemilla xanthochlora*) located at about 2,200 m a.s.l. (45.50 N, 7.12 E). Climate scenarios were used with radiative forcing for the high (RCP8.5) and medium (RCP4.5) representative concentration pathways (Vuuren *et al.*, 2011) in three 30-year time slices centred on the years 1990s (1981-2010, near past), 2020s (2011-2040, near future) and 2050s (2041-2070, medium future).

Results and discussion

With the warmest scenario (RCP8.5), snow cover is projected to decrease rapidly approaching the end of the century, while less relevant differences were observed with other RCP/time slice combinations (not shown). Accordingly, the length of the growing season is projected to increase (Figure 2). A range of response strategies to climate change is already part of the farmers' knowledge and capital base, including better management of the pastoral resources by means of pasture rotations and wide diversification of pastoral resources. However, options for buffering against the risks linked with climate extremes are

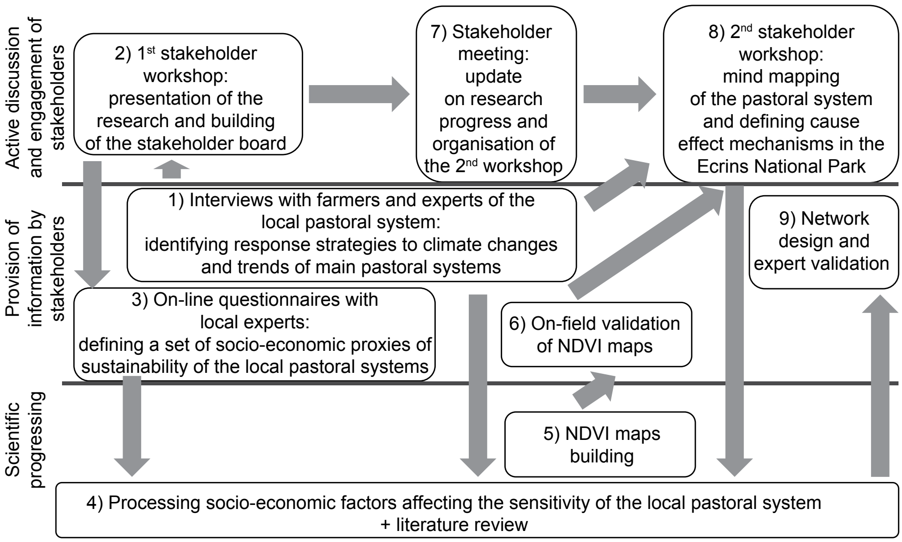

Figure 1. Combined participatory and data collection/processing progression for the development of the analytical network of the Écrins pastoral systems.

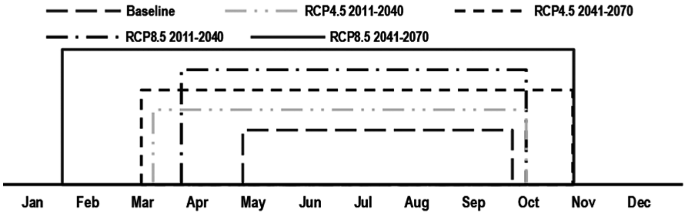

Figure 2. Projected changes of the length of growing season for baseline and future climate scenarios.

progressively reduced as a consequence of the abandonment of more fragmented or less productive pastoral resources. This points to the potential increase of vulnerability of local pastoral systems.

Conclusions

While the effects of the climate change on the pastoral resources are part of an *ad-hoc* modelling process, exploratory interviews and workshops with local experts and stakeholders confirmed the relevance, for an effective analysis of pastoral systems' vulnerability, of considering direct and indirect feedbacks that may exist between adaptation capacity and sensitivity to changes. Interconnections among drivers of response will be analysed further (and the analysis enlarged to the National Park Gran Paradiso) for their impact on the sustainability of Alpine pastures.

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References

Brien P. (2018) Analyse de l'évolution de l'utilisation des alpages sur le territoire du Parc National des Écrins (1996-2012), perceptions des tendances en cours et des enjeux à venir. L'exemple de la prédation et du changement climatique en alpage. Rapport de stage en entreprise, Université Savoie-Mont Blanc (in French)

Dibari C., Argenti G., Moriondo M., Staglianò N., Targetti S. and Bindi M. (2013) Climate change impacts on distribution and composition of the Alpine Natural Pasturelands. *Proc. 1st annual conference climate change and its implications on ecosystem and society*. Società Italiana per le Scienze del Clima, Lecce, Italy, pp. 578-586. (in Italian)

Dibari C., Bindi M., Moriondo M., Staglianò N., Targetti, S. and Argenti, G. (2016) Spatial data integration for the environmental characterization of pasture macrotypes in the Italian Alps. *Grass and Forage Science*, 71, 219-234.

Dobremez L., Della-Vedova M., Legeard, J.-P. (2013) Les alpages sentinelles. *Espaces naturels*, 41, 36-37 (in French)

Felber R., Bretscher D., Münger A., Neftel A. and Ammann C. (2016) Determination of the carbon budget of a pasture: effect of system boundaries and flux uncertainties. *Biogeosciences*, 13, 2959-2969.

Gobiet A., Kotlarski S., Beniston M., Heinrich G., Rajczak J. and Stoffel M. (2014) 21st century climate change in the European Alps – a review. *Science of the Total Environment*, 493, 1138-1151.

Gottfried M., Pauli H., Futschik A., Akhalkatsi M., Baranok P., Alonso J.L.B., Coldea G., Jan D., Erschbamer B., Calzado M.R.F., Kazakis G., Kraji J., Larsson P., Mallaun M., Michelsen O., Moiseev D., Moiseev P., Molau U., Merzouki A., Nagy L., Nakhutsrishvili G., Pedersen B., Pelino G., Puscas M., Rossi G., Stanisci A., Theurillat J.P. and Tomaselli M. (2012) Continent-wide response of mountain vegetation to climate change. *Nature Climate Change* 2, 111-115.

Nori S. and Gemini M. (2011) The common agricultural policy vis-à-vis European pastoralists: principles and practices. *Pastoralism: Research, Policy and Practice* 1, 27.

Orlandi S., Probo M., Sitzia T., Trentanovi G., Garbarino M., Lombardi G. and Lonati M. (2016) Environmental and land use determinants of grassland patch diversity in the western and eastern Alps under agro-pastoral abandonment. *Biodiversity and Conservation*, 25, 275-293.

Van Vuuren D.P., Edmonds J., Kainuma M., Riahi K., Thomson A., Hibbard K., Hurtt G.C., Kram T., Krey V., Lamarque J.-F., Masui T., Meinshausen M., Nakicenovic N., Smith S.J. and Rose S.K. (2011) The representative concentration pathways: an overview. *Climatic Change*, 109, 5-31.

Grassland plant species richness in dairy farming systems with different feeding strategies

Bettin K.[1,2], Komainda M.[1], Tonn B.[1,2] and Isselstein J.[1,2]
[1]Division of Grassland Science/Department of Crop Sciences, University of Goettingen, Germany; [2]Centre of Biodiversity and Sustainable Land Use (CBL), University of Goettingen, Germany

Abstract

Increasing concentrate levels in dairy cow rations have decreased the relevance of permanent grassland as a resource for German dairy farmers. We hypothesised that higher phytodiversity can be found on grassland of farms with lower concentrate feed levels, since dependence on permanent grassland as the main source for livestock nutrition may lead to a more differentiated grassland management. We conducted 388 vegetation surveys on permanent grassland of organic (ORG) and conventional (CON) dairy farms in three grassland-dominated regions of Germany and quantified overall species number per site (α diversity), per farm (γ diversity) and the heterogeneity between the grassland sites of each farm (β diversity). The sample comprised 28 control farms with typical regional-specific levels of concentrate feeding (HC) paired with farms feeding less concentrate (LC). Across regions, diversity indices were higher on grassland of LC than HC farms, an effect that was more pronounced on CON than ORG farms. Farming system affected α diversity alone, and only among HC farms or in the least species-rich region. Our results indicate that low concentrate feeding strategies may be a promising way to improve grassland diversity, especially in CON farming systems.

Keywords: concentrate, biodiversity, feeding strategy, phytodiversity

Introduction

Grassland ecosystem services range from feed provision to biodiversity protection (Solen *et al.*, 2019). During the past decades feeding of dairy cows has evolved into specialised high-input/high-output systems relying on a large quantity of forage from arable land and supplied to the animal in a total mixed ration with maize and concentrate (Knaus, 2016; Schingoethe, 2017). The swards grazed by dairy cattle in North-western Europe are dominated by a limited number of vascular plant species that are adapted to regular defoliation and nutrient input (Egan *et al.*, 2017). The provision of high-quality grass requires little spatial and structural heterogeneity of swards at harvest. These swards have consequently low potential to promote biodiversity and dairy production at the same time (Tallowin *et al.*, 2005). There is, consequently, a trade-off between management intensity or type of management (grazing vs cutting) and plant species richness on grassland (Van Vooren *et al.*, 2018). According to Van Dobben *et al.* (2019), the farming system, in terms of organic (ORG) vs conventional (CON) farming, has an impact on plant species richness. In the latter study, the level of nitrogen and phosphorus fertilisation differed markedly between the production systems and are, thus, major steering variables for plant diversity. As these nutrients can also enter the farm in the form of concentrates, the control of concentrates consequently represents one option to promote plant species richness in intensive dairy farming. Moreover, feeding less concentrate increases dependency on grassland as a main source for livestock nutrition, motivating a more differentiated grassland management, e.g. to utilise marginal grasslands for youngstock or dry cows. Here we hypothesize that an increased level of concentrate feeding reduces plant species diversity on farm level irrespective of the production region and farming system.

Materials and methods

We selected 28 farm pairs (n=56) in three German regions (Allgäu, southern Germany: n=20, low mountain ranges of central Germany: n=16, north-west German lowlands: n=20). 15 pairs were ORG, 13 pairs CON farms. Each farm pair consisted of one farm feeding little or no concentrate (LC) and one control farm with typical region-specific concentrate feeding levels (HC). The LC farms had fed less than 150 g concentrate per kg milk during 2014-2016, respectively 200 g kg^{-1} milk in the case of CON farms of central and north-west Germany. The HC farms exceeded these limits but were similar in farm structure to their paired LC farms. Two permanent grassland sites of each management, i.e. 'pasture', 'mown pasture', 'meadow' and 'extensive grassland', were chosen on each farm to conduct vegetation surveys (n=388), with presence and estimated biomass proportion recorded for all vascular plant species on one 9 m^2 quadrat per field.

Statistical analysis was carried out in R (version 3.6.0). We applied linear mixed-effects models (Pinheiro *et al.*, 2019) to analyse the effects of feeding strategy (HC vs LC) and farming system (ORG vs CON) on mean species number per plot (α diversity), per farm (γ diversity,) and on the heterogeneity between the grassland sites of each farm (β diversity, according to Lande (1996)). Farm pair was included as random effect and appropriate transformation and variance structure were chosen to ensure normality and homoscedasticity of residuals. We report the results of the most parsimonious models according to second order Akaike information criterion (AICc).

Results and discussion

All diversity indices differed significantly between the feeding strategies (Table 1). Both β and γ diversity were higher on LC than on HC farms; for α diversity, this was the case among CON, but not ORG farms (Figure 1). Differences between farming systems occurred in interaction with region or concentrate category for α diversity (Table 1). Within HC farms (14.7 vs 11.2 species per plot) as well as in the

Table 1. Test of linear mixed model effects on three parameters of grassland phytodiversity.

Effect[1]	Species per plot (α)		Species per farm (γ)		Beta-diversity (β)	
	F	*P*-value	*F*	*P*-value	*F*	*P*-value
Concentrate category (CC)	28.35	<0.0001	45.88	<0.0001	19.47	0.0001
Farming system (FS)	46.97	<0.0001	3.19	0.0869	–	–
Region (R)	50.70	<0.0001	21.25	<0.0001	14.18	0.0001
CC × FS	5.52	0.0267	8.21	0.0082	–	–
FS × R	4.43	0.0241	–	–	–	–

[1] CC = low vs control levels of concentrate feeding; FS = conventional vs organic farms.

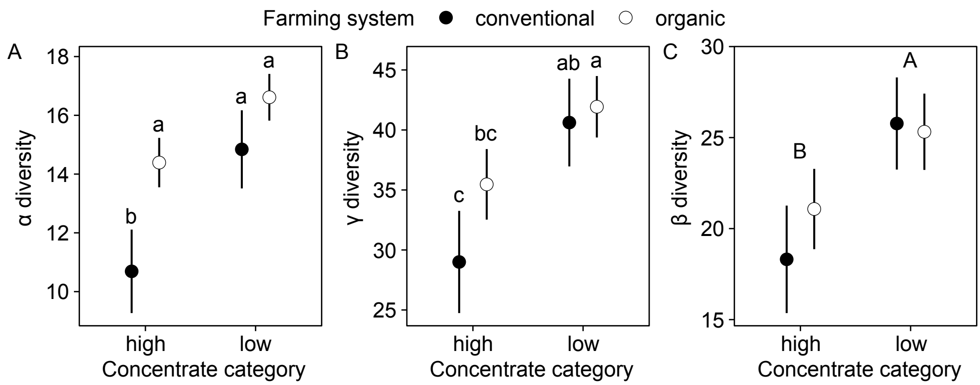

Figure 1. Effect of high and low levels of concentrate feeding on grassland phytodiversity parameters in conventional and organic farms. Mean ± standard deviation; letters indicate significant differences between means (lower case) or means across farming systems (upper case).

north-west German region (12.5 vs 7.9 species per plot), ORG farms had a higher α diversity than CON farms. All diversity indices were higher in central and south Germany than in north-west Germany, for α diversity this difference was more pronounced among CON than ORG farms.

We found a relationship between feeding strategy and grassland plant diversity that was independent of region, even though diversity differed significantly between the study regions themselves. This supports our hypothesis for grassland-dominated regions in Germany under differing environmental and structural conditions. Under CON farming, the relationships between feeding-strategy and α and γ diversity were stronger than they were under ORG farming, presumably because ORG farms comply with restrictions that lead to smaller differences in farming practices between HC and LC farms.

Conclusions

We showed that, among dairy farms, those who pursue a feeding strategy relying on grassland as the main source for livestock nutrition can promote higher phytodiversity in permanent grassland. Studying the direct and indirect drivers of phytodiversity across feeding systems will further substantiate how low concentrate feeding strategies can improve grassland diversity, especially in CON dairy farming systems.

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References

Egan M., Lynch M.B. and Hennessy D. (2017) Including white clover in nitrogen fertilized perennial ryegrass swards: effects on dry matter intake and milk production of spring calving dairy cows. *Journal of Agricultural Science* 155, 657-668.

Knaus W. (2016) Perspectives on pasture versus indoor feeding of dairy cows. *Journal of the Science of Food and Agriculture* 96, 9-17.

Lande, R. (1996) Statistics and partitioning of species diversity, and similarity among multiple communities. *Oikos*, 5-13.

Pinheiro J., Bates D., DebRoy S, Sarkar D. and R Core Team (2019) nlme: linear and nonlinear mixed effects models. R package version 3.1-139. Available at: https://CRAN.R-project.org/package=nlme

Schingoethe D.J. (2017) A 100-year review: total mixed ration feeding of dairy cows. *Journal of Dairy Science,* 100, 10143-10150.

Solen L.C., Finger R., Buchmann N., Gosal A.S., Hörtnagl L., Huguenin-Elie O., Jeanneret P., Lüscher A., Schneider M.K. and Huber R. (2019) Assessment of spatial variability of multiple ecosystem services in grasslands of different intensities. *Journal of Environmental Management* 251, 109372

Tallowin J.R.B., Smith R.E.N., Goodyear J. and Vickery J.A. (2005) Spatial and structural uniformity of lowland agricultural grassland in England: a context for low biodiversity. *Grass and Forage Science* 60, 225-236.

Van Dobben H.F., Quik C., Wieger Wamelink G.W. and Lantinga E.A. (2019) Vegetation composition of *Lolium perenne*-dominated grasslands under organic and conventional farming. *Basic and Applied Ecology* 36, 45-53.

Van Vooren, L., Reubens, B., Broekx, S., Reheul, D. and Verheyen, K. (2018) Assessing the impact of grassland management extensification in temperate areas on multiple ecosystem services and biodiversity. *Agriculture, Ecosystems & Environment* 267, 201-212.

Grasslands, biodiversity and business: Boreal grassland products as value-added agriculture

Birge T.
Department of Agricultural Sciences and HELSUS Helsinki Institute of Sustainability Science: University of Helsinki, P.O. Box 27, 00014 Helsinki, Finland

Abstract

The term 'values-based supply chains' refers to creating closer connections between producer and consumer in the supply chain and to enhancing product value through a product's identity characteristics. I examined the potential added values of grassland products from primarily Boreal grasslands and how these can be made visible to consumers to support biodiversity objectives. The aim of the study is to support Latvian Fund for Nature's GrassLIFE project objective of improving the economic aspects of sustainable grasslands management. It consists of: (1) literature review of five grassland product categories (meat, dairy, honey, grass products, medicinal products), (2) set of 20 case studies, and (3) framework and set of tests for assessing added values. Grassland and semi-natural grassland products particularly can be differentiated by nutritional and other qualities in many cases (e.g. meat and dairy), but there are substantial knowledge gaps in differentiation of grassland and non-grassland products (e.g. medicinal plants). The framework for added values (nutrition, environment, social, animal welfare) provides a tool for visualising the added values but parameters have to be defined. The framework scale has 4 levels, ranging from no to high additionality, compared with standard production.

Keywords: semi-natural grasslands, High Nature Value farmland, values-based supply chains, grassland products, case studies, embedded values

Introduction

European grasslands are important habitats and provide a variety of ecosystem services, but especially semi-natural grasslands and grazed woodlands have declined due to intensification or abandonment (Oppermann *et al.,* 2012). Thus, it is important to strengthen the economic viability of sustainable grassland production. 'Values-based supply chains' can increase producer revenue by creating closer connections between producer and consumer and enhancing product value through a product's identity characteristics (Lu and Dudensing, 2015). Environmental, nutritional, animal welfare and social benefit are qualities that can be communicated when products are differentiated on the market. This paper is based on the report *Grasslands, Biodiversity and Business* (Birge, 2019), which aimed to provide support for improving the economic aspects of sustainable grassland use in Latvia specifically, and the Boreal region broadly, by identifying potential added values of grassland products, especially from semi-natural grasslands.

Materials and methods

The three-part desk study, conducted in 2019, focused on grassland products: meat, dairy, honey, grass products, and wild medicinal plants, as well as textiles for case-studies only. For the literature review I combined primary search terms 'grassland' and 'semi-natural grassland' with geographic areas (Boreal, Denmark, Estonia, Finland, Latvia, Lithuania, Norway, Sweden, Northern Europe), product categories, and terms such as 'added' or 'embedded' value. I also used grey literature and snowball method. I considered search results relevant if they related to (semi-natural) grassland products with focus on agricultural product, biodiversity benefits, marketing measures, or tests that can be used to compare and validate semi-natural grassland products. The review covered only literature published in English, and results from outside the main geographic area were included in some cases, e.g. for the product categories.

I described 20 case studies of grassland products and product differentiation. Of these, 11 are from the Boreal region and 9 from outside the region: Denmark (2); Poland (1); Romania (1); UK (1); multi-country (4). The cases are distributed as: meat and dairy (9), textiles, honey, and grass products (2 each), and medicinal and aromatic plants (5). I identified the cases through prior knowledge, online searches, and requests to colleagues in Boreal countries for examples from their countries. Representatives from 13 of the cases provided feedback or photographs.

The conceptual framework for identifying additionality was based on pre-defined added values: environmental benefits, animal welfare, social benefits, and nutritional qualities (Figure 1). I supplemented the framework with a table of tests from the literature review that can be carried out to evaluate product quality and test for potential added value.

Results and discussion

The literature review showed that meat and dairy product composition varies, depending on fodder and pasturage, although there are significant knowledge gaps. Grass pasturage results in more favourable nutrient composition of meat, particularly in terms of the type and percentage of fatty acids and vitamin E (Daley *et al.,* 2010). Pasturage affects the quality and composition of final products such as cheese (Dumont *et al.,* 2013). Added values are used in marketing meat and dairy from grasslands: four of the cases are of national or international grass-based certification, and the remainder also emphasize grassland added values of their products.

Sophisticated analysis is available for differentiating honey, but there are substantial knowledge gaps in identification of honey from particular production systems (Soares *et al.*, 2017). Meadow or grassland honey is not readily identifiable through literature review or online search. The honey case studies are Estonian alvar honey and Norwegian heather honey.

There is a growing body of research about grass as biofuel. The literature focuses mainly on quantity, energetic potential, and environmentally harmful components inherent in the raw feedstock. One of the case studies highlights an energy plant in Estonia that uses biomass from semi-natural grasslands to produce heat. Thatch material and pressed 'pet pellets' (case study) are other uses.

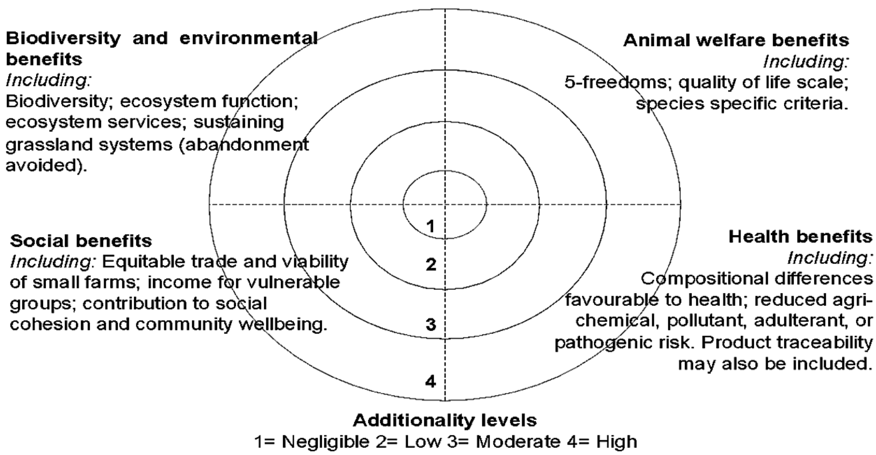

Figure 1. Framework for assessing additionality in grassland products: four types of added values.

Significant knowledge gaps exist on the potential differences in composition of wild medicinals collected from semi-natural grasslands compared with cultivars, but some variation has been identified (Dias *et al.*, 2013). Sustainable harvesting has positive social and conservation outcomes but over-harvesting is a risk. A case study of FairWild shows how certification can promote sustainable use. The cases also describe grassland-derived plants used in cosmetics and culinary uses.

Context-specificity and knowledge gaps create challenges for setting parameters for an added-values assessment framework. However, a general framework for assessing and visualising additionality can be adapted to various conditions and contexts (Figure 1).

Conclusions

This review identified added values of grassland products but also substantial knowledge gaps. Products must be differentiated for compensation of the added value in the market. The framework could aid in identifying and providing support for claims of product additionality.

Acknowledgements

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References

Birge T. (2019) *Grasslands, biodiversity and business.* Report to the GrassLIFE project, Latvian Fund for Nature. 116 pp. Available at: grasslife.lv/about-grasslife-english/for-further-reading/

Daley C.A., Abbott A., Doyle P.S., Nader G.A. and Larson S. (2010) A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition Journal Nutrition Journal* 9, 10.

Dias M.I., Barros L., Dueñas M., Periera E., Carvalho A.M., Alves R.C. Oliveira M.B.P.P., Santos-Buelga C. and Ferreira I.C.F.R. (2013) Chemical composition of wild and commercial *Achillea millefolium* and bioactivity of the methanolic extract, infusion and decoction. *Food Chemistry* 141, 4152-4160.

Dumont B., Thórhallsdóttir A.G., Farruggia A. and Norderhaug A. (2013) Livestock grazing and biodiversity in semi-natural grasslands. *Grassland Science in Europe* 18, 314-326.

Lu R. and Dudensing R. (2015) What do we mean by value-added agriculture? *Choices* 30(4), 1-8.

Oppermann R., Beaufoy G. and Jones G. (eds.) (2012) *High nature value farming in Europe: 35 European countries – experiences and perspectives*. Verlag regionalkultur. 544 pp.

Soares S., Amaral J., Oliveira M.B. and Mafra I. (2017) Comprehensive review on the main honey authentication issues: production and origin. *Comprehensive Review of Food Science and Food Safety* 16, 1072-1100.

Conservation strips can improve biodiversity in intensively used grassland

Boob M.[1,2], Grant K.[2], Thumm U.[1] and Elsaesser M.[1,2]
[1]*Institute of Crop Sciences / Biobased Products and Energy Crops, University of Hohenheim, Germany;*
[2]*Agricultural Centre Baden-Wuerttemberg, Grassland and Forage Production, Germany*

Abstract

Biodiversity in grassland is declining, i.e. because grassland use has been intensified. For example, excessive cutting prevents plants from flowering and pollinators such as butterflies no longer find enough food. In 2018, a field trial was set up to at two sites in south Germany to explore measures to improve biodiversity on the edges of intensively used meadows (min. four cuts per year). Here, five conservation strip variants are tested in 5-m-wide strips of min. 50 m length. These conservation strips are compared with the middle of the corresponding field, which is used intensively. The conservation strips have not been fertilised since 2019 and are used less frequently. Additionally, seed mixtures were sown in 2018 to increase the number of flowers. The results show that already in the first year in some cases the number of plant species was significantly higher in the conservation strips than in the middle of the intensively used meadows. However, there was no significant difference in the number of butterfly species.

Keywords: grassland margin, insect diversity, species richness

Introduction

In the last decades, the abundance and species numbers of insects have declined considerably (e.g. Habel *et al.*, 2016; Seibold *et al.*, 2019). In grassland, the reasons for this decline include advances in mowing machinery (Humbert *et al.*, 2010) and increase in number of cuts. Another reason is the intensification of grassland management, including fertilisation, which leads to reduced plant diversity (Socher *et al.*, 2013). The massive decline in arthropods not only disturbs food webs, but also harms ecosystem services, such as pollination. Measures to improve biodiversity are common in extensively used grassland, but have so far rarely been tested in intensively used meadows (Fritch *et al.*, 2017). For this reason, we set up a field trial to investigate whether uncut grass strips and resown strips enhance biodiversity, in particular pollinator species, such as butterflies, which rely on plants as a food source.

Materials and methods

Five different variants (Table 1) were tested as conservation strips on the edge of intensively used meadows at two sites (A and B). The meadows at site A and B, with a mean pH value of 6.6, high soil carbon content of 12-16% dry matter (DM) and mean total N content of 1.2% DM soil (0-10 cm), are cut five times per year to produce forage for dairy cows.

Table 1. Conservation strip variants.

Conservation strip variant (C)		Seed mixture (number of species)	Number of cuts per year	Time of cut
C1	Reduction in number of cuts	None	2	June, October
C2	Seed mixture	Forbs and legumes (29)	1	August
C3	Seed mixture	Grasses, forbs and legumes (42)	2	June, October
C4	Seed mixture	Legumes (2)	2	June, October
C5	Rotating uncut grass strip	None	4	Second to fifth cut

In September 2018, variants 2, 3 and 4 were installed by hand sowing mixtures of perennial grassland species (Table 1) after harrowing with a rotary tiller. Variants 1 and 5 were uncut grass strips and installed in 2019. All conservation strips are 5 m wide and min. 50 m long and usually at the edge of the meadow alongside ditches.

Vegetation surveys took place in April 2019. All plant species were recorded on three subplots (2×2 m^2) within each conservation strip (C) and in the middle of the adjacent meadow (M) to compare species numbers. To assess insect diversity, a survey of butterflies was conducted within transects of 50 m. As suggested by Pollard and Yates (1994), butterfly species were counted and identified visually for 30 min on 5 days (May-August) within each conservation strip and in the middle of the intensively used meadow.

Statistical analyses were performed using R (R Core Team 2019) and Excel 2013 (Microsoft Office). Linear mixed-effect models (package nlme) were fitted to each response variable and checked for homogeneity of variance by Q-Q plots. Where the residual distribution was not normal, data were boxcox-transformed (package MASS). Multiple comparisons tested a generalized linear hypothesis for linear mixed-effects models (package multcomp). The full mixed-effects model used for each response tested a two-way interaction between variant and M/C. Site and subplot number were included as random factors.

Results and discussion

In some conservation strip variants, the mean number of plant species was significantly higher (Figure 1). In variants C2 and C3 this was probably due to the sown species-rich mixture. The mixture of C4 contained only 2 species (Table 1), therefore there was no significant increase in plant species.

In 136 butterfly survey visits at the two sites, a total of 268 individuals belonging to 17 different species were observed. The most frequently recorded were the widespread species *Pieris napi* and *Vanessa cardui* (Table 2). There were no significant differences in species richness and abundance between C and M (n=5) at either site.

The field trial showed that so far mainly generalist butterfly species are to be found in both conservation strip and meadow middle. Enhanced flower availability in conservation strips can provide extra nectar

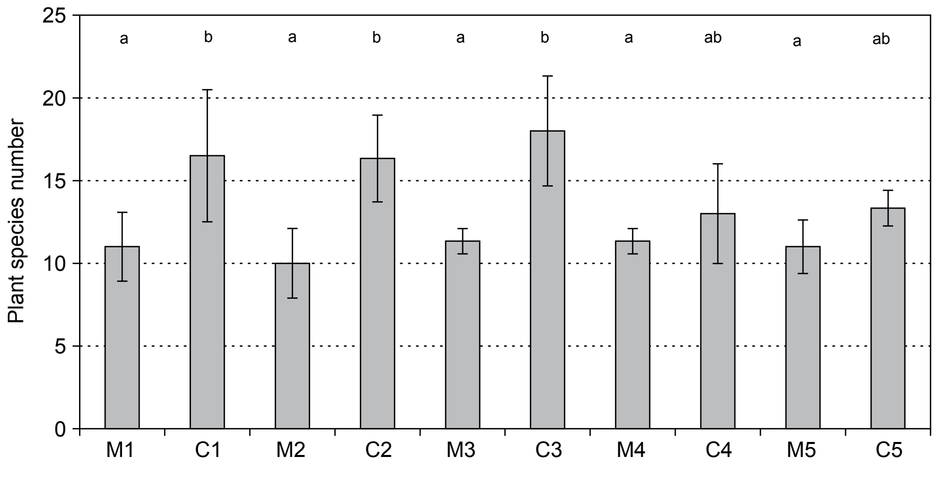

Figure 1. Mean number of plant species (4 m^2) of all variants (C = conservation strip variant, M = middle of the adjacent meadow) at both sites with standard deviation. Identical letters indicate non-significant differences between variants for each site (P<0.05).

Table 2. Total numbers of butterfly individuals and species observed at site A and B within conservation strip (C) and meadow middle (M).

Site	Transect	Individuals	Species	Most frequent species (count)
A	C	68	11	*Pieris napi* (16)
	M	58	8	*Pieris napi* (12)
B	C	92	12	*Vanessa cardui* (30)
	M	50	8	*Vanessa cardui* (18)

and pollen for adults, but butterfly larvae need habitats and feeding plants as well. An experiment with wax models of butterfly larvae showed that mowing can be detrimental (Humbert *et al.*, 2010), leading to a population sink rather than enhanced biodiversity. Although adult butterflies can move several kilometres, the surrounding landscape plays an important role in finding new habitats (Korpela *et al.*, 2013). Perhaps butterfly species need more time to colonise the conservation strips. Therefore, it is important to install perennial strips and continue the surveys in subsequent years.

Conclusions

Conservation strips can promote biodiversity in intensively used meadows. It is possible that they need to be kept in place for several years to allow colonisation by butterflies.

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References

Fritch R.A., Sheridan H., Finn J.A., McCormack S. and ÓhUallacháin D. (2017) Enhancing the diversity of breeding invertebrates within field margins of intensively managed grassland: effects of alternative management practices. *Ecology and Evolution* 7, 9763-9774. https://doi.org/10.1002/ece3.3302

Habel J.C.,Segerer A., Ulrich W., Torchyk O., Weisser W.W. and Schmitt T. (2016) Butterfly community shifts over two centuries. *Conservation Biology* 30(4), 754-762. https://doi.org/10.1111/cobi.12656

Humbert J.-Y., Ghazoul J., Sauter G.J. and Walter T. (2010) Impact of different meadow mowing techniques on field invertebrates. *Journal of Applied Entomology* 134 (7), 592-599.

Korpela E.L., Hyvönen T., Lindgren S. and Kuussaari M. (2013) Can pollination services, species diversity and conservation be simultaneously promoted by sown wildflower strips on farmland? *Agriculture, Ecosystems & Environment* 179, 18-24.

Pollard E. and Yates T.J. (1994) *Monitoring Butterflies for Ecology and Conservation*. Springer, 274 pp.

Seibold S., Gossner M.M., Simons N.K., Blüthgen N., Müller J., Ambarlı D., Ammer C., Bauhus J., Fischer M., Habel J.C., Linsenmair K.E., Nauss T., Penone C., Prati D., Schall P., Schulze E.D., Vogt J., Wöllauer S. and Weisser W.W. (2019) Arthropod decline in grasslands and forests is associated with landscape-level drivers. *Nature* 574, 671-674. https://doi.org/10.1038/s41586-019-1684-3

Socher S.A., Prati D., Boch S., Müller J., Baumbach H., Gockel S., Hemp A., Schöning I., Wells K., Buscot F., Kalko E.K.V., Linsenmair K.E., Schulze E.-D., Weisser W.W. and Fischer M. (2013) Interacting effects of fertilization, mowing and grazing on plant species diversity of 1500 grasslands in Germany differ between regions. *Basic and Applied Ecology* 14, 126-136.

Investigating the impact of soil acidity on grazed Scottish grassland swards

Boyko R.[1,2], Paton G.[1], Walker R.[2], Watson C.[2] and Norton G.[1]
[1]School of Biological Sciences, The University of Aberdeen, AB24 3UL, Scotland, United Kingdom;
[2]Scotland's Rural College Aberdeen, AB21 9YA, Scotland, United Kingdom

Abstract

Pasture lime applications are found to be inadequate to maintain optimum soil pH values in the UK since the removal of the liming subsidy in the 1970s, with the rate of annual lime applied having decreased by 86,000 Mg per year in the past two decades. Lower pH value soils result in reduced sward quality and fewer legumes (e.g. white clover), impacting sward total crude protein and other nutritional properties. To achieve optimum nutritional quality, this research asks whether farmers should improve soil pH values or modify the sward varieties to suit more acidic soils? This is tested through observing the impact of soil pH values on the nutritional quality of grass-clover swards on relevant Scottish soils. An established field demonstration and greenhouse experiment were used to determine the impact of pH and soil type on the sward composition and subsequent nutritional quality. Experiments confirm sward composition is significantly positively impacted by soil pH values resulting in improved yield and crude protein.

Keywords: acidity, lime, legumes, grassland, pH, botanical composition

Introduction

Trends of increasing soil acidity in UK agricultural land have been observed, likely due to the removal of the lime subsidy in the 1970s and the subsequent reduction in lime applications. Liming rates have decreased at around 86,000 Mg per year over the last two decades (PNMG, 2018). Managing a soil to optimum pH values can increase the species diversity within a grassland (Schuster and Diekmann, 2003). With species diversity can come increased yields and nutritional quality benefits, particularly in the case of swards with legumes which can improve sward yield 1.7-3.5 times that of a monoculture (Sturludottir *et al.*, 2014). Legumes can increase the crude protein (CP), macro and micronutrient concentrations in a sward, and are often preferentially eaten by animals due to their higher digestibility as compared to grasses, resulting in greater feed intake (Dewhurst *et al.*, 2001). The aim of this research is to quantify the true impact of soil acidity on Scottish grassland swards and the subsequent impact on grazing animals using a commonly grown grass-clover mixture. This research will be supplying the necessary missing data for nutritional quality, species composition and yield of a commonly used white clover (WC) and perennial ryegrass (PRG) sward along a managed soil pH gradient.

Materials and methods

To measure how nutritional quality may be impacted by soil acidity, plant material was collected from the established SRUC Craibstone pH long-term experiment (LTE), created in 1961. The demonstration mimics an eight-crop rotation which includes three years of 'grazed' PRG-WC sward and 7 incremental plots with pH values from 4.5 to 7.5. Further description can be found in Walker *et al.* (2011). To mimic grazing quality, three 32×32 cm quadrats were cut at regular intervals from each plot from the three cropping years that represent grassland years 1, 2 and 3. These samples were separated into species, oven dried (48 h at 80 °C) and stored for further processing. Three years of such data will be collected. To compare species proportions as they change on a single soil, a greenhouse experiment was created to compare multiple soil types with contrasting pH values and the same sward. Both experiments analyse CP, N, C and fibre on annual material combined and at a single mid-season cutting. Data analysis (one-way ANOVA) is performed with SigmaPlot.

Results and discussion

For the year 2019, results from the Craibstone pH-plot plant material collection show sward composition is observed to significantly change due to soil pH value changes (Figure 1) (P<0.01, both PRG and WC yield). At pH 4.5, year 2 WC yields zero Mg ha^{-1} whereas at pH 6.0, yield increases to 3 Mg ha^{-1} and overall yield increases from 5 to 6 Mg ha^{-1}. Similarly, in year 3, overall yield increases from 5.8 to 8.2 Mg ha^{-1} from 4.5 to 5.5-6.5 pH values. Figure 2 shows soil pH significantly impacts CP yields. This is observed in the combined CP yield of pH 4.5 to 5.5/6.0 for both the second year and third year since establishment (P<0.01 combined yield of PRG, WC and for both grassland years 2 and 3). Contrasting the overall yielded material (Figure 1) to the CP results (Figure 2), the data confirm both that WC is higher in protein and that in managing soil pH to enhance the growing environment for WC, greater CP will be found in the overall intake in a mixed sward. Additional trends in this first season of data collection confirm that WC grows to become a greater proportion of the sward and more dominant within the sward as years since establishment progress, confirming past literature. The same is true for PRG in this experiment.

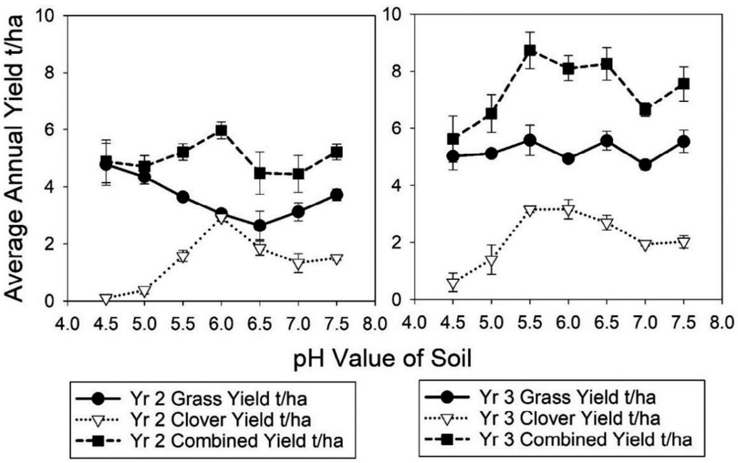

Figure 1. Year 2 and year 3 annual yield of grass and clover from the SRUC Craibstone pH plot demonstration for the year 2019.

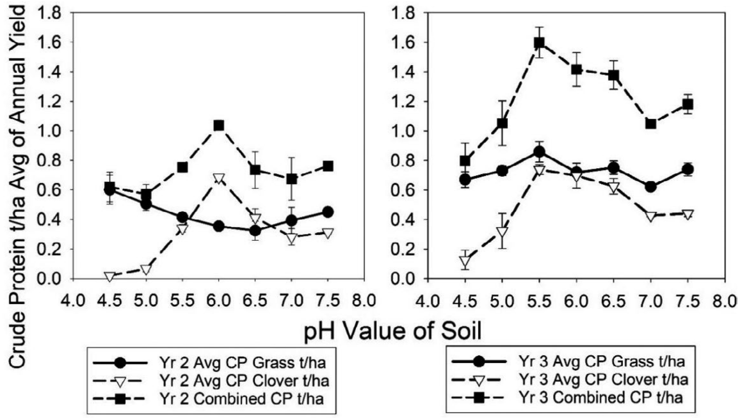

Figure 2. Year 2 and year 3 crude protein of grass and clover from the SRUC Craibstone pH plot demonstration for the year 2019.

Conclusions

Improving pH values of soil to an optimum range can increase the proportion of clover in a sward, increase overall yield and therefore increase the crude protein content of the sward. These benefits are shown to be achieved by maintaining the soil pH values within the optimum range (5.5-6.5). It is therefore suggested to farmers that liming is required for both efficiency and optimum yields on this soil type, though further nutritional quality data is to follow.

References

Dewhurst R.J., Scollan N.D., Youell S.J., Tweed J.K. and Humphreys M.O. (2001) Influence of species, cutting date and cutting interval on the fatty acid composition of grasses. *Grass and Forage Science* 56: 68-74.

Professional Nutrient Management Group (PNMG) (2018) Update report on nutrient management planning. Available at: http://www.nutrientmanagement.org/tried-and-tested/2018-pnmg-report/.

Schuster B. and Diekmann M. (2003) Changes in species density along the soil pH gradient — evidence from German plant communities. *Folia Geobotanica* 38, 367-379.

Sturludóttir E., Brophy C., Bélanger G., Gustavsson A., Jørgensen M., Lunnan T. and Helgadóttir Á. (2014) Benefits of mixing grasses and legumes for herbage yield and nutritive value in Northern Europe and Canada. *Grass and Forage Science* 69: 229-240.

Walker R., Watson C. and Edwards A.C. (2011) The long-term effects of widely differing soil pH on the yields of an eight course crop rotation established in 1961. Conference: Making long term crop rotations fit for the future. *Aspects of Applied Biology* 113, 111-115.

Reducing ammonia emission from manure application on grassland using lime suspensions: proof of principle

Bussink D.W., Thijssen D. and Verweij S.
Nutrient Management Institute, Nieuwe Kanaal 7C, 6709 PA Wageningen, the Netherlands

Abstract

Application of cattle slurry (CS) with the trailing shoe machine (narrow band application) on peat and clay grassland is no longer permitted in the Netherlands due to high NH_3 emission (emission factor 26% of the NH_4 nitrogen). CS must now be diluted with 50% water or can be applied by slice injection (19% emission). However, the latter gives a risk of sod damage. Diluting with water increases the costs for the dairy farmer. We have investigated whether CS application in a single operation with the trailing shoe machine and simultaneously spraying a lime suspension over the CS strip, can structurally reduce NH_3 emission. A first comparative field trial was carried out in August 2019. On peat grassland, 20 m^3 CS ha^{-1} were applied both with shallow injection and with the trailing shoe machine. In the latter, a total of 1.1 m^3 ha^{-1} of lime suspension was applied in two concentrations corresponding to 375 and 185 kg CaO ha^{-1}. It turned out that the NH_3 emission at both treatments was lower than that of shallow injection. For highest lime level it reduced NH_3 emission up to 35%.

Keywords: ammonia emission, cattle slurry, lime, injection

Introduction

In the Netherlands, ammonia (NH_3) emissions must be further reduced. These are now 120,500 Mg per year, of which 114,000 Mg comes from agriculture (Van Bruggen *et al.*, 2019). About 35% of the NH_3 emission results from slurry spreading on grassland and arable land, mainly cattle slurry (CS). Since this year, only slice injection (trenches of 5 cm depth at about 20 cm from each other) is allowed on grassland. The exceptions are peat and clay grasslands, where CS can also be applied with a trailing shoe machine (narrow band application, CS is laid in strips at about 20 cm from each other between the grass) under the condition that the CS is at least 50% diluted with water. The emission is then equivalent to that of slice injection (19% of the NH_4-N in CS). On peat and (heavy) clay grassland, slice injection is less suitable because of the risk of damage to the sod. Furthermore, under dry conditions on heavy clay, it is not even possible to make a trench because the soil is too hard. The application of diluted CS with the trailing shoe machine is then a solution. The disadvantage, however, is that 1.5 times as much CS must be applied. This results in more driving on the fields and also higher costs. The question is whether it is possible to use additional spraying to reduce NH_3 without using a lot of water. We have investigated whether the application of very finely ground lime (more than 50% <2 microns) in the form of a suspension to slurry strips during application can reduce the emission. Preliminary measurements in the lab clearly showed an emission reduction.

Materials and methods

The test was carried out in the last week of August 2017 on peaty grassland near Wageningen. The grass was mowed the week before so that the stubble height was 5-7 cm. The NH_3 emission trial consisted of 4 treatments for the application of CS to grassland. The reference treatment was slice injection with a target application of 20 m^3 ha^{-1}. The three other treatments concerned the application of CS with the trailing shoe machine in combination with lime suspension, namely:

- TS_700: 20 m^3 CS ha^{-1} plus 700 litres of lime suspension diluted with 350 litres of water;
- TS_350: 20 m^3 CS ha^{-1} plus 350 litres of lime suspension diluted with 700 litres of water;
- TS-mix: 20 m^3 CS ha^{-1} where 700 litres of lime suspension are mixed with the manure.

These quantities were selected based on previous tests in which the emission was measured under controlled conditions using a closed chamber diffusion method. The lime suspension has a specific gravity of 1,455 kg m^3 and has a neutralizing value of 25 per 100 kg product. The trailing shoe machine had a separate tank for spraying diluted lime suspension directly over the CS strip with a pressure of 3 bar via a spray nozzle.

Recent research indicates that it is possible to work with small fields (<20 m radius) (Vilms Pedersen *et al.,* 2018), which offers important advantages for the required field area. Therefore, small circular plots – to obtain a constant path length for the emission measurement – with a diameter of 25 m were used. The experimental plots were at least 75 m apart (centre distance) in order to minimize mutual interference. The CS was applied in parallel strips of different lengths in order to approach a circular shape The slice injection machine had a working width of 5 m (resulting in 5 parallel passes) and the trailing shoe machine of 3.6 m (resulting in 7 parallel passes).

The NH_3 emission was measured using an aerodynamic method based on a one-point measurement at a characteristic height of 90 cm in the middle of the field (the ZINST method Wilson, 1982). The height of this point is determined by the surface roughness (≈2 cm). For the measurement battery-operated anemometers and Wind101A data loggers (MadgeTech, Inc., Warner, N.H.) along with 3 passive ALPHA samplers (Sutton *et al.,* 2001) were installed in the centre of each field at the ZINST height. In addition, at the edge of the field the wind speed and the NH_3 background concentration were also measured. The samplers were changed in the morning and evening (at 07:00 and 19:00) except for the start day, where the samplers were changed around 16:00 and 20:00 in the afternoon. Within 2 km there was an official meteorological station for additional met data. By using backward Lagrangian stochastic dispersion modelling using the Windtrax software (Thunder Beach Scientific, Halifax, NS, Canada) the emission was calculated from the wind and ALPHA sampler data.

Results and discussion

Application of CS started on 26 August at noon. All treatments were established within 2 hours (Table 1). The amount applied was higher for slice injection and lower for trailing shoe application than planned. During the trial it was very hot with daily maximum temperatures of 31, 33 and 28 °C on 26, 27 and 28 August, respectively. The trial was stopped after 3 days due to heavy rainfall (>10 mm) on August 29. The cumulative emission over the measurement period was 16% for slice injection and 10, 12 and 23% respectively for treatment TS_700, TS-350 and TS_mix. For the latter treatment, much less lime was applied than planned. The highest emission was measured on the 1st day (Figure 1). The measurements indicate that spraying lime suspension over the CS strips can be an effective measure to reduce NH_3 emissions. The low dosage (TS_350) also gives less emission than slice injection. The lime suspension is applied on top of the CS strip. The reason for the emission reduction is probably the application of extra moisture on the CS strip (0.4 mm) in combination with an adsorbent 'coating'. The fineness of the lime suspension (<2 micron) provides a large specific surface on which NH_3 can adsorb. The measured

Table 1. Results of the emission measurement trial.[1]

Treatment	Start time	CS	DM	NH_4-N	total N	TAN	NH_3-N emission	
	h	m^3 ha^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	kg ha^{-1}	kg ha^{-1}	% of TAN
Slice-inj	12:00	28.4	90	2.0	4.70	56.8	8.82	15.5
TS_700	12:45	17.5	89	2.0	4.78	35.0	3.47	9.9
TS_350	13:00	17.5	90	2.0	4.64	35.0	4.07	11.6
TS_mix	14:10	17.5	89	2.0	4.65	35.0	7.97	22.8

[1] CS = cattle slurry; DM = dry matter; TAN = total ammonical nitrogen.

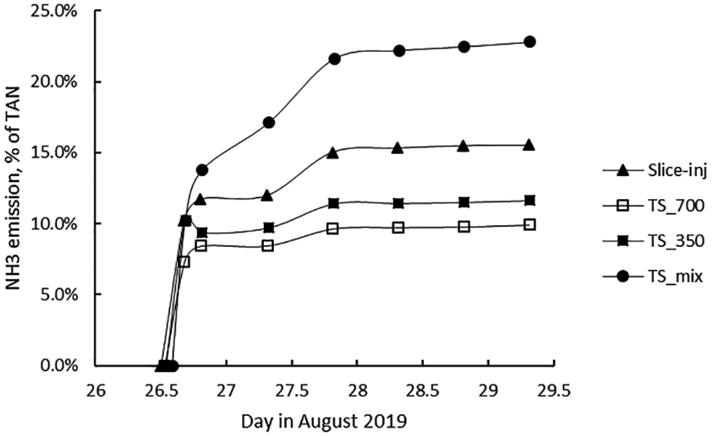

Figure 1. The emission pattern after application of cattle slurry on 26 August 2019. TAN = total ammonical nitrogen.

emissions were relatively low, in particular given the high air temperatures during the experimental period. This can be due to crust formation after manure drying (confirmed by visual observations) as well as less effective NH_3 sorption by Alpha samplers. In the spring of 2020 more measurements will take place at different weather conditions (lower temperatures) so that a better picture can be obtained.

In conclusion application of lime suspension looks a promising method to reduce NH_3 emission.

References

Carozzi M., Loubet B., Acutisa M., Ranac G. and Ferrarac R.M. (2013) Inverse dispersion modelling highlights the efficiency of slurry injection to reduce ammonia losses by agriculture in the Po Valley (Italy). *Agricultural and Forest Meteorology* 171-172, 306-318.

Sutton M.A., Miners B., Tang Y.S., Milford C., Wyers G.P., Duyzer J.H. and Fowler D. (2001) Comparison of low-cost measurement techniques for long-term monitoring of atmospheric ammonia. *Journal of Environmental Monitoring* 3, 446-453.

Vilms Pedersen S., Scotto di Perta E., Hafner S., Siegfried Pacholski A. and Sommer S.G. (2018) Evaluation of a simple, small-plot meteorological technique for measurement of ammonia emission: feasibility, costs, and recommendations. *American Society of Agricultural and Biological Engineers. Transactions* 61, 103-115.

Van Bruggen C., Bannink A., Groenestein C.M., Huijsmans J.F.M., Lagerwerf L.A., Luesink H.H., Van der Sluis, S.M., Velthof G.L. and Vonk J. (2019) *Emissions into the atmosphere from agricultural activities in 2017. Calculations using the NEMA model.* Wageningen, The Statutory Research Tasks Unit for Nature and the Environment (WOT Natuur & Milieu). WOt-technical report 147. 131 p (In Dutch).

Wilson J.D., Thurtell G.W., Kidd G.E. and Beauchamp E.G. (1982) Estimation of the rate of gaseous mass transfer from a surface source plot to the atmosphere. *Atmospheric Environment* 16, 1861-1867.

Biomass production and soil carbon and nitrogen content with innovative cropping systems

Chen J., Lærke P.E., Manevski K. and Jørgensen U.
Aarhus University Centre for Circular Bioeconomy, Department of Agroecology, 8830 Tjele, Denmark

Abstract

To meet the growing challenges from food security, renewable resources and climate change, innovative cropping systems should aim to maximise biomass production while minimising carbon (C) and nitrogen (N) footprints. However, the effects of various innovative cropping systems on biomass production and soil C and N content remain poorly understood. In this study, multiple innovative cropping systems were compared. Averaged across the five years, results showed that biomass production varied between the cropping systems, but soil C and N contents were relative stable. There was generally no clear relationship between average biomass production and changes in soil C and N content. Our results suggest that, over 5 years, increased production of biomass without reducing C and N content in surface soil is feasible through optimising cropping systems; however, long-term observations are also required.

Keywords: biomass production, soil carbon and nitrogen content, traditional agriculture, crop rotation, perennial grass, grass-legume

Introduction

Achieving climate-smart sustainable agriculture is one of humanity's grand challenges, because of the potential conflicts between climate change and biomass production (Tilman *et al.*, 2002; Walter *et al.*, 2017). It has sometimes been assumed that higher biomass production could reduce soil C and N content, accelerating climate change and threatening agriculture sustainability. However, this hypothesis has not been rigorously examined across various cropping systems (e.g. perennial grasses vs annual crops in mono-agriculture or in rotation). The development of innovative cropping systems that aim to increase biomass production as well as soil C and N content are strongly desired. Nonetheless, there have been few attempts to systematically investigate the effects of various cropping systems on biomass production and soil C and N content in the long term, as well as the links between biomass production and soil C and N content. Our objectives are to: (1) study the effects of various cropping systems on biomass production and soil C and N content, and (2) investigate the potential links between average biomass production and changes in soil C and N content.

Materials and methods

The study was conducted at Research Centre Foulum, Denmark (9°35 ′E, 56°30 ′N, 48 m a.s.l.). Soils were Typic Hapludult and sandy loam texture. The mean annual temperature was 7.8 °C, the mean annual precipitation was 740 mm. The experiment was established in 2012 with four replicates. Five cropping systems with a variable number of subsystems were compared (see Figure 1 for the detailed information of specific species for each cropping system). Detailed information on multiple cropping systems, species, fertilisation and harvest can be found in Manevski *et al.* (2017, 2018). In brief, aboveground biomass was documented after each harvest from 2012 to 2017. Surface soils (0-20 cm) were sampled in 2012 and 2017 and analysed for soil C and N content using a Vario MAX cube (Elementar Analyzer AG). Linear mixed-effects model was adopted to evaluate the effects of cropping systems including the sub-system on biomass production and soil C and N content. All analyses were conducted in R 3.5.1.

Results and discussion

Across the five years, biomass production highly varied across various crop species (Figure 1A) e.g. traditional mono-agriculture (TA)1 (see Figure 1 for abbreviations and detailed information for each cropping system) produced 60% more biomass than TA2. Optimised crop rotations (OCRs) generally produced more biomass than TA2, despite biomass production varied across the four subgroups of OCRs. Regarding perennial grass, we observed more biomass for tall fescue and *Festulolium* and less biomass for Sibirian. There was less biomass production for DLF and SLU as compared to other cropping systems due to the absence of N fertilisation.

Across the five years, TA1, OCR4 and Sibirian significantly decreased soil C content by 6, 4, and 2%, respectively, whereas tall fescue significantly increased soil C content by 7% (Figure 2). As for soil N, tall fescue and DLF significantly increased soil N content by 7 and 12%, respectively. We did not observe any significant changes in soil C and N content for other crops. One likely explanation was that five years was insufficient to document significant changes in soil C and N content, since C accumulation is a relatively slow process (West and Post, 2002). Another explanation might be the removal of plant residues. Furthermore, there was no clear relationship between average biomass production and changes in soil C and N content (Figure 1B). Our results indicate that it is feasible to increase biomass production without reducing soil C and N content in the short-term.

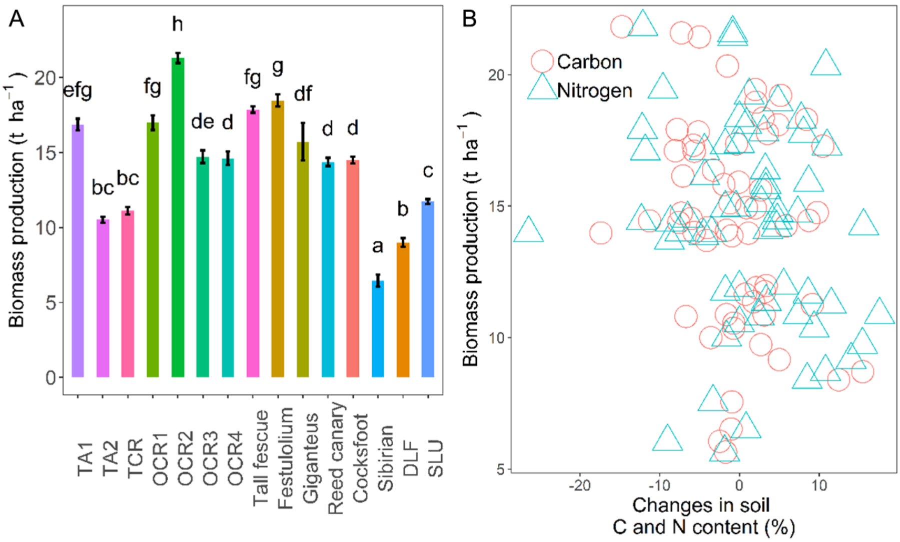

Figure 1. (A) Average biomass production from the various cropping systems. (B) Relation between biomass production and changes in soil C and N content. Two traditional mono-agriculture (TA1-2), TA1, *Zea mays*; TA2, *Triticosecale*. One traditional crop rotation (TCR, *Hordeum vulgare* and *Brassica napus*). Four optimised crop rotations for longer growing seasons and higher radiation use efficiency (OCR1-4). Four intensively fertilised perennial grasses (tall fescue, *Festulolium*, reed canary, cocksfoot). Two low fertilised *Miscanthus* genotypes (*M×giganteus and M. sacchariflorus Sibirian*). Two grass-legume mixtures without N fertilisation (DLF and SLU). Detailed information on each crop can be found in (Manevski *et al.*, 2017, 2018). Values in Figure 1A without common letters indicated significant difference at P<0.05. No relation was found in Figure 1B even when examined for each cropping system.

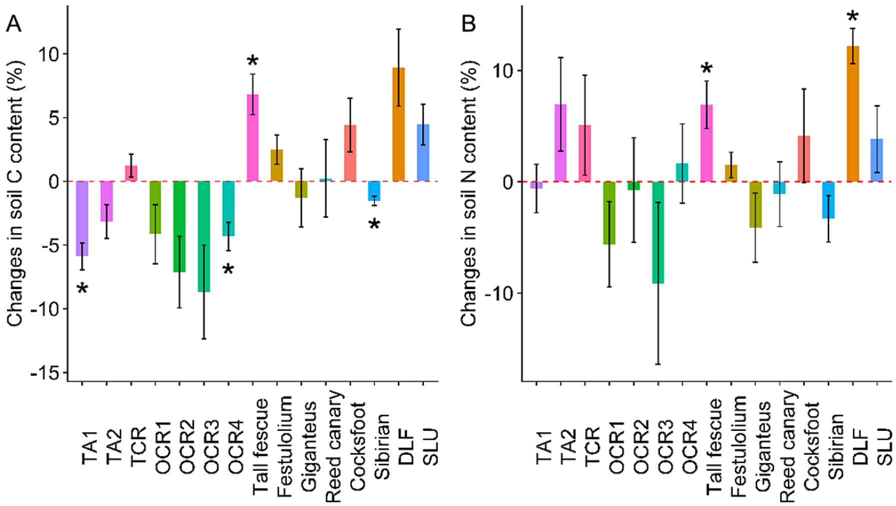

Figure 2. Changes in soil C and N content across various cropping systems. Asterisks indicate significant difference at *P*<0.05. See Figure 1 for more information on each cropping system.

Conclusions

Our short-term results showed that biomass production varied considerably, whereas soil C and N content remained relatively stable across multiple cropping systems. These results suggest that it is feasible to increase biomass production without reducing soil C and N content through optimised cropping systems. Across all crops, tall fescue resulted in significantly increased soil C and N content, both by 7%, while sustaining higher harvest yields. Nevertheless, continuous field observations of above- and below-ground biomass production and soil C and N content are required to clarify long-term C and N flows within the cropping systems.

Acknowledgements

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References

Manevski K., Lærke P.E., Jiao X., Santhome S. and Jørgensen U. (2017) Biomass productivity and radiation utilisation of innovative cropping systems for biorefinery. *Agricultural and Forest Meteorology*, 233, 250-264.

Manevski K., Lærke P.E., Olesen J.E. and Jørgensen U. (2018) Nitrogen balances of innovative cropping systems for feedstock production to future biorefineries. *Science of the Total Environment*, 633, 372-390.

Tilman D., Cassman K.G., Matson P.A., Naylor R. and Polasky S. (2002) Agricultural sustainability and intensive production practices. *Nature*, 418, 671.

Walter A., Finger R., Huber R. and Buchmann N. (2017) Opinion: Smart farming is key to developing sustainable agriculture. *Proceedings of the National Academy of Sciences*, 114, 6148-6150.

West T.O. and Post W.M. (2002) Soil organic carbon sequestration rates by tillage and crop rotation. *Soil Science Society of America Journal*, 66, 1930-1946.

Influence of microplastics on the establishment of different grassland species

Cornelsen H. and Wrage-Mönnig N.
Grassland and Forage Science, Faculty of Agricultural and Environmental Sciences, University of Rostock, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany

Abstract

Occurrence and effects of microplastics (MP, <5 mm diameter) belong to the highly discussed, anthropogenically induced environmental topics. Increasing production rates in the plastics industry in combination with inadequate recycling and disposal measures lead to a constant accumulation of differently sized synthetic polymer fragments of various origins in marine, fluvial and terrestrial environments. Reliable information on concentrations and dynamics of the plastic fragments in soil is still insufficiently available, and especially information on the impact of MP in grasslands is lacking. Here, we tested the effect of polyvinyl-chloride powder (PVC) on the germination and development of *Lolium multiflorum* ssp. *Westerwoldicum* (LM) and *Trifolium repens* (TR) in germination trials and a field experiment. A laser-scanning microscope was used for analysis of the fresh plant material; dried plant material was analysed using an isotopic ratio mass spectrometer. The germination rate decreased for both plant species with MP and it had a significant effect on the δ^{13}C-signature and plant dry matter yield. These results underline the importance of analysing MP concentrations in soil and conducting impact analyses on the influence of MP on plant development.

Keywords: microplastics, environmental hazard, grassland, polymers, germination trials

Introduction

Fate and impact of microplastics (MP) in aquatic environments have been studied for several years, exposing potentially negative effects for a whole range of aquatic organisms (Cole *et al.,* 2011). Although the origin of MP is mostly terrestrial, detailed information on their dynamics concerning this environment are lacking (Bergmann *et al.,* 2019). More recently, studies affecting the interaction between MP and terrestrial ecosystems are gaining scientific attention because of the variety of processes that may be affected by MP (Horton *et al.,* 2017; Rillig 2012). Regarding the role of soil parameters like biodiversity and aggregate stability for food production, agricultural soils especially come into focus. Important entrance points for MP are via aerial transport or rain (Bergmann *et al.,* 2019) as well as from the use of sewage sludge and plastic mulching (Steinmetz *et al.,* 2016), with indistinct consequences for productivity and food chains (Nizzetto *et al.,* 2016). This study aims at testing the influence of MP on the performance of grassland species.

Materials and methods

MP was simulated using polyvinyl-chloride powder (PVC), ranging in particle size from 1 to 63 µm. It was applied in germination trials and a pot experiment with seeds of *Lolium multiflorum* ssp. *Westerwoldicum* (LM) and *Trifolium repens* (TR). The germination trials involved seeds grown in two replicates in petri dishes on moistened filter paper under laboratory conditions; no soil was involved here. Four different amounts of MP were added for the different treatments. They ranged from 0-2 g with increments of 0.5 g. The germinated seeds were counted regularly.

For the pot experiment, Mitscherlich vessels were filled with 6 kg of soil each and three concentration levels of MP were added to the soil as follows: 0 mg; 720 mg; 6,000 mg per 6 kg of soil (n=4). The harvested plants were analysed separately for root and shoot parts, using a confocal laser-scanning microscope (CLSM) for the fresh material. The dried and ground plants were analysed for different yield parameters and $\delta^{13}C$ signatures via isotopic ratio mass spectrometry (IRMS). All analyses and figures were developed using Microsoft Excel and the statistical environment 'R' (3.3) (R Development Core Team, 2012).

Results and discussion

For the germination trials, the germination rate was highest for seeds in petri dishes without any addition of MP (Figure 1). The larger the amount of MP (in g per petri dish), the smaller was the germination rate. Most of the seeds, especially in the groups with larger amounts of MP, showed growth depression in various ways and germinated abnormally according to the ISTA Handbook on Seedling Evaluation (Don, 2013). The negative impact of MP on the germination process seemed more distinct for LM than TR. The exact mechanism of how MP hinders germination needs to be clarified in future research.

No visible effects of MP on the growth habitus of LM and TR could be seen in the pot experiment and no differences among the concentration levels were visible using CLSM (Figure 2). There were significant effects of MP observed on dry matter (DM) and $\delta^{13}C$-signatures. An increase in the amount of MP in the soil resulted in decreasing rates of DM, visible mainly for LM. The $\delta^{13}C$-signatures for both species showed enrichment in concentration level two. The enrichment could be a sign of the plants incorporating MP ($\delta^{13}C$ signature of pure PVC was -13.41 ‰) but may it also show that less water was available for the plants.

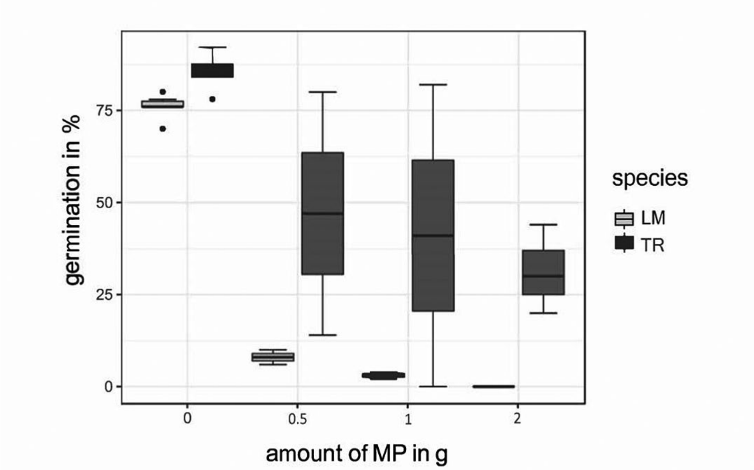

Figure 1. Germination rate (as %) of the seeds presented over the amounts of MP (microplastics) in g on moistened filter paper in petri dishes, differentiated according to the plant species LM (*Lolium multiflorum*) and TR *(Trifolium repens)*.

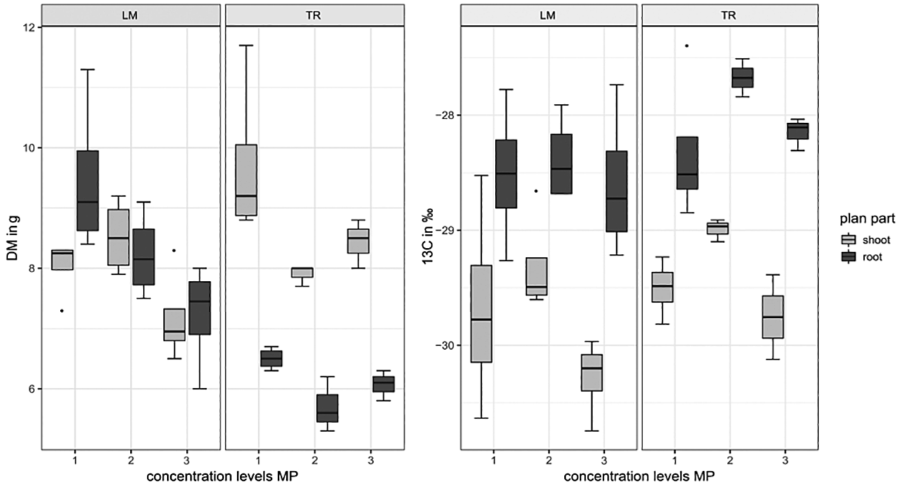

Figure 2. Influence of the three different concentration levels of MP (microplastics): 1 = 0 mg; 2 = 720 mg; 3 = 6,000 mg per 6 kg of soil on dry matter (DM) in g (left) and ^{13}C-signatures in ‰ (right), differentiated for root and shoot (plant part) of LM *(Lolium multiflorum)* and TR *(Trifolium repens)*.

Conclusions

Plants growing under MP conditions showed depressions in germination and reduced plant yield. The IRMS method showed sensitivity towards MP, but no adequate universal reference methods exist to make the results comparable. Further research is needed to quantify and characterize both the effect of MP on plant growth as well as the real amounts of MP in the soil. We are currently working on germination trials concerning the impact of different sizes and kinds of polymers on plant behaviour.

References

Bergmann M., Mützel S., Primpke P., Tekman M.B., Trachsel J. and Gerdts G. (2019) White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. *Science Advances* 5 (8), eaax1157. DOI: 10.1126/sciadv.aax1157.

Cole M., Lindeque P., Halsband C. and Galloway T.S. (2011) Microplastics as contaminants in the marine environment: a review. *Marine Pollution Bulletin* 62, 2588-2597. https://doi.org/10.1016/j.marpolbul.2011.09.025

Don R. (2013) *ISTA Handbook on Seedling Evaluation*. 3., rev. ed., including Amendments 2006, 2009 and 2013. Bassersdorf: International Seed Testing Association.

Horton A.A., Walton A., Spurgeon D.J., Lahive E. and Svendsen C. (2017) Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Science of the Total Environment* 586, 127-141. https://doi.org/10.1016/j.scitotenv.2017.01.190

Nizzetto L., Futter M.N. and Langaas S. (2016) Are agricultural soils dumps for microplastics of urban origin? *Environmental Science & Technology* 50, 10777-10779. https://doi.org/10.1021/acs.est.6b04140

R Development Core Team (2012) R: a language and environment for Statistical computing. R Foundation for Statistical Computing.

Rillig M.C. (2012) Microplastic in terrestrial ecosystems and the soil? *Environmental Science & Technology* 46, 6453-6454. https://doi.org/10.1021/es302011r

Steinmetz Z., Wollmann C., Schaefer M., Buchmann C., David J., Tröger J. *et al.* (2016) Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? *Science of the Total Environment* 550, 690-705. https://doi.org/10.1016/j.scitotenv.2016.01.153

Which grass for wildlife management on airports?

Cougnon M., Van Oost E. and Reheul D.
Plant and Crop Department, Ghent University, Coupure Links 653, 9000 Gent, Belgium

Abstract

For safety reasons, wildlife is not wanted on the grass fields of airports. Appropriate habitat management is the most sustainable measure to prevent wildlife problems. In a field trial, comparing swards of nine different grass species or mixtures of species, we addressed the following question: which species or mixture is least grazed by rabbits? The trial area was stocked with rabbits, and during two growing seasons the amount of forage grazed on the different swards was measured using the cage method. Swards based on tall fescue (*Festuca arundinacea* Schreb.) were, independently of the variety or the presence of endophytes, grazed less than swards based on perennial ryegrass or red fescue. Taking into account sward height recommendations, the forage type of tall fescue seems to be most suited as a grass cover for airports.

Keywords: rabbits, tall fescue, endophytes, alkaloids

Introduction

For safety reasons, wildlife is not wanted on the grass fields of airports. Whereas birds form a direct hazard when they get in the aircrafts' engines, rabbits form an indirect threat, attracting birds of prey, which can cause severe bird strikes.

Appropriate habitat management is the most sustainable measure to prevent wildlife problems. Applying a long grass policy, i.e. keeping sward height between 150 and 200 mm, as prescribed by Brough and Bridgman (1980), discourages most birds species that feed or rest on grass. Geerts and Korevaar (2004) found that at Schiphol airport, in the Netherlands, tall fescue (*Festuca arundinacea*) and tufted hairgrass (*Deschampsia cespitosa*) were species best suited to this long-grass policy. In New Zealand, tall fescue infected with a particular strain of the endophyte (*Neotyphodium coenophialum*) proved to be very successful in reducing the number of feeding birds (Pennell *et al.*, 2017a) and rabbits (Pennell *et al.*, 2017b) on these swards. The present study was established in cooperation with Brussels Airport, where there is a recurrent problem with high numbers of rabbits along the runways. The aim is to find out which species or mixture of species could be suited to renovate the airports' grassland, taking into account this particular problem.

Materials and methods

In spring 2017 nine different species or mixtures of species (Table 1) (called 'mixtures' hereafter), were sown in a randomised complete block design with three replicates at the experimental farm in Melle, Belgium. Individual plot size was 25.2 m^2 (6×4.2 m) and sowing density was 3,000 seeds m^{-2}. In summer 2017, the young swards were fertilised with 50 kg N ha^{-1}. There was no subsequent fertiliser application. The swards were mulched twice in the establishing year. In spring 2018, the experiment was fenced and stocked with 15 rabbits. The rabbits' preference for the mixtures was measured by placing cages of 1.5 m^2 on each plot. The rabbits were unable to graze under the cages. Before the cages were placed, the whole trial was mulched at a height of ca. 15 cm. The cages remained in place for 4-5 weeks, after which the grass biomass inside and outside the cages was measured by clipping four randomly selected squares of 0.2×0.2 m at a height of ca. 2 cm. After drying and weighing the harvested samples, the proportion of grass regrowth that had been grazed was calculated as: 1 – (biomass out cage / biomass in cage). This procedure was repeated three times in 2018 and two times in 2019, resulting in five sampling periods.

Table 1. Seed companies providing the seeds, and composition of the mixtures compared.[1]

Name	Company	Composition
Control	DSV (DE)	Perennial ryegrass, red fescue, creeping bentgrass, white clover
Avanex®	PGG (NZ)	Tall fescue 'Jackal' with endophyte AR601
Barairport	Barenbrug (NL)	Tall fescue 'Bardavinci', tufted hairgrass 'Barscampsia'
Bardavinci	Barenbrug (NL)	Amenity type tall fescue
Barvado	Barenbrug (NL)	Amenity type tall fescue
Caius	Carneau (FR)	Forage type cocksfoot
airportmix	DLF (DK)	Amenity type of tall fescue with perennial ryegrass
Femelle	Ugent (BE)	Forage type tall fescue
Solario	DSV(DE)	Red fescue 'Jasperina', 'Finesto'&'Doriana', smooth stalked meadow grass 'Limousine', perennial ryegrass 'Vesuvius'

[1] Perennial ryegrass (*Lolium perenne*), red fescue (*Festuca rubra*), creeping bentgrass (*Agrostis stolonifera*), white clover (*Trifolium repens*), tall fescue (*Festuca arundinacea*), smooth stalked meadow grass (*Poa pratensis*), cocksfoot (*Dactylis glomerata*).

The data were tested for homoscedasticity using the *leveneTest()* function in R (3.0.2) and, if found to be homoscedastic, subjected to one-way anova using the *aov()* function to test the effect of the different mixtures on the proportion of grazed grass. Grass height in each plot was measured weekly from April to November (10 measurements/plot) with a plate meter (Jenquip, NZ).

Results and discussion

Only in two out of five sampling periods, was there a significant effect of the mixture on the preference. Standard deviations were often high, reflecting the heterogeneity in the swards caused by the rabbit presence. On some plots that were not grazed at all, the biomass measured outside the cage was higher than the biomass inside the cages (probably due to the shading of the cages), resulting in negative proportions (Table 2).

The control mixture, composed of the species that have been dominating Brussels airports' swards up to now, and Solario, a mixture dominated by red fescue, were obviously the most preferred mixtures (Table 2). The mixtures containing only tall fescue, especially the forage types Femelle and Avanex® were clearly the least preferred. There was no clear advantage of the endophyte-presence in Avanex®, compared to Femelle, an endophyte-free variety; the preference of both was comparable. There was no measurable effect of tufted hairgrass on the rabbit preference: although it was clearly present in the Barairport swards, the preference for this mixture was not different from that of Bardavinci, the tall fescue variety present in Barairport.

Table 2. Proportion of grass regrowth grazed in 4-5 weeks periods, averaged over the replicates.[1]

	May 2018	Aug 2018	Oct 2018	May 2019	Sept 2019
Control	0.79 (0.029)	0.75 (0.174)	0.93 (0.029)	0.44 (0.196)	0.34 (0.098)
Avanex®	0.25 (0.126)	0.04 (0.071)	0.14 (0.068)	0.36 (0.252)	0.1 (0.149)
Barairport	0.27 (0.340)	0.26 (0.181)	0.34 (0.151)	0.49 (0.190)	0.08 (0.100)
Bardavinci	0.26 (0.039)	0.03 (0.062)	0.13 (0.212)	0.29 (0.291)	-0.04 (0.225)
Barvado	0.43 (0.180)	0.03 (0.113)	0.23 (0.409)	0.49 (0.179)	0.12 (0.166)
Caius	0.14 (0.205)	0.27 (0.112)	0.37 (0.151)	0.49 (0.162)	-0.04 (0.046)
airportmix	0.47 (0.044)	0.65 (0.144)	0.58 (0.206)	0.54 (0.061)	0.42 (0.227)
Femelle	0.31 (0.264)	-0.07 (0.048)	0.37 (0.346)	0.06 (0.312)	-0.09 (0.166)
Solario	0.63 (0.080)	0.81 (0.169)	0.66 (0.293)	0.6 (0.100)	0.59 (0.140)
P-value	0.0061	<0.001	0.102	0.133	<0.001

[1] The values between brackets are standard deviations. P-values indicate the significance of the effect of the mixtures.

The presence of perennial ryegrass in the airportmix swards resulted in a high preference for this mixture. The cocksfoot, Caius, had a low preference, mostly comparable to that of the forage tall fescues. However, we noticed that when the grass herbage of the most preferred mixtures became limited, the rabbits first grazed the cocksfoot, before they started to graze the forage types of tall fescue (Figure 1).Taking into account that long grass 150-200 mm has a negative effect on wildlife presence, the forage type tall fescues Femelle and Avanex® are clearly the most interesting mixtures. Near the end of the season, when grass became scarce, the rabbits started to graze the soft leaved tall fescue rather than the erect growing harsh-leaved Avanex®, resulting in a decreasing sward height for Femelle (Figure 1).

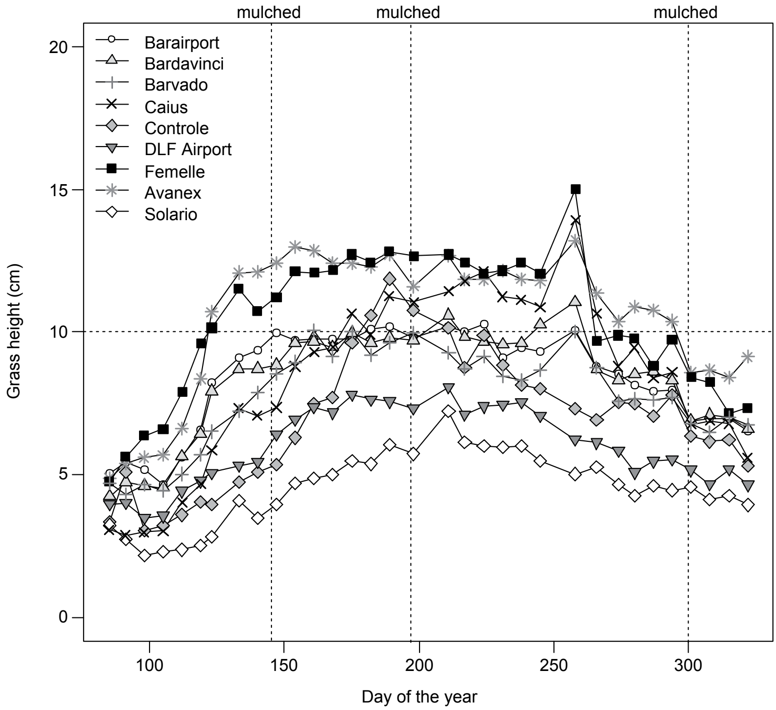

Figure 1. Evolution of grass heights of swards grazed by rabbits.

Conclusions

Based on this trial, forage types tall fescue, preferably not soft leaved varieties, have the best prospects for controlling wildlife problems on grass at airports. Endophyte infection of the tall fescue had no great effect on rabbit preference.

References

Brough T. and Bridgman C.J. (1980) An evaluation of long grass as a bird deterrent on British airfields. *Journal of Applied Ecology* 17, 243-253.

Geerts R. and Korevaar H. (2004) *Lang gras beheer op luchthaven Schiphol. Resultaten van een praktijkproef met alternatieve grasveldmengsels*. WUR Nota327, Wageningen, Nederland.

Pennell C.G.L., Rolston M.P., Van Koten C., Hume D.E. and Card S.D. (2017a) Reducing bird numbers at New Zealand airports using a unique endophyte product. *New Zealand Plant Protection* 70, 224-234.

Pennell C.G., Rolston M.P., Latham A.D.M., Mace W.J., Vlaming B., Van Koten C. and Card S.D. (2017b) Novel grass-endophyte associations reduce the feeding behaviour of invasive European rabbits (*Oryctolagus cuniculus*). *Wildlife Research* 43(8), 681-690.

Permanent meadows and climate change in dairy system areas in Emilia-Romagna (Italy)

Dal Prà A.[1], Valli L.[1], Davolio R.[1], Pacchioli M.T.[1], De Monte A.[2] and Scotti C.[2]
[1]Centro Ricerche Produzioni Animali – CRPA S.p.A., Viale Timavo 43/2, 42121 Reggio Emilia, Italy; [2]I.Ter, Via Zacconi 12, 40127 Bologna, Italy

Abstract

Permanent meadows (PM) are important for the production of Parmigiano Reggiano PDO cheese and support the agro-ecosystems functions (landscape, wildlife, recreation). The aim of this research was to evaluate (with soil-C models) whether and to what extent PM are able to sequester carbon in the soil and how much this C-stock can reduce the carbon footprint of milk production. Moreover, the floristic composition of the PM in the climate change scenario was assessed, as the forage quality and digestibility greatly affect enteric methane emissions from the cows. The study was performed on five dairy farms with PM located in Reggio Emilia. In each farm, the floristic composition of the first, third and fourth cut was evaluated for three sites showing that the forage yield and quality is conditioned by intensity of mowing as well as by the extreme events due to climate change. The simulation of soil carbon dynamics has shown that PM is a carbon sink and that it can mitigate the carbon footprint of milk by 5-25%, depending on soil properties and agricultural practices.

Keywords: grassland, species richness, climate change scenario, LCA, dairy cattle

Introduction

Permanent meadows (PM) located in the provinces of Parma and Reggio Emilia (Northern Italy) are important for the production of Parmigiano Reggiano PDO cheese and support the agro-ecosystems functions (landscape, wildlife, recreation). The management of the PM – never ploughed and regularly applications of cattle slurry and farmyard manure – should be able to preserve over time the organic matter content of the soil. One of the objectives of the project was to assess how the carbon stock of the soil was able to counterbalance greenhouse gases (GHG) emissions from the dairy cows, quantified with a life cycle assessment (LCA) approach. Moreover, the management and the floristic composition of the PM were investigated in order to assess if the environmental changes related to the climate crisis affect the forage quality.

Materials and methods

The carbon footprint (CFP) of the milk produced in the five dairy farms was assessed, taking as a functional unit 1 kg FPCM (fat and protein corrected milk) and the cradle-to-gate system boundaries. The GHG emissions, both those of the farm (enteric emissions, manure management, energy use, cultivation of the forages), and those induced by the production of the technical inputs used in the farm (grain and concentrate feed, mineral fertilisers, fuel, electric energy, etc.) were included. The methane and nitrous oxide emissions were assessed according to IPCC 2006 methodology (IPCC, 2006) and the values of the GHG emissions for manufacturing of the farm inputs were taken from the Ecoinvent database (https://www.ecoinvent.org/database/introduction-to-ecoinvent-3/introduction-to-ecoinvent-version-3.html). To assess the soil carbon dynamic in the PM of the five farms, two of the most popular international soil-C models (Roth-C and DNDC) were used.

Analysis of the floristic composition was carried out using the Daget-Poissonet method (1971), on three PM for each farm, with three 15 m transects for each PM, 15 points for each transect, 2 years of investigation, for a total of 270 points for each farm. Data were analysed by ANOVA and Tukey's range test of SPSS (V26), with farm as main effect.

Results and discussion

The carbon footprint of 1 kg of FPCM for the five investigated farms was, on average 1.3 kgCO_2-eq (kg FPCM)$^{-1}$ (Figure 1). The major share of the overall emissions was the enteric methane emissions, which on average contribute 43% of the total emissions. These emissions are strongly influenced by the digestibility of the animals' diet, in particular the quality of the forage, which is also influenced by its botanical composition. The second most important share of emissions was associated with production of the purchased feed (25%), and the third was from emissions of methane (CH_4) and nitrous oxide (N_2O) from the manure management (16% overall). The results are in agreement with most studies that report the carbon footprint of milk in the range of 0.9-1.4 kg CO_2-eq kg milk (De Vries and De Boer, 2010).

The simulation of the carbon sequestration potential of the PM made by the two C-dynamic models demonstrated that the PM was able to increase the soil organic matter, increasing the carbon sequestration. The extent of this C-accumulation was conditioned, above all, on the contribution of organic matter inputs, both of the livestock manure and of the crop residues. According to the models, values ranged from 0.7 to 10 Mg C ha^{-1} y^{-1} (that is 0.05-0.36 kg CO_2-eq kg FPCM^{-1}) achieving a reduction of the milk's carbon footprint by 5-25%, depending on soil properties and agricultural practices.

In the botanical analysis of PM, 62 species (Table 1) were recorded. *Lolium multiflorum* L., 1799 was the most frequent species, followed by *Trifolium repens*, L. 1753, *Convolvulus arvensis* L., 1753 and *Taraxacum officinale*, Weber ex F.H.Wigg. 1780. Herbage composition showed a prevalence of '*Poaceae*', except for farm A, where there were more 'Other species', Farms D and E, which recorded the highest cuts, also showed a high presence of '*Fabaceae*' The volume of irrigation water is the main factor that limits forage yield, while botanical composition is conditioned by many factors like intensity of mowing, nitrogen rates, harvesting time, soil compaction. The reduction of the cutting frequency favours the species ('Other species') of limited fodder interest. Compared to a previous study in the same area (Dall'Olio and Mantovi, 2008), the floristic composition of PM shows a lower number of species and an increase of drought and heat wave stress-tolerant plants.

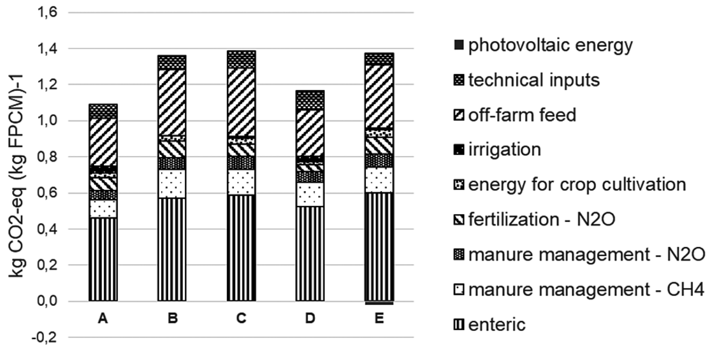

Figure 1. Carbon footprint of milk produced by the farms in Emilia-Romagna region, Italy. Based on life cycle assessment (LCA).

Table 1. Characterisation of farms and herbage botanical composition.[1]

Item	Farm					SE	*P*-value
	A	B	C	D	E		
Soil	Silty clay loam	Clay loam	Silty clay loam	Silty clay loam	Clay loam	-	-
Irrigation							
Irrigation method	Flood	Flood	Flood	Flood	Flood	-	-
Irrigation volume (m^3 ha^{-1})	4.955^a	4.825^a	11.041^c	9.376^b	13.785^d	552	<0.001
Irrigation (number)	3.0^a	2.5^a	6.0^b	5.5^b	8.5^c	0.203	<0.001
Management							
Cuts (number)	4.0^a	3.5^a	5.0^b	5.5^b	5.5^b	0.039	<0.001
Yield (Mg DM ha^{-1})	10.4^b	7.4^a	17.2^c	15.5^c	24.1^d	0.627	<0.001
Fabaceae (%)	7.2^a	1.1^a	20.3^b	8.1^a	17.8^b	2.586	<0.001
Poaceae (%)	39.8^a	51.2^a	57.4^b	54.5^b	61.1^b	4.13	<0.001
Other species (%)	53.0^c	47.7^b	22.3^a	37.4^b	21.1^a	4.179	<0.001
Main botanical composition							
Lolium multiflorum (%)	8.17^{ab}	15.17^b	6.28^a	13.0^{ab}	8.72^{ab}	3.12	0.032
Trifolium repens (%)	5.33^{ab}	0.39^a	12.67^c	6.94^b	12.39^c	1.908	<0.001
Convolvulus arvensis (%)	7.94^{ab}	12.72^b	3.0^a	6.11^a	4.56^a	2.028	<0.001
Taraxacum officinale (%)	9.44^{cd}	1.44^a	7.11^{bc}	11.50^d	3.67^{ab}	1.451	<0.001

[1] Between column different letters indicate significant difference at $P<0.05$. SE = standard error; DM = dry matter.

Conclusions

Agricultural activities are not only a source of greenhouse gases, but they can also contribute to counteract the causes of climate change, as when they are able to increase soil organic matter content. This is the case of PM used to feed the dairy cows in the Parmigiano-Reggiano PDO cheese area. Such grasslands do not require soil cultivation and receive the organic input of the livestock manure. The floristic composition of PM, which affects forage quality and, consequently, cattle enteric methane emissions, is also conditioned by climate change and agricultural practices.

Acknowledgements

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References

Daget P. and Poissonet T. (1971) Un mèthode d'analyse phytologique des praires. *Annales Agronomiques* 22, 5-41.

Dall'Olio N. and Mantovi P. (2008) Prati con graminacee a tutela delle acque. *Agricoltura*, 89-91.

De Vries M. and De Boer I.J.M. (2010) Comparing environmental impacts for livestock products: a review of life cycle assessments. *Livestock Science* 128, 1-11.

IPCC (2006) IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme, IGES, Japan.

Effect of protein level in the diet of dairy cows on the slurry composition to be used as fertiliser

Elouadaf D., Martínez-Fernández A., Soldado A. and Vicente F.
Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), P.O. Box 13, 33300 Villaviciosa (Asturias), Spain

Abstract

Less than 30% of the nitrogen (N) ingested by dairy cattle is retained in milk. Large amounts of N are excreted. In the slurry this has potential environmental impact if it is applied incorrectly. The objective of this work was to evaluate effects of protein reduction in the diet on the nitrogen balance of dairy cows and to improve the quality of slurry as a co-product that can be used as fertiliser. Six Holstein cows were divided among three isoenergetic diets but with different protein levels (17, 15 and 13%) in a double 3×3 Latin square model. Dry matter intake, milk production and composition were not affected by protein level. The excretion of total N in faeces was not affected by protein content in the diet. However, ammonia-N in the urine was almost four times lower in the diet with the lowest protein level, and total-N excreted in the urine was also significantly lower in the 13% treatment. 33% of phosphorus and 21% of potassium was excreted in the faeces, while 0.16% of phosphorus and 25% of potassium were excreted in the urine. In conclusion, the diet containing 13% of protein meets the protein requirement for lactating cows producing 31 kg day^{-1}, with less nitrogen excreted in the urine.

Keywords: legumes, protein level, milk, nitrogen excretion

Introduction

The evolution of agriculture in the last 75 years has been marked by a high fertilisation with nitrogen (N), a determining factor in the agricultural productivity increase. Livestock transform ingested vegetable N into animal products, eliminating the unused part in the form of excreta rich in N. Dairy cattle use up to 25-35% of the dietary N for milk synthesis, while the remainder is excreted in faeces and urine (Wanapat *et al.*, 2009). As a consequence, the development of livestock farming has led to a concentration of excreted N in the environment when the slurry has been mismanaged. Ruminants have a low efficiency of N utilisation. Diets with different protein concentrations have similar N faecal excretion but differ in urinary N excretion (Edouard *et al.*, 2016). Urinary N is the result of the catabolism of body proteins and the surplus of dietary N neither used by the ruminal microbiota nor by the animal after its intestinal absorption (Calsamiglia *et al.*, 2010). The objective was to evaluate the effect of different protein levels in the ration of dairy cows on the quality of slurries used as fertiliser (N-P-K) without affecting milk production and quality.

Materials and methods

Six Holstein dairy cows with 677±37.3 kg live weight, and 35.4±6.38 kg milk per day in the first third of lactation were selected and distributed within a replicated 3×3 Latin square design. Treatments consisted in three isoenergetic diets (9.63 MJ metabolizable energy (ME) kg^{-1} dry matter (DM)) with three crude protein (CP) levels: (1) High protein (HP: 170 g CP kg^{-1} DM); (2) Medium protein (MP: 150 g CP kg^{-1} DM); and (3) Low protein (LP: 130 g CP kg^{-1} DM). The evaluation time was 42 days, divided into three periods with 10 initial days of adaptation to diet, and four sampling days. Research on animals was conducted in accordance to the European Union Animal Welfare Directive Number 2010/63/EU with approved by Research Ethics Committee of the University of Oviedo (Ref. PRONAE 26-2018). The animals were housed singly in pens and given access to drinking water and feeding *ad libitum*. The cows were milked twice daily with an interval of 12 hours. Individual dry matter intake and milk production were recorded daily. Feed samples were taken daily during sampling days in each experimental period. Faeces were also collected daily from the collector box and a proportionate aliquot taken from each animal to get a composite sample for each animal and period. Urine was collected daily in 1 litre of a sulphuric acid solution (10% v/v) by means of external separators stuck on the vulva. Daily samples were pooled on individual animal bases, after recording the weight and the specific gravity. Milk was sampled from each animal the second and fourth sampling day. Morning and evening samples from individual cows were mixed according to its milk yield to get a representative sample by day and cow. Feed samples were dried (60 °C, 24 h), milled (0.75 mm), and analysed by NIRS. Total N and ammonia N contents of faeces and urine were determined by the Kjeldahl method. Potassium was determined by AA spectrophotometry, and phosphorus by UV-VIS spectrophotometry in all samples. Milk samples were analysed by MilkoScan. Results were contrasted by analysis of variance using a mixed model considering diet and period as fixed effects and cow as a random effect with R (R Core Team, 2017).

Results and discussion

The daily average dry matter intake was 20.7, 20.9 and 19.7 kg d^{-1} for HP, MP and LP diets respectively, with no differences among treatments. Milk production and composition also did not show significant differences, with 31.9±2.57 kg d^{-1}, 41.7±2.37 g fat kg^{-1} and 29.4±0.38 g protein kg^{-1}. Table 1 shows the daily faecal and urinary excretions of N-P-K.

For an average daily excretion of 9.03±0.59 kg DM of faeces, the excretions of total N and N-NH_3 did not differ significantly (P>0.05) with the protein level in the diet, with 230, 219 and 203 g N d^{-1} and 18.2, 13.8 and 16.0 g N-NH_3 d^{-1} for high, medium and low protein diets respectively. Dairy cows had an average intake of 150 g P d^{-1}, substantially greater than the approximate rate of 55 g P d^{-1} reported by NRC (2001) as required by lactating dairy cows with similar yield. They excreted 48.98 g P d^{-1} in faeces, which implies that 33% of the phosphorus ingested was excreted in the faeces. Likewise, the cows ingested 3.77 g K d^{-1}, which was three times requirements (NRC, 2001), and excreted 21% in faeces. The dietary protein level affected significantly the excretion of total-N in the urine, with lower total-N excreted in urine when the cows were fed with the LP treatment (P<0.05). When the level of protein in the diet was increased, the excretion of N-NH_3 in urine was increased (P<0.05). This could mean greater ammonia emission with high protein diets. There were no differences in the excretion of P and K, with a mean of 0.24 g P d^{-1} and 0.96 g K d^{-1} respectively.

Table 1. Daily faecal and urinary excretions of N-P-K with three crude protein levels in diet: High protein (HP), Medium protein (MP), and Low protein (LP).[1]

		HP	MP	LP	SD	*P*-value
Faeces	Dry matter (kg d^{-1})	8.97	9.25	8.88	0.586	ns
	Nitrogen (g d^{-1})	229.58	219.36	203.19	14.231	ns
	N-NH_3 (g d^{-1})	18.22	13.77	15.95	4.542	ns
	Phosphorus (g d^{-1})	49.11	53.10	44.72	4.144	ns
	Potassium (g d^{-1})	0.81	0.77	0.84	0.105	ns
Urine	Litres day^{-1}	25.21	24.35	23.76	1.256	ns
	Nitrogen (g d^{-1})	143.70^a	148.53^a	111.67^b	9.565	*
	N-NH_3 (g d^{-1})	2.66^a	1.85^{ab}	0.66^b	0.601	*
	Phosphorus (g d^{-1})	0.23	0.35	0.13	0.086	ns
	Potassium (g d^{-1})	0.99	0.94	0.94	0.077	ns

[1] Statistical analysis not performed for the chemical composition. SD = standard deviation; ns P>0.05; * P<0.05.

Protein concentration in milk is low for the protein levels in HP and MP treatments, both with similar energy content. In general, the first limitation to milk protein synthesis is the amount of metabolizable energy available (Walker *et al.*, 2004). However, all diets had an adequate level of energy. Thus, the low protein concentration of the milk of the HP and MP treatments might be due to greater intake of undegradable protein and, as a result, a decrease in the rate of microbial protein synthesis (Latham *et al.*, 1974), resulting in decreased rates of synthesis of milk protein (Sutton, 1989). The results confirm that a similar faecal nitrogen excretion is obtained with varying levels of crude protein (Edouard *et al.*, 2016). The nitrogen excreted in faeces is derived from non-digestible microbial protein, endogenous protein and non-digestible dietary proteins. Likewise, these authors reported that when the level of the protein in the diet increased, the N excreted in the urine also increased. But in our study, the differences between HP and MP treatments are too low. This might be because the crude protein intake in both treatments were similar (3.5 and 3.1 kg d^{-1}), while the LP treatment had low crude protein intake (2.5 kg d^{-1}) and, consequently it had lower urine N excretion. Phosphorus is excreted mainly in faeces; however, potassium excretion is mainly urinary and secondarily faecal (Meschy, 2010).

Conclusions

It is therefore concluded that the diet containing 130 g CP kg^{-1} DM meets the protein requirement for lactating cows producing 31 kg of milk per day. The additional nitrogen in higher crude protein treatments resulted in a substantial increase of $N\text{-}NH_3$ in the urine.

Acknowledgements

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References

Calsamiglia S., Ferret A., Reynolds C.K., Kristensen N.B. and Van Vuuren A.M. (2010) Strategies for optimizing nitrogen use by ruminants. *Animal* 4, 1184-1196.

Edouard N., Hassouna M., Robin P. and Faverdin P. (2016) Low degradable protein supply to increase nitrogen efficiency in lactating dairy cows and reduce environmental impacts at barn level. *Animal* 10, 212-220.

Latham M.J., Sutton J.D. and Sharpe M.E. (1974) Fermentation and microorganisms in the rumen and the content of fat in the milk of cows given low roughage rations. *Journal of Dairy Science* 57, 803-810.

Meschy F. (2010) Nutrition minérale des ruminants, Quae, Versailles, France, 208 pp.

NRC (2001) *Nutrient requirements of dairy cattle*, 7th revised edition, National Research Council, National Academy Press, Washington, DC, 381 pp.

R Core Team (2017) *R: a language and environment for statistical computing*. R Foundation for Statistical computing, Vienna, Austria.

Sutton J.D. (1989) Altering milk composition by feeding. *Journal of Dairy Science* 72, 2801-2814.

Walker G.P., Dunshea F.R. and Doyle P.T. (2004) Effects of nutrition and management on the production and composition of milk fat and protein: a review. *Australian Journal of Agricultural Research* 55, 1009-1028.

Wanapat M., Pilajun R. and Kongmun P. (2009) Ruminal ecology of swamp buffalo as influenced by dietary sources. *Animal Feed Science Technology* 151, 205-214.

Improvement of nutrient efficiency and energy use in grassland farms on marginal lands

Elsaesser M.[1], Hummler T.[1], Dentler J.[1], Kiefer L.[2] and Bahrs E.[2]
[1]*Agricultural Center Baden-Wuerttemberg (LAZBW), 88326 Aulendorf, Germany;* [2]*University Hohenheim, 70599 Stuttgart, Germany*

Abstract

South Germany envisages problems in maintaining grasslands on marginal land, which are partly in designated Nature 2000 areas, in scattered fruit tree orchards and on steep slopes in mountainous areas. Dairy farms as main users of grassland in such areas are diminishing. This study is part of an EIP project, the main objective of which was to identify factors that could improve the economic benefits and environmental sustainability in dairy farming. Therefore, the nutrient status and energy use of 15 grassland farms in such areas were investigated over a 3-year period (2014-2017). Nitrogen efficiency varied between 17 and 51%, whereas on unproductive land the value was even lower. Mean phosphorus efficiency was 110%, with the highest value on an organic farm being 242%. Such high values of P-efficiency lead to future soil infertility if P is not replaced. Global energy efficiency was on average 1.2 and varied between 0.3 and 2.1. Three out of 15 farms had a positive energy balance. Increases in N-efficiency, conserving C storage in soils and reduction of livestock are possible solutions to improve the sustainability of dairy farms on marginal lands.

Keywords: N-efficiency, P-efficiency, energy consumption, dairy farms, marginal land

Introduction

High nutrient efficiency and energy use are the main factors of an EIP-agri project for sustainable grassland use on marginal lands in Baden-Wuerttemberg South Germany (Bahrs *et al.*, 2019). Due to hilly landscapes, steep slopes and unfavourable climatic conditions, such as summer drought and severe winters, a big segment of the grassland in Baden-Wuerttemberg can be classified as marginal land (55% of the total grassland area = 246,476 ha). Moreover, existing Natura 2000 areas designation adds further management limits. The grassland yields here are low and of poor quality. However, the maintenance of these grasslands is of high importance in order to keep essential functions such as safeguarding against erosion, ground water protection and sustaining elevated biodiversity in the region (Thomet *et al.*, 2011). But these ecosystem functions vary dramatically depending on the intensity of grassland management (Schellberg and Pötsch, 2014). Although society is highly interested in the ecological values of grassland, the grassland areas are decreasing mainly in marginal regions (Elsäßer, 2018) due to changes in human nutrition, the economic situation of farms and low productivity. Objectives of this study were to identify the factors of economic and ecological sustainability on dairy farms producing in areas of marginal quality. Here the nutrient status and energy use situation of the project farms are addressed.

Materials and methods

In the EIP project 'Sustainable use of grasslands in marginal areas', scientists from University of Hohenheim and LAZBW Aulendorf, and some additional participants including 24 grassland farmers, established an operational group, located in the Black Forest region or in Swabian Alb. Farms are located at an altitude between 250 and 1,100 m asl. On average the farms had 51 milking cows, 1.3 ha forage area per cow and they feed 0.9 Mg concentrates per cow. Some 15 farmers (EIP1 to EIP13 are organic farms, EIP14 and 15 are conventional farms) took part in a more in-depth investigation on nutrient use and energy consumption over three years (2014-17). Data were collected and evaluated using the method ′farmgate balance′ and for energy consumption, the ′AgriClimateChange Tool′ of Solagro (2014).

Nutrient balances were calculated as difference between total input (fertiliser, concentrates, etc.) minus output (milk, meat, etc.). N- and P-efficiency is the relation between input and output.

Results and discussion

During the experimental period, we analysed nitrogen and phosphorus fluxes. The calculated nitrogen balances (Figure 1) showed that farms in these regions usually have low intensity management. Mean N-efficiency was 39.3% with a range of 17 to 51%. Low productive grassland swards are noteworthy here. In particular, farm `EIP6` consists of large unused areas with bushes, steep slopes and unproductive grassland (specified as *Nardetum*), which are responsible for this unfavourable situation. The very high P-efficiency in farm ´EIP12´ up to 242% (Figure 2) shows a situation often observed in organic farms where phosphorus fertilisation and use of concentrates are restricted. Eight farms showed negative P balances. This seems to be unsustainable, even if phosphorus mobilisation from the soil is taken into account. However, the P-efficiency was on average 110%.

The global energy efficiency of 15 farms was on average 1.2 (Figure 3), whereas the energy efficiency varied between 0.3 (farm EIP 6) and 2.1 (farm EIP 12). In total 12 farms showed values higher than 1, which is the target line. These farms produced more energy than they consumed. Average energy consumption was 15.25 GJ ha^{-1}. The main factors for energy consumption were fuel usage (28%), electricity (22%) and concentrates (21%). Carbon dioxide emissions were on average 182.9 Mg ha^{-1} a^{-1}. Only 14% of the CO_2 emissions were caused by the use and management of the soils. Reduction of greenhouse gas emissions is the main target for Germany. In order to reach these environmental objectives, a clear reduction of N-balances, the increase in N-efficiency, the sustaining carbon sequestration of the soils and the optimisation of grassland management and fertilisation should be targeted.

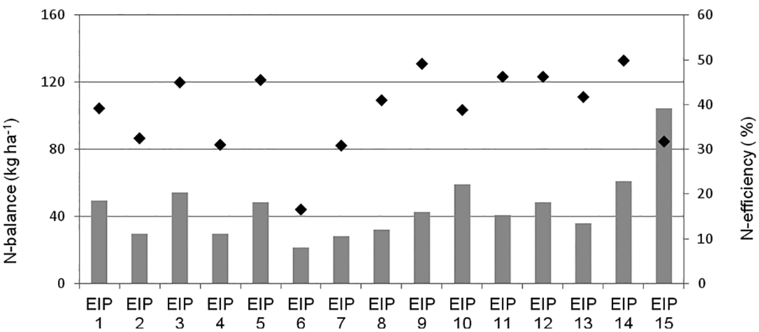

Figure 1. N-balances (bars) and N-efficiency (points) of 15 EIP-farms (average 2014-2017).

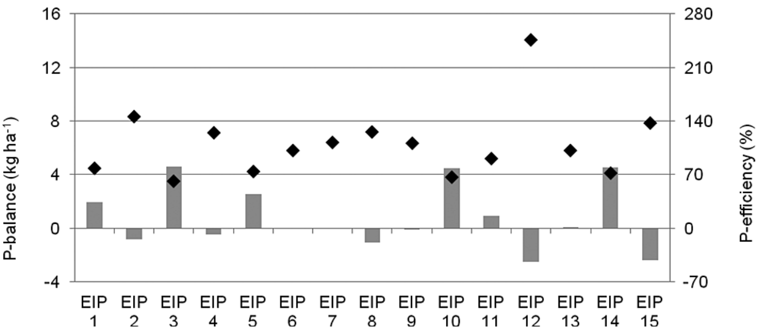

Figure 2. P-balances (bars) and P-efficiency (points) of 15 EIP-farms (average 2014-2017).

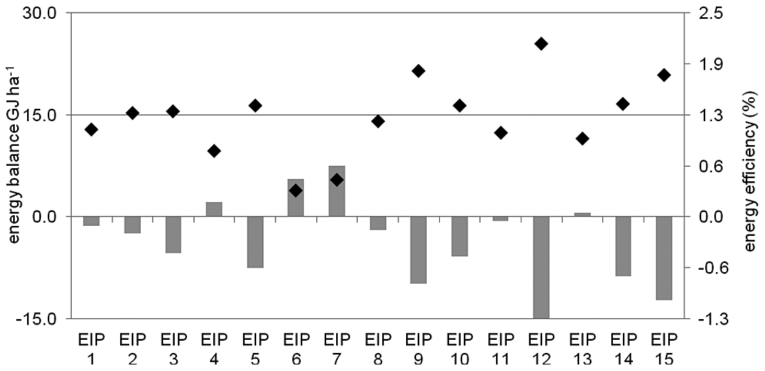

Figure 3. Energy balances (bars) and energy efficiency (points) of 15 EIP farms (average of 2014-2017).

Conclusions

Large differences in all investigated parameters show that even in regions of marginal grassland conditions, farms differ widely. The management intensity and level of fertilisation are the main factors for nutrient and energy efficiency. Analysing N and P fluxes on grassland farms would appear to be worthwhile on grassland farms, especially on organically managed farms. Moreover improvement of grassland swards if possible and permissions under nature protection restrictions can help to increase the nutrient balances and energy efficiency.

References

Bahrs E., Kiefer L., Dentler J., Hummler T. and Elsaesser M. (2018) *Nachhaltige Grünlandnutzung in ausgewählten Problemgebieten Baden-Württembergs.* Final Report EIP project, University Hohenheim.

Elsaesser M. (2018) Grünlandnutzung und Biodiversität – Synergien und Antagonismen. *Landwirtsch. Hochschultag Hohenheim, Landinfo,* 13-17.

Schellberg J. and Poetsch E. (2014) Multiple functions of organisms and their interaction – a different approach to multifunctionality of permanent grassland. *Tagungsband 58. Jahrestagung AG Grünland und Futterbau*, Arnstadt, pp. 20-30.

Solagro (2014) The agriclimate change tool (ACCT). Available at: https://extranet.solagro.org/ACCTool.

Thomet P., Cutullic E., Bisig W., Wuest C., Elsaesser M., Steinberger S. and Steinwidder A. (2011) Merits of full grazing systems as a sustainable and efficient milk production strategy. *Grassland Science in Europe* 16, 273-285.

Using legume-grass commercial seed mixes to improve pasture of dehesa farms: production, persistence and diversity

Fernández-Habas J., Hidalgo Fernández M., Leal Murillo J.R., García Moreno A. and Fernández-Rebollo P.

Department of Forestry, ETSIAM, University of Cordoba, Córdoba, Spain

Abstract

Pasture improvement using legume-grass commercial seed mixes is a common practice in dehesa farms to increase pasture productivity for livestock production. Normally, the introduction of these mixtures is carried out after previous tilling of the land and basal dressing, mainly with phosphorus. The persistence of the introduced species, and their effect on pasture diversity at plot scale, has not received much attention. This study aims to analyse the effectiveness of this approach to improve the productivity of the pasture and its effect on plant diversity. The study was carried out on two dehesa farms from the south of Spain devoted to beef cattle. Natural pasture and sown pasture with legume-grass commercial seed mixes sown in 2017 were sampled during spring of 2019 to assess: (1) persistence of introduced species, (2) biomass production and (3) plant diversity. Sown pasture showed more herbage yield than natural pasture at least two years following the sowing. Most of the introduced species were present after two years of seeding but their abundance was low. The sowing of pasture had little effect on native plant diversity.

Keywords: annual grassland, species composition, grazing, intensification, Spain

Introduction

The dehesa is a savanna-like ecosystem that covers 3 million hectares in the Iberian Peninsula. The main human activity in this ecosystem is livestock breeding (Fernández-Rebollo *et al.*, 2008). This relies heavily on grassland production (Porqueddu and González, 2011). In recent years, sowing of legume-grass commercial seeds by dehesa farmers has increased, with aims of improving grassland performance to sustain the livestock feeding. The seeds of the most of the available annual grassland species are selected and multiplied mainly in Australia. However, these cultivars have often been poorly adapted to the complexity of management systems and variable climatic conditions (Salis *et al.*, 2012). Sowing in the dehesa grassland might also have a negative effect on plant diversity, given that the relationship between production and diversity is hump-shaped, with richness first rising and then declining with increasing productivity (Fraser *et al.*, 2015). The objectives of this study were to: (1) assess the persistence of the sown species after two years; (2) assess the sowing effect on biomass production, and (3) compare the plant diversity of the sown and natural dehesa grasslands.

Materials and methods

The study was carried out in two dehesa farms devoted to beef cattle located in the north and north-west of Andalusia (Spain). This area has a typical Mediterranean climate with long and dry summers. The annual average rainfall is 784 and 560 mm at the first and second locations, respectively. Soils are acidic, with a loamy-clay soil texture at the first location and loamy-sandy at the second.

In autumn 2017, on each farm, a large field (between 20-30 ha) was sown with a mixture of seeds after previous tillage and fertilisation with 150 kg ha^{-1} of lime superphosphate (18% P_2O_5). The legume-grass seed mix was composed of: *Lolium multiflorum, Ornithopus sativus, Ornithopus compressus, Trifolium incarnatum, Trifolium michelianum, Trifolium resupinatum, Trifolium subterraneum,* and *Trifolium vesiculosum.* After sowing, the pasture was grazed at low intensity at the end of winter and during summer. Grazing was restricted during the spring to allow flowering and seed production. In the second year after sowing, sampling was carried out on the two improved fields and two adjacent natural pastures, all of them reserved for summer grazing. In each field (sown and natural pastures) from both farms, at the end of November, February and May, twelve sampled quadrats of 0.4×0.4 m, placed around three points, were clipped to assess pasture yield (DM). Sampled biomass was oven-dried at 60 °C for 48 h and then, weighed for dry matter determination. In the May sampling, the biomass was manually sorted into the different species before drying, to assess species composition as yield proportion. Diversity was computed through species richness (S) and Shannon-Wiener Index (H). Additionally, curves of species accumulation were computed in EstimateS software following the interpolation method proposed by Colwell *et al.* (2012). ANOVA was performed to compare pasture yield between fields (sown and natural pasture), considering sowing as a fixed factor, locations as a random factor and the interaction of both factors. Pearson correlations were performed to assess the relationship between yield and plant diversity.

Results and discussion

Sown pasture exhibited higher production than natural pasture in spring ($P<0.05$) (Figure 1). However, production reached similar levels in autumn and winter. Pasture herbage production (natural and sown) of the farm located in the more humid environment (higher precipitation and loamy-clay soils) was also higher. Most of the sown species were registered during the sampling, although their abundance, expressed as a percentage of biomass, was low. The sown species that persisted after two years were *T. subterraneum, T. vesiculosum, O. compressus, O. sativus, T. michelianum and L. multiflorum.* Sown species contributed 6% to the total DM of spring sampling, of which 3% were legumes and 3% grasses.

The average number of species was 53 in natural pasture, and 49 in sown pasture. Figure 2 shows species richness per type of pasture for each location (derived from curves of species accumulation for n=12). Natural pasture hosted more species than the sown pasture on the farm located in a more humid environment, whilst it was the opposite for the farm with lower precipitation and sandy soils. Concerning the H index, the mean value was 2.66 and 2.50 for natural and sown pasture respectively. In both locations, H index of natural pasture was higher than that of sown pasture. Furthermore, H index of pasture with most precipitation was lower. There was no significant correlation between production and diversity (Pearson coefficient R=-0.12 and -0.09 for H and S respectively). Nevertheless, in the location of lower rainfall, we found a positive significant correlation between S and DM (Pearson coefficient R=0.46).

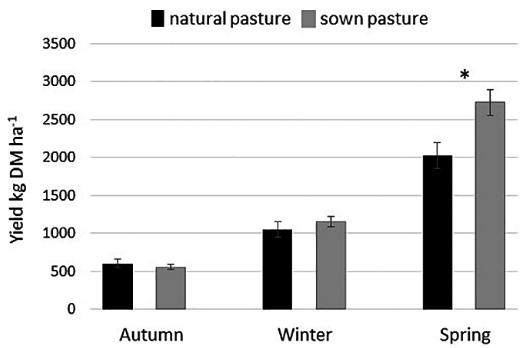

Figure 1. Dry matter (DM) of natural and sown pastures at each sampling date. An asterisk denotes a significant difference between natural and sown pastures ($P<0.05$).

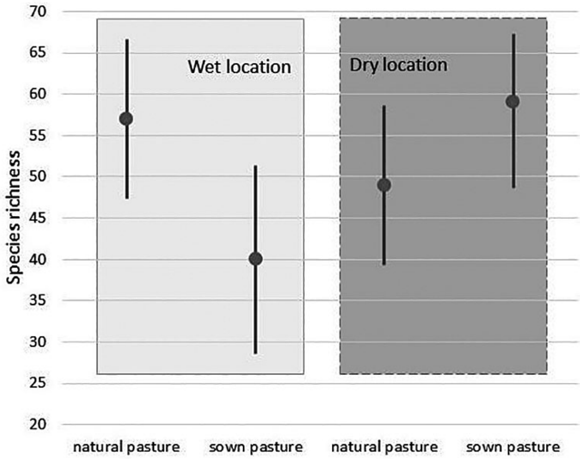

Figure 2. Mean of species richness of vascular plants at each location according to the type of pasture. Lines represent the 95% confidence interval.

Conclusions

The introduction of new pasture species in dehesa farms increased grassland production for at least two years following the sowing. Most of the introduced species were present after two years of seeding but their abundance was low. The sowing of pasture had little effect on native plant diversity. These results show that pasture improvement through the introduction of new species/varieties is possible in the dehesa system, with minimal impact on pasture diversity. However, it should be clarified whether similar increases in productivity can be obtained with other types of improvement, such as phosphoric fertilisation, which is less costly for farmers. More experimental work on farms is necessary in order to reach sound conclusions.

Acknowledgements

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References

Colwell R.K., Chao A., Gotelli N.J., Lin S.-Y., Mao C.X., Chazdon R.L. and Longino J.T. (2012) Models and estimators linking individual-based and sample-based rarefaction, extrapolation, and comparison of assemblages. *Journal of Plant Ecology* 5, 3-21

Fernandez-Rebollo P., Carbonero M.D. and Blazquez A. (2008) *The dehesa in the north of Cordoba. Future perspectives for its conservation*. Servicio de Publicaciones, Universidad de Cordoba, Cordoba, ES, pp. 443.

Fraser L.H., Pither J., Jentsch A., Sternberg M., Zobel M., Askarizadeh D. and Boldgiv B. (2015) Worldwide evidence of a unimodal relationship between productivity and plant species richness. *Science* 349, 302-305.

Olea L. and San Miguel-Ayanz A. (2006) The Spanish dehesa. A traditional Mediterranean silvopastoral system linking production and nature conservation. *Grassland Science in Europe* 11, 3-13.

Porqueddu C. and González F. (2011) Role and potential of annual pasture legumes in Mediterranean farming systems. *Pastos* 36, 125-142.

Salis L., Sitzia M., Vargiu M., Mulè P., Re G.A. and Sulas L. (2012) Adaptation of Australian self-reseeding forage legumes to three environments of Sardinia. *Options Mediterraneennes* 102, 265-269.

Drought resistance and water use efficiency of three genotypes of *Bituminaria bituminosa*: preliminary results

Fernández-Habas J.[1], Hidalgo-Fernández M.T.[1], Leal-Murillo J.R.[1], García-Moreno A.M.[1], Reina-Belmonte J.A.[1], Quero J.L.[1], Fernández-Rebollo P.[1], Vanwalleghem T.[2] and Mendez P.[3]

[1]Department of Forestry, School of Agricultural and Forestry Engineering, University of Córdoba, Campus de Rabanales, Edif Leonardo da Vinci, Crta. Madrid-Cádiz Km. 396, 14071 Córdoba, Spain; [2]Department of Agronomy, Hydraulic Engineering Area, School of Agricultural and Forestry Engineering, University of Cordoba, Campus de Rabanales, Edif. C4 Celestino Mutis, Ctra Madrid Km 396. 14071 Córdoba, Spain; [3]Canary Institute of Agronomy Research, La Laguna, Ctra. de El Boquerón, s/n, San Cristobal de La Laguna 38270 Valle Guerra, Tenerife, Spain

Abstract

The increase in temperature and reduction of rainfall due to climate change is threatening the provision of environmental services (ES) by permanent grasslands. This is especially serious in the Mediterranean Basin, where the higher temperatures and the lack of water in summer months together with the weather variability, compromise the provision of highly important ES such as pasture for livestock production. The use of drought tolerant legumes is one of the most promising approaches to tackle these threats. *Bituminaria bituminosa* (L.) Stirton, a perennial legume, is increasingly being investigated due to its potential to be productive for livestock in drier conditions. Genotypes from the Canary Islands have shown their suitability in farms in Australia because of their better palatability and considerable biomass production. This study aims to assess the drought resistance and water use efficiency of three genotypes of *Bituminaria bituminosa: B. bituminosa* var. *bituminosa,* var. *albomarginata* and var. *crassiuscula.* In a greenhouse study, the water use efficiency was compared under two different watering treatments through measurements of plant water use, instantaneous leaf water-use efficiency, and relative leaf water content. Var. *albomarginata* reported higher leaf relative water content than the other two genotypes. However, var. *bituminosa* and var. *crassiuscula* seem to perform better under conditions of high-water availability.

Keywords: *Bituminaria,* climate change, drought, permanent grasslands, Canary, water

Introduction

Tedera (*Bituminaria bituminosa* (L.) C.H. Stirton is a traditional forage resource in the Canary Islands and is promising fodder plant for other territories. Genotypes from the Canary Islands are adapted to the large diversity of environments from these Islands, with annual rainfall varying from 150 up to 600 mm (Méndez *et al.,* 2006). These genotypes have been used as fodder for livestock by the farmers of the Canary Islands (Méndez and Fernández, 1990) and recently introduced in Australia given their good response to drought stress, especially the genotype var. *albomarginata* (Foster *et al.*, 2012, 2015; Oldham *et al.,* 2013).

Although significant advances have been made on selecting ancestors for breeding programmes (Real and Verbyla, 2010), further research is needed to understand the physiological adaptations of the three genotypes of *B. bituminosa* from the Canary Islands that could lead to their suitable use in the Mediterranean Basin as a fodder plant for livestock.

The objective of this study is to compare the drought resistance and water use efficiency of three genotypes from the Canary Islands.

Materials and methods

Three genotypes from wild populations of the Canary Islands (Spain) were compared; *B. bituminosa* var. *albomarginata* (Malpaso, Lanzarote), var. *crassiuscula* (Chavao-Cañado, Tenerife) and var. *bituminosa* (Tamarco, Tenerife). Plants were grown in seedlings trays and after 17 days transplanted to pots (diameter 16.8 cm, depth 37 cm and volume 6 L). The soil used to fill the pots was a mix of Gramosemi peat (Gramosemi GF-Anz./Verm from Gramoflor) and sand (9:1) with 2 g of 19-19-19 NPK fertiliser per pot. The experiment was randomised complete block design of two watering treatments [well-watered (WW) or drought-stressed (DS)] with six replicates. WW plants were watered every day with 50 ml (~2.3 l m^{-2}) for the first 47 days. Thereafter, when the plants had a higher demand for water, watering was changed to 75 ml (~3.4 l m^{-2}) for the rest of the experiment (16 days). To reduce watering ~50%, DS plants were watered every two days with the same amount of water and following the same planning of watering than WW plants. The experiment was conducted in a greenhouse from 1 April to 3 June 2019 when all plants were harvested. The temperature was set to 30°C/15°C (day/night).

Relative leaf water content (RWC) on fully expanded leaves was calculated as RWC (%) = (FW – DW) / (SFW – DW) × 100, where FW is fresh weight, DW is dry weight and SFW is saturated fresh weight. Net photosynthesis rate (A) and stomatal conductance (g_s) were measured in fully expanded leaves using a portable infrared CO_2 gas analyser (LiCor Li6400XT, Li-Cor, Inc., Lincoln, NE, USA) fitted with a 2 cm^2 leaf cuvette and PAR of 1000 μmol photon m^{-2} s^{-1}. Water-use efficiency (WUE) was derived from the ratio A / g_s. Four pots per genotype and treatment were weighed three times per week from 30 April to 29 May to estimate plant water evapotranspiration (ET). Total dry matter (DM) was measured at harvest time.

Differences among genotypes and treatment were tested by two-way ANOVAs. When differences were significant, comparison of means test (Tukey's test) was carried out.

Results and discussion

Total DM was significantly (P<0.05) reduced in DS plants. Var. *albomarginata* had the lowest DM production while differences in DM were not found between var. *bituminosa* and *var. crassiuscula* (Table 1). RWC was not significantly reduced in DS plants. However, var. *albomarginata* had higher RWC than the other genotypes. WUE did not show significant differences among treatments or genotypes. Both treatments showed similar ET until 13 May. Thereafter, WW plants had significant higher ET. Var. *albomarginata* reported significantly lower ET from 1 May to 13 May than the two other genotypes. From then on, when water content had decreased ~40% and ~32% in DS and WW respectively, all genotypes had the same ET pattern.

These results differ from previous studies where var. *albomarginata* was highly productive in drought stressed conditions (Martínez-Fernández *et al.*, 2010; Real and Kidd, 2012). However, these studies agree on var. *albomarginata* reporting high RWC for drought conditions (Foster *et al.*, 2015; Martínez-Fernández *et al.*, 2010, 2012). Initial soil moisture could have affected the early development of var. *albomarginata* leading to a lower DM of plants of var. *albomarginata,* which naturally grow in a very low rainfall environment (170 mm). However, var. *bituminosa* and var. *crassiuscula* might be less susceptible to this initial water content.

Conclusions

Var. *albomarginata* showed high RWC, which demonstrates its resistance to water stress. Var. *bituminosa* and var. *crassiuscula* showed lower resistance to water stress, although they could perform better than var. *albomarginata* under conditions of high-water availability.

Table 1. Mean values and standard error (se) of three genotypes of *Bituminaria bituminosa* under well-watered (WW) and drought stressed (DS) treatments.[1,2]

Genotype	Treatment	Dry mass (g)		RWC (%)		WUE (μmol CO_2 (mol H_2O)$^{-1}$)	
		Mean	SE	Mean	SE	Mean	SE
var. *albomarginata*	WW	8.7a	1.4	85.5a	1.5	105.6	11.3
	DS	4.2c	1.1	92.1a	1	104.9	21.4
var. *bituminosa*	WW	19.6b	1.9	83.5b	2.5	117.2	18
	DS	9.8d	1.8	77.3b	4.7	124.7	21.6
var. *crassiuscula*	WW	20.5b	2.2	81.9b	3.4	138.9	28.4
	DS	10.6d	0.5	74.8b	4.2	86.3	13.6

[1] Mean values not sharing a common letter differ significantly (P<0.05) according to Tukey's test.

[2] RWC = relative leaf water content; WUE = water use efficiency; SE = standard error.

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References

Foster K., Lambers H., Real D., Ramankutty P., Cawthray, G.R. and Ryan M.H. (2015) Drought resistance and recovery in mature *Bituminaria bituminosa* Var. *Albomarginata*. *Annals of Applied Biology* 166, 154-169. https://doi.org/10.1111/aab.12171.

Foster K., Ryan M.H., Real D., Ramankutty P. and Lambers H. (2012) Drought resistance at the seedling stage in the promising fodder plant tedera (*Bituminaria bituminosa* Var. *Albomarginata*). *Crop and Pasture Science* 63, 1034-1042. https://doi.org/10.1071/CP12216.

Martínez-Fernández D., Walker D.J., Romero P., Martínez-Ballesta M.C. and Correal E. (2012) The response of the leguminous fodder plant *Bituminaria bituminosa* to water stress. *Journal of Agronomy and Crop Science* 198, 442-451. https://doi.org/10.1111/j.1439-037X.2012.00515.x.

Martínez-Fernández D., Walker D.J., Romero P. and Correal E. (2010) The physiology of drought tolerance in tedera (*Bituminaria bituminosa*). *Options Méditerranéennes. Séries A. Mediterranean Seminars* 159, 155-159. http://om.ciheam.org/om/pdf/a92/00801235.pdf.

Méndez P., Fernández M. and Santos, A. (1990) Variedades de '*Bituminaria bituminosa*' (L.) Stirton (Leguminosae) en el archipiélago canario. Pastos. *Revista de La Sociedad Española Para El Estudio de Los Pastos* 2021, 157-166.

Méndez P., Santos A., Correal E. and Ríos S. (2006) Agronomic traits as forage crops of nineteen populations of *Bituminaria bituminosa*. In: *Proceedings of the 21st General Meeting of the European Grassland Federation, Badajoz, Spain. Grassland Science in Europe*, 11, 300-302.

Oldham C., Real D., Bailey H.J., Thomas D., Van Burgel A., Vercoe P., Correal E. and Rios S. (2013). Australian and Spanish scientists are collaborating in the domestication of tedera: young merino sheep grazing a monoculture of tedera in autumn showed preference for certain accessions but no signs of ill health. *Crop and Pasture Science* 64, 399-408. https://doi.org/10.1071/CP13059.

Real D. and Kidd D. (2012). Forage production of the drought tolerant Mediterranean forage legume tedera (*Bituminaria bituminosa* Var. *Albomarginata*) in the medium rainfall zone of South Western Australia as affected by plant density and cutting frequency. *Options Méditerranéennes. Séries A: Mediterranean Seminars* 102, 387-90. Available at: http://om.ciheam.org/om/pdf/a102/00006985.pdf.

Real D and Verbyla A.P. (2010). Maximizing genetic gains using a 'plant' model in the tedera (*Bituminaria bituminosa* Var. *Albomarginata* and Var. *Crassiuscula*) Breeding Program in Australia'. *Options Méditerranéennes. Séries A. Mediterranean Seminars* 9, 87-95. Available at: http://om.ciheam.org/om/pdf/a92/00801221.pdf.

Semi-natural grasslands in boreal Europe: the rise of a socioecological research agenda

Herzon I.[1,2], Raatikainen K.J.[3,4], Wehn S.[5,6], Rūsiņa S.[7], Helm A.[8], Cousins S.A.O.[9] and Rašomavičius V.[10]

[1]Department of Agricultural Sciences, P.O. Box 27, 00014 University of Helsinki, Finland; [2]HELSUS, P.O. 20, Box 65, 00014 University of Helsinki, Finland; [3]University of Jyväskylä, Department of Biological and Environmental Science, School of Resource Wisdom, University of Jyväskylä, Finland; [4]University of Turku, Department of Geography and Geology, Geography Section, Turku, Finland; [5]Norwegian institute of Bioeconomy Research, Norway; [6]Multiconsult, Norway; [7]Faculty of Geography and Earth Sciences, University of Latvia, Riga, Latvia; [8]Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia; [9]Department of Physical Geography, Stockholm University, Stockholm, Sweden; [10]Nature Research Centre, Institute of Botany, Vilnius, Lithuania

Abstract

The European continent holds substantial areas of semi-natural grasslands, among which are many endangered and species-rich habitats. Their continuation is dependent on some form of human activity, either for agricultural production or conservation, or both. We quantified the areas of semi-natural grasslands in the managed grasslands in the region. We reviewed literature across the region to compile evidence on research scope and directions in relation to such grasslands by aspects such as ecology, land use change, socioeconomics and others, explored drivers of the research agenda, and outlined future research needs. There are challenges in defining and quantifying semi-natural grassland habitats even across a restricted region. Agricultural and other policies had a clear impact on the research agenda in different countries. There are recent signs of a shift from classical ecological studies towards more multidisciplinary and integrated research. To address sufficiently the threats to semi-natural grasslands, political and research frameworks in the European Union should pay more attention to the socioecological context inherent in their management. When aiming at meeting future demands for grassland production, the many facets of grassland values to humanity and biodiversity should be considered.

Keywords: conservation, land-use statistics, literature review, low-input production

Introduction

The European continent holds substantial areas of semi-natural grasslands (SNG), defined as 'habitats inhabited by native and spontaneously colonizing species that are dependent on traditional management … to prevent invasion of woody species' (Westhoff, 1983). This management includes grazing, mowing, and the removal of trees and shrubs, but precludes ploughing, substantial fertilising, drainage or herbicide use. SNG are increasingly valued for such public goods as unique biodiversity, carbon sequestration, water retention, heritage, and reduced fire risks (Bengtsson *et al.*, 2019). Despite their relatively low productivity, they remain a key source of forage in High Nature Value farming systems in Europe. Here we focus on European Union (EU) countries within the boreal region, i.e. Sweden, Finland, Estonia, Latvia, and Lithuania, as well as Norway. The aims were: examining the current status of SNG within the managed grassland cover of the region, and exploring the development of research agendas in time and across the region.

Materials and methods

We retrieved data from national land-use registers and evaluations and Eurostat on covers of grassland types. Permanent grasslands are defined as those remaining in place for a minimum of five consecutive years. We further conducted a literature review based on searches in Scopus and Web of Science. We categorised the papers by relevant research aspects: (1) ecological, (2) agronomic (e.g. biomass quantity and quality, animal welfare and growth), (3) cultural (cultural values, traditional land use, heritage, artisanal products), (4) socioeconomic (profitability, motivations, challenges, opportunities, solutions), (5) political (subsidies, regulations), (6) functioning and regulating ecosystem services (pollination, flood management, soil, etc.), (7) land-use change, (8) techniques (for management or identification), (9) innovative land-use solutions (bioenergy, education, tourism etc.), and (10) restoration. In the review, we included all types of semi-natural habitats, including those not grassland in *sensu stricto*. These have a high share of shrubs, trees, and non-gramineous species, and include silvopastures, burned and/or grazed heaths, mowed mires and fens.

Results and discussion

There are challenges in defining and quantifying SNG even across a restricted region (e.g. including or excluding old arable fields). Comparable data on semi-natural habitats other than grasslands are particularly scarce because these are not always included in agricultural land use or receive agricultural subsidies. Bearing in mind somewhat different definitions, their coverages vary dramatically, from 4,000 hectares for all types in Lithuania to 60,000 ha of coastal heath and 4 million ha of grazed forest in Norway. Common definitions and periodic inventories, also using modern technology, are necessary in light of the critical importance of semi-natural habitats for conservation and their role in low-input livestock production.

Estonia and Sweden have the highest proportion of SNG in relation to total grassland cover (over 10%), while Finland has the lowest (ca. 3%). Finland and Sweden differ in their share of temporary grassland compared with the other countries (Figure 1). Strong national public policies (also before the EU accession) heavily subsidised the process of intensification of grassland-based production at the expense of their ecological values.

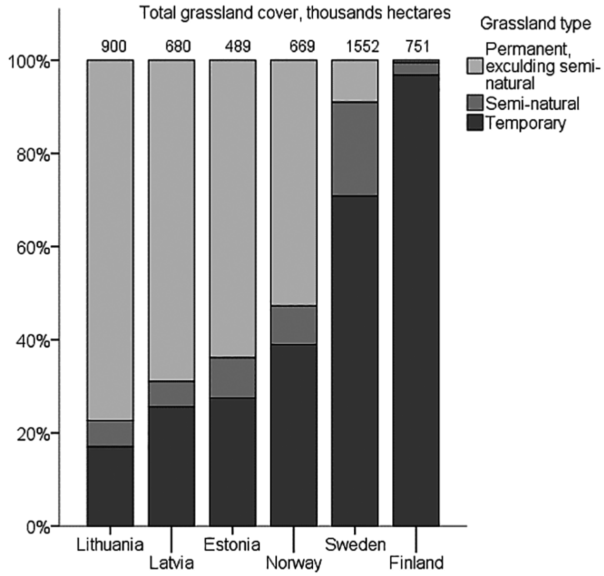

Figure 1. Relative areas for permanent (excluding semi-natural grassland), semi-natural, and temporary (rotational, five or less years on one field parcel) grasslands in the boreal countries of northern Europe. Only areas that are managed, either for production or as part of agricultural policy requirements, are included.

Of 1,009 papers identified in the literature search, 561 were relevant to our research topic. Of these, 43% were from Sweden, 18% from Norway, 17% from Finland, 15% from Estonia, 3% from Lithuania, and 1% from Latvia. The research focused most frequently on ecological aspects (51%), followed by management techniques or identification (15%) and land-use change (14%). Ecology and land-use change were nearly the only focus areas prior to 1996. After 1996, papers on management and restoration became common. After 2000, the number of publications doubled and research focus diversified into aspects with socioeconomic dimensions (e.g. policy and cultural ecosystem services) and other ecosystem services. Innovative ways of using SNG is the most recent research, the focus on socioeconomics especially expanded from a few studies per year in the early 2000s to nearly 20 in 2016.

Only the topics of ecology and land-use change were represented in all countries. Of research on production, ca. 60% were from Norway, followed by Sweden (15%). In Norway, strong national policies focusing on maintaining rural viability, with an emphasis on the positive links between food production and other public goods (biodiversity and food quality) stimulated this. The rise in research can be attributed to the Convention on Biological Diversity in 1993, integration of biodiversity objectives into national and EU legislation and agricultural subsidies, as well as the EU accession. Under policy influences research has expanded into the socioeconomic aspects of management and restoration due to public payments for SNG, and a political focus on ecosystem services. Research into farmers' acceptance of the payments, their role in the farm economy, and targeting the payments to deliver multiple benefits proliferated.

Conclusions

The national political framework seems to determine the remaining share of SNG in a country, while international policies and specific national priorities influence research directions. In order to address sufficiently the threats that face SNG, research should focus at the socioecological context inherent in SNG management. When aiming at meeting future demands for grassland production, the many facets of grassland values should be considered.

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References

Bengtsson J., Bullock J.M., Egoh B. *et al.* (2019) Grasslands – more important for ecosystem services than you might think. *Ecosphere* 10(2), e02582. https://doi.org/10.1002/ecs2.2582

Westhoff V. (1983) Man's attitude towards vegetation. In: Holzner W., Werger M.J.A. and Ikusima I. (eds.) *Man's impact on vegetation*. W. Junk, Den Haag, pp. 7-24.

The influence of management and fertilisation quality of leachate from foothill meadow

Kacorzyk P.[1], Białczyk B.[1] and Kasperczyk M.[2]

[1] *University of Agriculture in Krakow, al. Mickiewicza 21, 31-120 Krakow, Poland;* [2] *The Jan Grodek State Vocational Academy in Sanok, ul. A. Mickiewicza 21, 38-500 Sanok, Poland*

Abstract

The aim was to assess the impact of fertilisation in foothill meadow land dominated by *Holcus lanatus* L. on the quality of leachate. Vegetation formed under the influence of fertilisation had a significant impact on the chemical composition of water moving through the 0-20 cm soil layer. The lowest concentration of NO_3-N in leachate water was found in the first research period (intensive vegetation), and the highest in the third (after vegetation). Between treatments, NO_3-N concentration was the highest on the mineral fertilisation treatment and lowest on the control. The highest P concentration in leachate water was in the third research period at the treatment receiving mineral fertilisation. Low P concentrations were recorded in leachate water from the control meadow and the unused meadow. From the point of view of the environment, the best alternatives are not fertilised and mowed meadows.

Keywords: meadow, floristic composition, leachate waters

Introduction

Poland is poor in terms of its water resources, which are on average 3 times smaller than in other European Union countries. Reasons for the small amounts water resources in Poland are: low precipitation, a predominance of light soils with a small water holding capacity, and a small amount of retention reservoirs. A review of the literature shows that the development of quantitative and qualitative water resources is significantly affected by the way the basin is used (Kornaś and Grześkowiak, 2011; Śmietanka, 2014; Smoroń, 2010; Twardy, 2015). Grasslands, whose turf protects the soil against erosion, slow down the outflow of rainwater, and they retain significant amounts of nutrients, and have a particularly beneficial effect. The limitation of agricultural activity in Poland after the political changes in the 1990s resulted in changes in the management of water resources. This phenomenon was particularly visible in the foothill and mountainous areas. The aim of the study was to assess the impact of fertilisation in the foothill meadow land dominated by *Holcus lanatus* L. on the quality of leachate moving through a 0-20 cm thick soil layer. The study also revealed how the use of the meadow, or the lack of it, changes the quality of leachate. Understanding these relationships will contribute to conscious shaping of the soil cover and improvement of leachate water quality.

Materials and methods

The experiment was conducted in the years 2012-2015 in the foothill region. The soil texture (0-20 cm layer) of the experimental area was 57% sand (2-0.05 mm fraction), 33% silt (0.05-0.002 mm fraction) and 10% clay (<0.002 mm fraction). According to World reference base, the soil was classified as Haplic Cambisol (Dystric) (PTG, 2011).

Three research options were considered: control meadow (unfertilised and mowed), mowed meadow, which was fertilised with mineral fertilisers (phosphorus (P) 18 kg ha^{-1}, potassium (K) 50 kg ha^{-1} and nitrogen (N) 120 kg ha^{-1}), unused meadow (unfertilised, not mowed). Nitrogen fertilisation in the form of ammonium nitrate was applied on each fertilised plot in 3 replicates, in two proportions: 60% for the first regrowth and 40% for the second regrowth. Phosphorus in the form of superphosphate and potassium in the form of potassium salt were applied entirely at the beginning of growing season.

The floristic composition of the sward was assessed by the Klapp estimation method as presented by Peratoner and Poetsch (2019). It was calculated from fresh matter of an area of 25 m^2. Each plot had three lysimeters, placed at a depth of 0-20 cm. The surface area of each circular-shaped lysimeter (diameter 50 cm) was 1,952 cm^2. Water samples were taken after rainy days (4 samples per plot per period). Every time 10% of the total water amount was taken for the analysis. The chemical composition of water was assessed in three research periods: (1) from 1 April until 30 June (intensive growth), (2) from 1 July until 31 October (slow growth), and (3) from 1 November until 31 March (no-growth).

Concentrations of NO_3-N, and P in leachate (Table 1) are the average values from three replicates. The concentration changes were calculated using the standard deviation SD and coefficients of variation (V%). Variation is given for three lysimeters placed on each treatment plot.

Results and discussion

On the control treatment, the main species was *H. lanatus* L. and it constituted 48% of the meadow sward. *Arrhenatherum elatius* L., *Trisetum flavescens* Pers., *Festuca pratensis* Huds., *Dactylis glomerata* L. were found in smaller amounts. Dicotyledons constituted 13%. On the fertilised plot the dominant species were *A. elatius* L. (30%) and *D. glomerata* L. (18%). These changes occurred at the expense of *H. lanatus* L. The share of other species was at a similar level as on the control plot. On the unused meadow plot, *A. elatius* L., and *T. flavescens* Pers. became the dominant species during the experimental years accounting for over 50% of the fresh matter yield at the end the period. The share of dicotyledonous plants increased by 2% compared with the control treatment.

Vegetation formed under the influence of fertilisation seemed to have a statistically significant impact on the chemical composition of water moving through the 0-20 cm soil layer. The lowest concentration of NO_3-N in leachate water was found in the first period (intensive growth). This was due to the intensive nutrient uptake of the growing vegetation (Table 1). The highest nitrogen concentrations in lysimeter water were found during the third period (no-growth). Probably this was due to the lower uptake of vegetation, and a relatively large amount mineralized from organic matter. Sapek and Kalińska (2004) also found high mineralization of organic matter in the autumn. Between the treatments, NO_3-N concentration was highest on the meadow receiving mineral fertilisation, and lowest on the control. The highest phosphorus concentration in leachate water (0.77 mg l^{-1}) was found in the third period (no-growth) on the plot receiving mineral fertilisation. Low phosphorus concentrations in the leachate were

Table 1. NO_3-N and P concentration in leachate waters from unfertilised and mowed (control), mineral fertilised, and unused (fallow) meadows.[1]

Variant	Period	Concentration NO_3-N, mg·l^{-1}	SD[1]	V%[2]	Concentration P, mg·l^{-1}	SD	V%
Control	1	0.45	0.15	35.33	0.33	0.19	55.33
	2	2.05	0.71	34.89	0.43	0.29	67.35
	3	2.72	0.57	21.22	0.44	0.19	27.25
	Mean	1.74	-	-	0.40	-	-
Mineral $P_{18}K_{50}N_{120}$	1	0.50	0.16	32.21	0.45	0.07	15.36
	2	2.10	0.81	38.42	0.40	0.27	67.58
	3	5.35	0.75	13.92	0.77	0.57	73.64
	Mean	2.65	-	-	0.54	-	-
Fallow	1	0.33	0.14	40.00	0.26	0.17	61.25
	2	2.02	0.76	37.55	0.50	0.13	24.65
	3	4.33	1.08	25.00	0.27	0.06	21.31
	Mean	2.23	-	-	0.34	-	-

[1] SD = standard deviation; V% = variability coefficient.

measured from the control meadow in all periods and from the unused meadow during the intensive and no-growth periods.

The content of N and P in leachate was the result of a floristic composition shaped under the influence of fertilisation. The highest N and P concentrations in the leachate resulted from fertilisation with ammonium nitrate and superphosphate.

Conclusions

Based on this study of foothill meadow we conclude that the vegetation formed under the influence of fertilisation has a significant impact on the quality of leachate water. The plant development phase also has a significant impact on the amount of nutrients found in leachate waters. The lowest concentration was found in the period of intensive vegetation, and the highest in the non-vegetative period. From an environmental point of view, the best alternative is not fertilised and mowed (control) meadow.

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References

Kornaś M. and Grześkowiak A. (2011) Wpływ użytkowania zlewni na kształtowanie jakości wody w zbiornikach wodnych zlewni rzeki Drawa. *Woda – Środowisko – Obszary Wiejskie* 11, 1 (33), 125-137.

PTG (2011) *Systematyka gleb Polski.* Roczniki Gleboznawcze 62.

Peratoner G. and Poetsch E. (2019) Methods to describe the botanical composition of vegetation in grassland research. *Die Bodenkultur: Journal of Land Management, Food and Environment* 70, 1-18.

Sapek B. and Kalińska D. (2004) Mineralizacja organicznych związków azotu w glebie w świetle długoletnich doświadczeń łąkowych. *Woda – Środowisko – Obszary Wiejskie* 4, 1 (10), 183-200.

Smoroń S., Twardy S. and Kuźniar A. (2010) Bilans azotu i fosforu w rolniczych obszarach karpackich o niekorzystnych warunkach gospodarowania. *Woda – Środowisko – Obszary Wiejskie* 10, 4 (32), 225-236.

Śmietanka M. (2014) The influence of permanent grasslands on nitrate nitrogen loads in modelling approach. *Journal of Water and Land Development* 21, 63-70.

Twardy S., Kopacz M. and Kurnicki R. (2015) *Charakterystyka zlewni górnego Dunajca w aspekcie czynników środowiskowych determinujących zrównoważony i trwały rozwój*. Red. S. Twardy. Wyd. ITP, Falenty-Kraków, 129.

Nitrogen and phosphorus gate balances on Finnish pilot dairy farms

Kajava S. and Sairanen A.
Natural Resources Institute Finland (Luke), Maaninka, Finland

Abstract

The aim of this work was to study nitrogen (N) and phosphorus (P) nutrient farmgate balances on 12 Finnish pilot dairy farms: 9 conventional farms (CF) and 3 organic farms (OF). The results were based on yearly production balance: input N and P (purchased animals, fertilisers and feeds, and biological N fixation) – output N and P (sold milk, meat, animal and crop products, and manure). Most N and P imports to CF came from fertilisers and concentrates. The median N balance of CF was 118 kg ha^{-1} and of OF 49 kg ha^{-1} respectively. The N utilisation (output/input) of CF was 2% and of OF 32%. The median P balance of CF was 9.5 kg ha^{-1} and of OF 4.2 kg ha^{-1}. The P utilisation of CF was 46% and of OF 49%. Farmgate balances were most affected by animal density and production intensity, and balances would be lower in all dairy farms if nutrient cycling were more closed. The farmgate balance levels of Finnish pilot farms were similar to other Nordic regions, yet balance was lower compared with some other European countries. However, milk production per ha also remains quite low in Finland.

Keywords: nutrient gate balance, nitrogen, phosphorus, resource efficiency, dairy farming

Introduction

The optimisation of nitrogen (N) and phosphorus (P) nutrient cycles has positive impacts on both the environment and the farm economy: a low nutrient input reduces leaching, and optimal inputs maintain the farm economy. N and P farmgate balances (GB-N, GB-P) can be used as an indicator in dairy farm nutrient management and environmental performance (e.g. Oenema *et al.,* 2003). The GB calculates the difference between nutrient inputs and outputs at farm level. A positive balance illustrates total nutrient loss and/or nutrient sequestration. In this study, the main objective was to study the differences in the GB on 12 Finnish pilot dairy farms which participated in the EuroDairy project.

Materials and methods

The study's data were collected in 2016, 2017, and 2018. The dairy farms were in eastern Finland and Kainuu. The key figures describing the farms are represented in Table 1. The average size of the pilot farms was larger than the Finnish average. The results are therefore not entirely representative of Finnish milk production.

Table 1. Descriptive numbers of the dairy farm data used in the study (2016-2018).[1]

	Conventional farms (n=9)		Organic farms (n=3)[2]	
	Mean	SD	Mean	SD
Cropping area, ha	160	48	320	165
Dairy cows ha^{-1}	0.62	0.16	0.40	0.10
Animal density, animal units ha^{-1}	0.90	0.25	0.59	0.17
Milk yield, kg year	10,000	900	9,700	800
Milk, kg ha^{-1}	6,200	2,000	3,900	700
Concentrates, kg cow^{-1}	10.6	1.5	9.4	1.1

[1] SD = standard deviation.

[2] In these figures reported one pilot farm which fields were under organic production and animals under conventional production.

The farm GB was calculated yearly, starting each January. The nutrient inputs of N and P consisted of mineral or organic fertilisers, animal feeds, and purchased animals. The estimation of biological N fixation of legumes was based on the grass dry matter yields and proportion of leguminous plants used in cultivation. Stock variations were included in calculations. The nutrient outputs of N and P consisted of sold milk, meat, animal and crop products, and manure. The total farm GB and the efficiency of N and P utilisation were determined as the difference between nutrient inputs and outputs. Due to the small sample and variation between pilot farms structures, the results of GB-N and GB-P and nutrient utilisations are represented as medians.

Results and discussion

The median GB-N of conventional and organic farms was 118 and 49 kg N ha^{-1}, respectively, and N utilisation was 28 and 32% (Table 2). In earlier studies, the GB-N of Finnish dairy farms has averaged 119-120 kg ha^{-1} (e.g. Marttila, 2005; Virtanen and Nousiainen, 2005). The efficiency of N utilisation was also at the same level as in previous experiments. The average N input of conventional farms was 163 kg ha^{-1}, and 50% of N input came from mineral fertilisers, and 38% from purchased concentrates. Organic farms had the largest amount of N accumulation through biological N fixation (50%), and the second highest amount was through purchased feed (45%).

The GB-P of conventional and organic farms was 9.5 kg P ha^{-1} and 4.2 kg P ha^{-1} respectively, and the efficiency of P utilisation was 46 and 49%. The previous GB-P of Finnish dairy farms has averaged 12-15 kg ha^{-1} (e.g. Marttila, 2005; Virtanen and Nousiainen, 2005; Nousiainen, 2011). The efficiency of P utilisation was also at the same level as in previous studies. The average P input of conventional farms was 18.6 kg ha^{-1}, and 79% of P input came from purchased concentrates, and 15% from mineral fertilisers. Organic farms also had the largest amount of input P from concentrates (87%).

GB-N and GB-P were mostly affected by animal density and production intensity (see also Virtanen and Nousiainen, 2005; Huhtanen *et al.,* 2011), which also explains the GB differences between organic and conventional farms. Typically, organic farms have a low animal/field ratio (Table 1). The nutrient input per animal is instead fixed at farm level, so the increase of the field area per animal results in a reduction in the GB. In practice, there are relatively few opportunities to adjust the GB on a farm, because the availability of the field area is typically a limiting factor in production. The GB would be lower on dairy farms if the self-sufficiency of feed production were higher. However, imported grain is often a more profitable alternative compared with home-grown grain due to the Finnish field-oriented subsidy policy. Thus, both the field availability and economy are potential reasons for a high GB. In this study, the average proportion of home-grown concentrates in dairy cows' nutrition on conventional farms was 21.6% (range 0-45%).

Compared with previous studies in Finland, with lower dairy production intensity (Virtanen and Nousiainen, 2005; Nousiainen 2011), the GB-N was at a similar level, and the GB-P was lower. This suggests some development in the efficiency of dairy farms' nutrient utilisation; improvements in P utilisation can be a consequence of reduced P fertilisation. Compared with other European countries,

Table 2. N and P balance (median) and use efficiency (%) on Finnish pilot dairy farms.

	N balance, kg N ha^{-1}	N use efficiency, (%)	P balance, kg P ha^{-1}	P use efficiency, (%)
Conventional dairy farms[1]	118	28	9.5	46
Organic dairy farms[2]	49	32	4.2	49

[1] n=9; median of three years of five farms, median of two years of four farms.

[2] n=3; median of three years of two farms, median of two years of one farm.

the GB of Finnish pilot farms was similar to other Nordic countries (e.g. Swensson 2003) but lower compared with other European regions with intensive dairy production (Nielsen and Kristensen, 2005; Raison *et al.,* 2006). However, although milk production intensity has increased in Finland, it remains quite low compared with that of many other countries. Additionally, the nutrient utilisation levels of N and P are often lower.

Conclusions

GB-N of the pilot farms was at the same level, and the GB-P was lower compared with previous studies reported in Finland. An improved P balance can be a consequence of reduced P fertilisation. Animal density and the self-sufficiency of feed production are the most important explanations for the differences between farms' GB. The highest GB was determined on the farm where environmental legislation limited the maximal use of slurry. The GB of the Finnish pilot farms were similar to other Nordic countries, but lower compared with other European regions with intensive dairy production.

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References

Huhtanen P., Nousiainen J. and Turtola E. (2011) Dairy farm nutrient management model: 2. Evaluation of different strategies to mitigate phosphorus surplus. *Agricultural Systems* 104, 383-391.

Marttila J. 2005. *Ravinnetaseet maatalouden vesistökuormituksen arviointikeinona*. Pro gradu-tutkielma. 69 s. + liitteet. Helsingin yliopisto.

Nielsen A.H. and Kristensen I.S. (2005) Nitrogen and phosphorus surpluses on Danish dairy and pig farms in relation to farm characteristics. *Livestock Production Science* 96, 97-107.

Nousiainen J., Tuori M., Turtola E. and Huhtanen P. (2011) Dairy farm nutrient management model. 1. Model description and validation. *Agricultural Systems* 104, 371-382.

Oenema O., Kros H. and De Vries W. (2003) Approaches and uncertainties in nutrient budgets: implications for nutrient management and environmental policies. *European Journal of Agronomy* 20, 3-16.

Raison C., Pflimilin A. and Le Gall, A. (2006) Optimisation of environmental practices in a network of dairy farms in the Atlantic area. In: Jarvis S. and Pfimlin A. (eds.) *Green dairy project: interreg atlantic area IIIB N°100. Proceedings of the Final Seminar*, Rennes, France, 13-14 December 2006, Rennes, France: Institut de l'Élevage. pp. 43-65.

Swensson C. (2003) Analyses of mineral element balances between 1997 and 1999 from dairy farms in the south of Sweden. *European Journal of Agronomy* 20: 63-69.

Virtanen H. and Nousiainen J. (2005) Nitrogen and phosphorus balances on Finnish dairy farms. *Agricultural and Food Science* 14, 166-180.

Sward layer and responses to 14 years of grazing and defoliation management

Kassahun T.[1], Pavlů K.[1], Nwaogu C.[1], Pavlů L.[2], Gaisler J.[2] and Pavlů V.[1,2]
[1]Department of Ecology, Czech University of Life Sciences Prague, 165 21, Czech Republic; [2]Department of Weeds and Vegetation of Agroecosystems, Crop Research Institute, 460 01, Czech Republic

Abstract

Response of different plant functional groups of two sward layers subjected to different grazing intensity and defoliation management was studied at the Oldřichov grazing experiment, in the northern part of the Czech Republic. The study was based on two randomised block experiment with the following treatments: (1) extensive grazing; (2) intensive grazing; (3) cutting in June followed by extensive grazing; (4) cutting in June followed by intensive grazing; and (5) unmanaged control. Two sward layers, bottom (0-3 cm) and upper (>3 cm), of vertical structure were identified from samples collected each year from 1998 to 2012. The results indicated grazing intensity had significant effects on the total biomass as well as on the upper sward layer of all functional groups except for mosses. A greater proportion of living biomass in the unmanaged treatment was allocated in the upper layer than in managed treatments. Overall, graminoids had the highest amount at both layers compared to forbs, mosses and legumes in the 14-year period. The introduction of management, coupled with the successional development, had affected the swards at both layers.

Keywords: vertical structure, functional groups, grazing, cutting

Introduction

Grassland managers often consider grazing as one of the most important management tools for manipulating the structural variables in vegetation. For instance, the proportion of flowers, stems and dead material in the different horizons (vertical structure) are one of the main components that are open to manipulation (Tallowin *et al.,* 2005). Few studies have been conducted in temperate regions describing the vertical structure of swards under different management in homogeneous swards. The aim of the study is to investigate the impact of different grazing and defoliation management of different functional groups at two vertical layers (<3 cm and >3 cm) in Czech upland grasslands.

Materials and methods

The study was carried out at the Oldřichov grazing experiment located in the Jizera Mountains in the northern part of the Czech Republic, in Oldrichov v Hájích village, at 420 m a.s.l. Different management regimes were applied in each paddock. The treatments were: (1) extensive grazing (EG), where the stocking rate (SR) was adjusted to achieve a mean target sward surface height of greater than 10 cm; (2) intensive grazing (IG), in which SR was adjusted to achieve a mean target sward surface height of less than 5 cm; (3) cutting in June followed by extensive grazing (ECG) for the rest of the growing season; (4) cutting in June followed by intensive grazing for the rest of the growing season (ICG) and (5) unmanaged control (U). The mean stocking rates were approximately 500 kg ha^{-1} (2 heifers) and 1000 kg ha^{-1} (4 heifers) for EG and IG treatments, respectively. In early May each year from 2001-2012, six samples from a 50×25 cm steel frame were randomly collected by electric shears at ground level in each paddock. Sward layers of vertical structure of vegetation stand were discerned in each sample: 0-3 cm and >3 cm. The height 3 cm and more represented a vegetation layer that is grazed by heifers. Samples were then sorted into graminoids, forbs, mosses, legumes, dead material in each layer, subsequently dried for 48 h at 70 °C, and weighed. The effect of the treatments on total biomass of each functional group and their ratios was analysed using General Linear Models (GLMs).

Results and discussion

The treatments had significant effects on total biomass of all functional groups and vertical structure of most groups except for legumes and mosses (Table 1; Figure 1). After 14 years of grazing and defoliation management at our experiment site, a clear pattern was seen with both IG and ICG treatments having a significant positive effect on total biomass of legumes, while dead biomass, forbs and graminoids were largely supported in ECG and EG.

Table 1. Result of General Linear Model on the effect of treatment on total biomass and ratio of biomass in the upper layer (>3 cm) to total biomass for all functional groups.[1,2]

Total biomass	***Df***	***F***	***P*-value**	**IG**	**ICG**	**EG**	**ECG**	**U**
Graminoid	44.0	6.51	<0.001	bc	bc	a	b	c
Forb	44.0	10.69	<0.001	d	c	c	a	bc
Legume	44.0	63.74	<0.001	a	a	c	b	d
Living	44.0	3.6	0.012	c	bc	ab	a	bc
Dead	44.0	113.96	<0.001	d	d	b	c	a
Moss	44.0	7.2	<0.001	b	b	c	a	bc
>3 cm/Total biomass	***Df***	***F***	***P*-value**	**IG**	**ICG**	**EG**	**ECG**	**U**
Graminoid	44.0	9.82	<0.001	c	c	b	b	a
Forb	44.0	19.41	<0.001	c	c	ab	b	a
Legume	46.1	1.15	0.344	-	-	-	-	-
Living	44.0	15.95	<0.001	c	c	b	b	a
Dead	44.0	6.97	<0.001	b	b	a	a	b
Moss	44.9	0.94	0.448	-	-	-	-	-

[1] Significant differences between treatments in Tukey test are indicated by different lower-case letters (alphabetic order represent decreasing values of means, i.e. 'a' represents the largest mean).

[2] EG = extensive grazing; IG = intensive grazing; ECG cutting in June followed by extensive grazing for the rest of the growing season; ICG = cutting in June followed by intensive grazing for the rest of the growing season; U = unmanaged control.

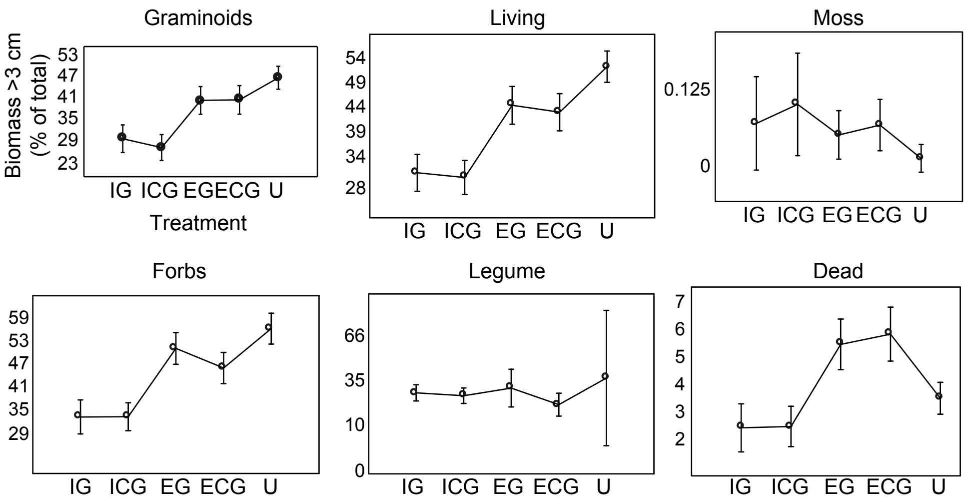

Figure 1. The effect of grazing intensity and defoliation management on the ratios of upper layer (>3 cm) to total biomass of each functional group. Error bars indicate confidence intervals. See General Linear Models results in Table 1.

Graminoids had the highest dry matter standing biomass throughout the study period, compared with forbs, legumes and mosses at both layers (Figure 2). One possible explanation could be that grasses in general are superior competitors and they may suppress other groups (Del-Val and Crawley, 2005). A high proportion of living biomass was revealed in the upper layer while large amount of dead biomass occurred in the bottom layer (Figure 2). As living biomass gradually reaches its ageing stage, more living biomass will be found in the upper layer and the dead biomass accumulates in the bottom layer.

The higher allocation of dead material to the upper layer in extensive grazing (Table 1) with taller sward was in agreement with Wright and Whyte (1989), who found a higher proportion of dead material with increasing sward height, as well as with Bircham and Hodgson (1983), who identified higher rates of senescence in tall swards, which is typical under extensive grazing management and also for ungrazed plots.

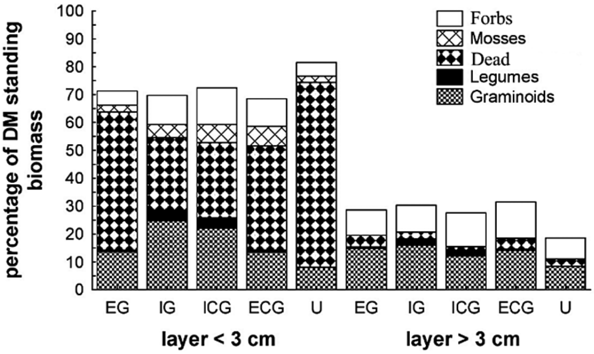

Figure 2. The effect of the different treatments on percentage of dry matter (DM) standing biomass.

Conclusions

The introduction of grazing and defoliation management at the study site have changed the sward structure with functional groups like graminoids and forbs dominating, while grazing intensity affected the vertical distribution of the groups in the sward.

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References

Bircham J.S. and Hodgson J. (1983) The influence of sward conditions on rates of herbage growth and senescence in mixed swards under continuous grazing management. *Grass and Forage Science* 38, 323-331.

Del-Val E. and Crawley M.J. (2005) What limits herb biomass in grasslands: competition or herbivory? *Oecologia* 142, 202-211.

Wright I.A. and Whyte I. (1989) Effect of sward surface height on the performance of continuously stocked spring-calving beef cows and their calves. *Grass and Forage Science* 44, 259-266.

Prolonged summer drought changes N dynamics in cut grassland

Kayser M.[1,2], Hoffmann M.[3], Ströer R.[1], Benke M.[4] and Isselstein J.[1]
[1]*Georg-August University Göttingen, Department of Crop Sciences, Germany;* [2]*University of Vechta, Geo-Lab, Germany;* [3]*Leibniz Centre for Agricultural Landscape Research, Germany;* [4]*Chamber of Agriculture Lower-Saxony, Germany*

Abstract

Climate change is expected to increase the probability of prolonged droughts in spring and summer in temperate climates. This might negatively affect grassland production, alter related nitrogen (N) dynamics, and require innovations for N management at the farm scale. There is a knowledge gap: effects of drought on residual soil N in autumn and on NO_3-N leaching are not well investigated. In Europe, the year 2018 was characterised by high temperatures and a long drought period in summer and might serve as an example of a climate change scenario. We hypothesise that N surpluses in cut grassland are increased in dry summers leading to an increased risk of N leaching. The extent of N leaching then depends on the amount of rainfall and temperature over winter. Using data from a field experiment in northwest Germany with different systems of N return, including mineral and organic fertiliser, we took N surplus, soil mineral N, and NO_3-N losses in leaching water to assess the effects of droughts on N cycling in the field. We evaluate whether N fertilisation, especially the return of excrement-N, could be adapted to changing conditions and what consequences this would have for dairy farms.

Keywords: climate change, nitrate, leaching, emissions

Introduction

There is an on-going debate on how nitrogen (N) dynamics in cut grassland systems might be affected by climate change and how they would react to periods of prolonged drought in summer. Productive cut grassland systems are generally characterised by large N offtake with harvested biomass resulting in relatively low N surpluses and small nitrate (NO_3) leaching losses even on coarse textured soils (Kayser *et al.,* 2015). N offtake in biomass serves as the main sink in matching N input from organic and synthetic N fertiliser. Droughts limit plant growth and N transformation processes and lead to a reduced N offtake resulting in N surpluses that are preserved until rewetting. The extent of N leaching over winter is then largely determined by the residual N in autumn and further N transformation processes and timing and amount of rainfall during winter as the driver of N translocation. The objective of our study was to quantify the effect of the interaction of drought conditions during summer and N fertilisation on NO_3-N leaching over winter in cut grassland systems.

Materials and methods

We used data for the 2018 growing period and the following winter period until April 2019 from a field experiment with five different treatments of N fertiliser. The experiment had been established in 2016 with a sward based on a ryegrass (*Lolium perenne* L.) dominated mixture with no clover. The experiment was located in a maritime climate in northwest Germany on a coarse-textured soil (sand to loamy sand). The long-term average temperature is 8.7 °C and the average annual rainfall 786 mm (Table 1). In a one-factorial design replicated in four blocks, we compared five different combinations of N input by cattle slurry and synthetic N fertiliser (Table 2). Plots were 12×4.5 m in size. Cattle slurry was applied by sliding shoe to all treatments, apart from the Control, with 120 kg N ha^{-1} in spring. For treatments with more than 120 kg N ha^{-1} N input, cattle slurry and CAN (calcium-ammonium-nitrate) were applied in doses of 60 kg N ha^{-1} after the first and second treatment. Details are given in Table 2. The sward was mown four times and grab samples were taken from each plot. Herbage was oven-dried, ground to <1 mm, and

Table 1. Precipitation and average daily temperatures for May 2018-April 2019 in three 4-month periods (denoted in Roman numerals); Long-term average 1981-2018.

	Precipitation [mm]				Temperature [°C]			
	V–VII	VIII–X	XI–I	II–IV	V–VII	VIII–X	XI–I	II–IV
May 2018 – April 2019	120.5	101.3	189.4	169.7	17.8	14.8	4.8	7.6
Long-term average	209.4	201.9	211.4	145.7	15.5	13.9	3.7	5.4

Table 2. Effect of N from fertiliser input (cattle slurry and synthetic fertiliser, calcium-ammonium-nitrate, CAN) on dry matter (DM) yield, N offtake with biomass (N yield), N surplus (N input fertiliser-N yield), and NO_3-N leaching; means of 4 replications, 2018-2019.[1]

Treatment	Cattle slurry kg N ha^{-1}	CAN kg N ha^{-1}	DM yield Mg ha^{-1}	N yield kg N ha^{-1}	N surplus kg N ha^{-1}	N leach[2] kg N ha^{-1}
Control	0	0	1.36^{a}	32^{a}	-32^{a}	22^{a}
SLR120	120	0	2.53^{b}	65^{b}	55^{b}	28^{a}
SLR240	240	0	2.68^{b}	70^{bc}	170^{c}	87^{b}
SLM240	120	120	2.99^{b}	91^{c}	149^{c}	146^{bc}
SLM360	240	120	3.97^{c}	117^{d}	243^{d}	221^{c}
P-value	-	-	<0.001	<0.001	<0.001	<0.001

[1] ANOVA: means with different letters differ at P=0.05.

[2] ANOVA and comparison of means based on log-transformed values (natural logarithm); back-transformed.

analysed for N. In each plot, six suction cups at 75 cm depth allowed sampling of leached water every 2-3 weeks. NO_3-N was analysed photometrically with a flow-injection analyser. Leaching as determined by a water-balance model (Hörmann, 1997) occurred from late December 2018 to early April 2019 and amounted to 201 mm.

Results and discussion

During spring and especially summer 2018 prolonged drought periods occurred: from May 2018 to October precipitation amounted to only 50-58% of the long-term average (Table 1). In mid-summer grass growth ceased and swards turned brown. Consequently, dry matter (DM) yields and N offtake for N inputs from 0-360 kg N ha^{-1} were reduced and amounted to about 1.4-4.0 Mg ha^{-1} and 32-117 kg N ha^{-1}, respectively (Table 2). N surpluses were high and N leaching losses unusually large, ranging from 22-221 kg N ha^{-1}. NO_3-N concentrations were high and did not drop below the threshold level of 11.3 mg N l^{-1} even for N inputs of 0 and 120 kg N ha^{-1} (Figure 1). Differences between the treatment with 240 kg from slurry alone (SLR240) and from the combination of slurry and synthetic N fertiliser (SLM240: 120 kg N from slurry and 120 from CAN) might be explained by higher NH_3 losses from slurry applied in summer for SLR240. The N from CAN as applied in SLM240 in summer was not reduced by volatile losses, but also not fully taken up by the sward.

Previous results from cut grassland on a neighbouring site showed DM yields as a four-year average for N inputs from 0-320 kg N ha^{-1} to be in a range of 5.6-11.8 Mg ha^{-1} for CAN and 4.9-9.3 Mg ha^{-1} for cattle slurry applied by sliding shoe. Corresponding NO_3-N leaching losses amounted to 6.0-15.6 and 7.8-13.9 kg N ha^{-1}, respectively; in no year did N leaching losses exceed 30 kg N ha^{-1} (Kayser *et al.*, 2015; see also Schröder *et al.*, 2010). The strong deviation in the year 2018/2019 from the cut grassland paradigm of small N leaching, even at N inputs of 360 kg N ha^{-1}, might be explained by three main interacting effects. Firstly, N offtake is the most important sink in cut grassland. Unproductive swards, poor soil conditions,

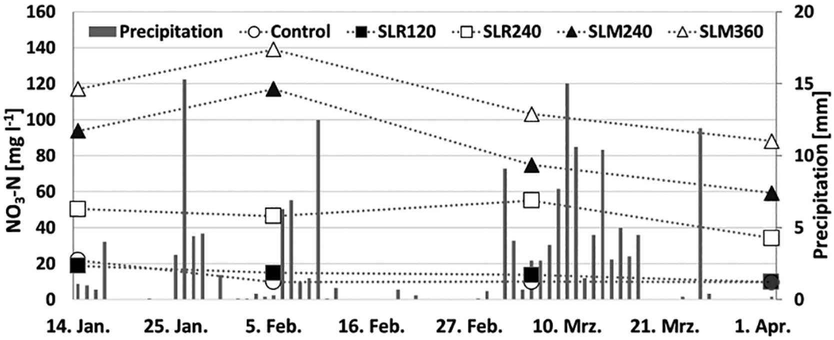

Figure 1. Nitrate concentrations (NO_3-N [mg l^{-1}]) and precipitation [mm] in the leaching period 2018-2019.

detrimental weather conditions in combination with large N inputs reduce the efficiency of the sink function and result in large residual N. Secondly, a severely damaged sward after prolonged drought will supply N from mineralization processes after rewetting of the soil. This might be in a range known from sward disturbance in the course of grassland renewal. Studies measuring the NO_3-N leaching losses by suction cups or using lysimeters determined N losses of 35-72 kg N ha^{-1} over the first winter following grassland renovation (Kayser *et al.*, 2018). Thirdly, these two effects will interact with a late start of the leaching period. Dry conditions prevailed during the autumn of 2018 and only in late December did the soil reach field capacity and leaching occurred. In January and February 2019 rainfall periods alternated with two freeze-thaw cycles and this was followed by mild temperatures of 6-10 °C and regular rainfall in March. Conditions like this would promote mineralization processes and further supply of N. Thus, a damaged sward, large residual N, and on-going mineralization resulted in exceptionally large NO_3-N leaching losses in 2018/2019.

Conclusions

Prolonged droughts during summer even extending into autumn can lead to large N leaching losses even from grassland. Not only is the main sink reduced, but the dynamics of N leaching are also altered. This reduction in N efficiency could have consequences for the N management and N balancing of dairy farms.

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References

Hörmann G. (1997) SIMPEL – Ein einfaches, benutzerfreundliches Bodenwassermodell zum Einsatz in der Ausbildung. *Deutsche Gewässerkundliche Mitteilungen* 41, 67-72.

Kayser M., Müller J. and Isselstein J. (2018) Grassland renovation has important consequences for C and N cycling and losses. *Food and Energy Security* 2018; e00146. https://doi.org/10.1002/fes3.146

Kayser M., Breitsameter L., Benke M. and Isselstein J. (2015) Nitrate leaching is not controlled by the slurry application technique in productive grassland on organic-sandy soil. *Agronomy for Sustainable Development* 35, 213-223.

Schröder J.J., Assinck F.B.T., Unek D. and Velthof G.L. (2010) Nitrate leaching form cut grassland as affected by the substitution of slurry with nitrogen mineral fertilizer on two soil types. *Grass and Forage Science* 65, 49-57.

Effects of organic farming on ecosystem services and multifunctionality in Switzerland: the *ServiceGrass* project

Klaus V.H.[1], Richter F.[1], Buchmann N.[1], Jan P.[2], El Benni N.[2] and Lüscher A.[3]
[1]Institute of Agricultural Sciences, ETH Zürich, Universitätstr. 2, 8092 Zürich, Switzerland; [2]Research Division Competitiveness and System Evaluation, Agroscope, Tänikon, 8356 Ettenhausen, Switzerland; [3]Forage Production and Grassland Systems, Agroscope, Reckenholzstrasse 191, 8046 Zürich, Switzerland

Abstract

Sustainable agriculture delivers not only market goods but also many public ecosystem services and non-market goods. Agricultural intensification undermines the delivery of many public ecosystem services at local level. Organic farming might decrease the environmental impact of intensive food and feed production on grasslands and could therefore be able to sustain both private and public ecosystem services. A systematic literature search of scientific publications revealed the absence of literature on the impact of organic farming on ecosystem services in grasslands. Thus, the project *ServiceGrass* was initiated to explore effects of organic grassland farming on several ecosystem services and their simultaneous provisioning, i.e. multifunctionality, in Switzerland. The principal aim of the project is to compare the ability of organic and conventional grasslands to deliver ecosystem services at plot and farm levels, including management intensification as a major driver. We aim at up-scaling results from grassland plots to farm- and sector-level in order to draw conclusions on the realized ecosystem services portfolio of both farming systems. Findings of this project will underline strengths and weaknesses of organic and conventional farming systems in delivering multiple ecosystem services for sustainable future grassland management.

Keywords: environmental impacts, economic assessment, grassland management, intensification, multifunctionality, sustainable agriculture

Introduction

Ecosystem services (ES) are the many indispensable benefits that humans gain from properly functioning ecosystems. Agricultural systems such as grasslands are supposed to deliver not only private ES, i.e. market goods, but also many public ES, i.e. non-market goods and services relevant for long-term food security and sustainable development. Given a growing world population and an increasing demand for meat and milk, understanding and managing the delivery of ES is of vast importance for human well-being (MEA, 2005; UN, 2015).

Grasslands are expected to provide an outstandingly high number of different ES, but quantitative measurements of many services are scarce (Allan *et al.*, 2015). In addition, the ongoing intensification of grassland management is the most important change affecting ES provision (Tscharntke *et al.*, 2005), mostly by increasing private services (forage yield and forage quality) but decreasing public services (Allan *et al.*, 2015). Thus, there are important trade-offs between individual ES and a sustainable intensification of food and feed production that increases productivity but maintains high levels of multifunctionality, i.e. the provision of multiple ES at once, is a major agricultural challenge. Organic farming is said to be among the most relevant strategies to decrease environmental impacts of intensive food and feed production (Reganold and Wachter, 2016), which in turn is assumed to maintain high levels of public ES (Sandhu *et al.*, 2010). Still, the environmental impact of intensification often differs when related to unit of land use area or to unit output (Jan *et al.*, 2019). It can be hypothesized that organic management of grasslands could be especially promising to promote multifunctionality because yields remain reasonably high under organic management (Klaus *et al.*, 2013). However, this has never been assessed.

Materials and methods

We assessed the published scientific literature on effects of organic farming with regard to ES and multifunctionality in grasslands to identify the number of publications on these topics. We screened existing literature via a systematic literature search using the search string [‘organic* farm*’ OR ‘organic* manage*’ OR ‘organic* prod*’] AND [grassland* OR pastur* OR ‘meadow*’ OR ‘hayfield*’ OR rangeland* OR ‘graz* land*’] as well as the same search plus AND ‘ecosystem service*’. We then replaced ‘ecosystem service*’ with [multi-functionality OR multifunc* OR ‘multi functionality’]. We reran the search string with replacing the previous grassland search string with either [‘permanent grassland*’ OR meadow*] or [‘temporary grassland*’ OR ley*] to assess results for both grassland types separately. We used the number of publications found as an indicator of how well different aspects of organic grassland management have been studied. To compare the amount of literature published on grasslands with that from croplands, we reran all searches with [‘arable land*’ OR cropland* OR ‘crop* rotation*’ OR ‘crop* syst*’] instead of the grassland strings. The search was done on 29 October 2019 using the *Web of Science* database from Clarivate searching in titles, abstracts, keywords in all publication entries of the Web of Science Core Collection from 1900 to 2019.

Results and discussion

The systematic literature search revealed considerably fewer scientific publications on organic farming in grasslands than on croplands (Figure 1). Searching specifically for temporary and permanent grasslands revealed particularly few results (Figure 1A). Concerning ES provision, only two studies addressing organically farmed permanent grasslands could be identified (Figure 1B). Studies on multifunctionality were generally very scarce and only when the search terms ‘grassland’ or ‘cropland’ were omitted could a significant number of studies be identified (Figure 1C). This suggests that currently, ES multifunctionality

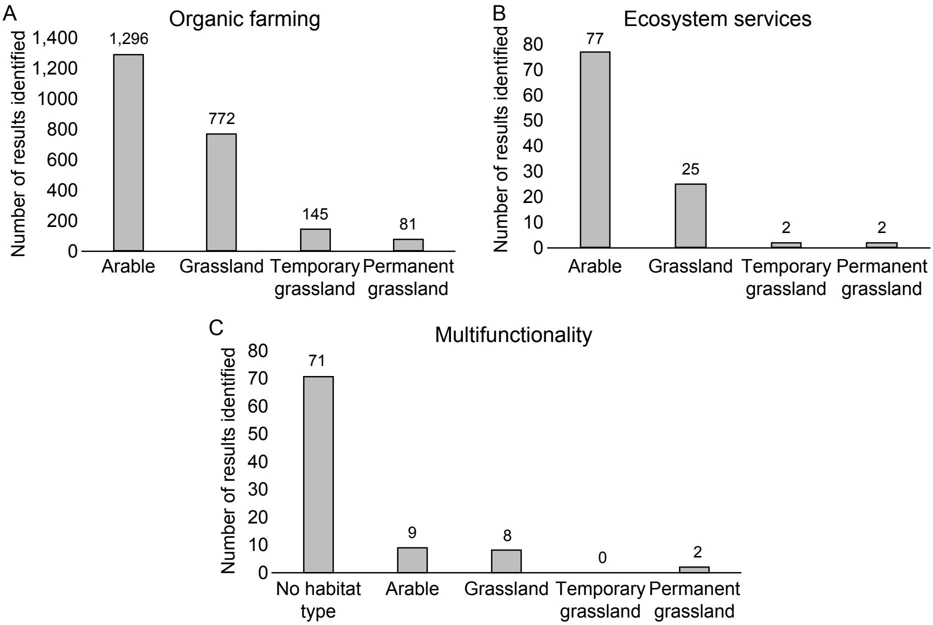

Figure 1. Outcomes of the systematic literature search comparing the number of scientific publications found on organic farming in croplands and grasslands. The latter was further separated into temporary grasslands (leys) and permanent grasslands. Panels show the number of (A) scientific publications in general, (B) publications including ecosystem services and (C) publications including multifunctionality. See methods section for details.

with regard to organic farming is rather a conceptual idea, and that evidence from published literature is scarce. However, it is possible that studies assessed more than one function but did not use the term 'multifunctionality' in the paper. In such cases, we would have underestimated the number of studies published. Despite the economic importance of grasslands, their high proportion of land cover, their potential multifunctionality and their biodiversity (Allan *et al.*, 2015; Le Clec'h *et al.*, 2019), a comprehensive assessment of ES and of the multifunctionality of organically managed grasslands has obviously never been made. The project *ServiceGrass* started in 2019 and aims at closing this knowledge gap. Its principal aims are (1) to measure and compare ES in organic and conventional grasslands in Switzerland, (2) to explain the impact of intensification on multifunctionality, and (3) to upscale plot-level results to farm- and sector-level. For the latter, we will combine measurements of ES from on-farm grassland plots with technical, structural and economic data from face-to-face interviews with Swiss farmers, farm accountancy and census data. This will enable us to draw conclusions on the effects of different management practices, as revealed by the farmers' interviews, on the delivery of grassland ESs. Project outcomes will be of high agricultural and societal relevance, as they will assess the effectiveness of grassland-based farming systems in ES provision and help improving organic and conventional farming systems to promote multifunctionality.

Conclusions

Effects of organic farming of grasslands, especially with regard to ES and multifunctionality, are widely under-studied, although grassland depicts the major share of land under organic management worldwide. To close this gap, the project *ServiceGrass* will intensively assess and evaluate ES provision from conventional and organic grasslands in Switzerland to promote the sustainable development of grassland management.

Acknowledgements

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References

Allan E., Manning P., Prati D., Grassein F., Alt F., Binkenstein J., … and Fischer M. (2015) Land use intensification alters ecosystem multifunctionality via loss of biodiversity and changes to functional composition. *Ecology Letters* 18, 834-843.

Jan P., Repar N., Nemecek T. and Dux D. (2019) Production intensity in dairy farming and its relationship with farm environmental performance: empirical evidence from the Swiss alpine area. *Livestock Science* 224, 10-19.

Klaus VH, Kleinebecker T, Prati D, Fischer M, Alt F, Boch S, … and Hölzel N (2013) Does organic grassland farming benefit plant and arthropod diversity at the expense of yield and soil fertility? *Agriculture, Ecosystems and Environment* 177, 1-9.

Le Clec'h S., Finger R., Buchmann N., Gosal A.S., Hörtnagl L., Huguenin-Elie, O., … and Huber, R. (2019) Assessment of spatial variability of multiple ecosystem services in grasslands of different intensities. *Journal of Environmental Management*, 251, 109372.

MEA (2005) *Millennium ecosystem assessment – ecosystems and human well-being* (Vol. 5). Island press, Washington DC, USA.

Reganold J.P. and Wachter J.M. (2016) Organic agriculture in the twenty-first century. *Nature Plants* 2, 15221.

Sandhu H.S., Wratten S.D. and Cullen R. (2010) Organic agriculture and ecosystem services. *Environmental Science Policy* 13, 1-7.

Tscharntke T., Klein A.M., Kruess A., Steffan-Dewenter I. and Thies C. (2005) Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. *Ecology Letters* 8, 857-874.

UN (2015) *RES/70/1 Transforming our world: the 2030 agenda for sustainable development*. Seventieth United Nations General Assembly, New York, 25.

A model-based assessment of C storage potential of French grasslands: a national study

Klumpp K.[1], Martin R.[1], Therond O.[2], Pellerin S.[3], Bamiere L.[4], Constantin J.[5], Launay C.[5], Gastal F.[6], Graux A.I.[7], Mosnier C.[8], Chambaut H.[9], Brune-Lafleure L.[9] and Rechauchere O.[10]
[1]INRAe, VetAgro Sup, UCA, UMR sur Écosystème Prairial (UREP), Clermont Ferrand, France; [2]INRAe, UMR LAE 'Laboratoire Agronomie et Environnement', Colmar, France; [3]INRAe, UMR ISPA 'Interactions Sol-Plante-Atmosphère', Bordeaux, France; [4]Economie Publique, INRA, AgroParisTech, Université Paris-Saclay, Thiverval-Grignon, France; [5]INRAe, UMR AGIR 'Agroécologie, Innovations, Territoires', Toulouse, France; [6]INRAe, UE Ferlus, Lusignan, France; [7]INRAe, UMR Pegase 'Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Élevage', Rennes, France; [8]INRAe, UMR UMRH 'Unité Mixte de Recherche sur les Herbivores', Clermont-Ferrand, France; [9]IDELE, Mon Voisin, Le Rheu, France; [10]INRAe, DEPE 'Délégation à l'Expertise scientifique collective, à la Prospective et aux Etudes', Paris, France

Abstract

From a climate change perspective, grassland soils have the ability to sequester C. However, there are still uncertainties on the magnitude of C sequestration potential, and their use in climate initiatives (i.e. 4p1000). Average values (± standard error) of 0.7±0.1 Mg C ha^{-1} yr^{-1} for permanent grassland (PG), and 0.4 to 0.8 Mg C ha^{-1} yr^{-1} for grass-ley (temporary grassland, TG), have been cited by different studies, while soil inventory reports only 0.05±0.3 Mg C ha^{-1} yr^{-1}. These discrepancies can be attributed to differences in pedo-climatic conditions, intensity and type of management, but also to age and lifetime of temporary grasslands (TG). To analyse in detail C sequestration potential of French grasslands, a national study '4p1000 France' was conducted to identify (1) C 'storing' practices; (2) their potential to be adopted as mitigation option; and (3) their cost of implementation. Along with a literature review, a modelling approach at fine spatial-scale resolution (1 km^2) was used to simulate key grassland managements for PG and TG identified from agricultural statistics. Results showed that insertion of TG gained additional +0.47 Mg C ha^{-1} yr^{-1}, while the replacement of mowing by grazing of intensively used PGs added +0.3 Mg C ha^{-1} yr^{-1} to soil, compared to baseline (0.26 and 0.21 Mg C ha^{-1} yr^{-1} for TG and PG). C storage under baseline and mitigation practices was able to offset field-based greenhouse gas emissions over French grassland areas.

Keywords: permanent grassland, temporary grassland, C sequestration, management, technical costs

Introduction

Following the Paris agreement (COP21, 2015), France aims to reach a carbon neutrality objective by 2050. Given the contribution to national greenhouse gas (GHG) emissions (20%), the French agricultural sector plays and important role in achieving this ambitious target. To attain carbon neutrality a number of mitigation actions are required: reduction in N_2O and CH_4 emissions, renewable energy production, and carbon storage in biomass and soil, the latter being less studied.

The 4per1000 initiative (https://www.4p1000.org/fr) suggests that an increase of 4‰ per year of soil C would theoretically compensate for anthropogenic CO_2 emissions and thus limit temperature rise to +1.5 °C by 2050. In France, the total stock of organic carbon in the 0-30 cm horizon of soils (excluding artificial surfaces) is of the order of 3.6 Gt of C (13.4 Gt CO_2e). An increase of 4‰ per year of this stock would, however, only offset about 12% of emissions French GHG (458 Mt CO_2e in 2016). By doing the calculation, more theoretical, on the horizon 0-100 cm, the percentage of compensation would reach 15%. According to this controversy, the '4per1000 initiative' has emphasized the need to quantify C storage potential of agricultural soils (baseline) and the related costs of additional C storage (mitigation potential) at the national level.

According to scientific literature, annual C sequestration rates of grassland can vary between -2.2 (loss) and +2.5 (gain) Mg C ha^{-1} yr^{-1}, reporting either a beneficial effect of grassland management on C sequestration (Conant *et al.*, 2017) or negative (Brynes *et al.*, 2018; Eze *et al.,* 2018). Depending on the measurement method (soil inventory versus gas exchange measurements), average (± standard error) values of 0.7±0.1 Mg C ha^{-1} yr^{-1} for permanent grassland (PG) (Soussana *et al.,* 2010), and 0.4 to 0.8 Mg C ha^{-1} yr^{-1} for grass-ley, have been cited by different studies, while long-term soil inventory reported only 0.05±0.3 Mg C ha^{-1} yr^{-1} (e.g. Meersmann *et al.,* 2011). Recent meta-analyses also underline the effect of climate, soil physical properties, grassland type and age (i.e. temporary or permanent), and grassland management (nutrient fertilisation, intensity of grassland use) on C storage potential of grasslands (Abdalla *et al.,* 2017). To fully examine C storage potential on national level, a study has been conducted (see Pellerin *et al.,* 2019) to (1) identify C 'storing' practices, (2) to map C storage potential of French grassland soils (baseline) and (3) estimate C storage potential and costs of implantation for chosen mitigation options.

Materials and methods

In order to analyse C storage and mitigation potential of EU grassland ecosystems, a literature analysis was carried out for timeframe of 1980 to present. Despite a large available literature reporting C storage potential, only 80 studies were retained, reporting direct measurements of soil C stock changes and management practices including grazing (type and pressure), mowing (yield and number of cuts), grassland age and nutrient fertilisation regimes.

In order to determine and map C storage potential of French grasslands, we run the crop model STICS, and a grassland model PASIM (Ma *et al.,* 2010), for temporary and permanent grasslands, respectively. Models were run on a fine spatial scale (1 km^2) for a 30-year period, using French soil mapping units (Figure 1), SAFRAN climate description and actual dominant grassland management base on agricultural statistics (30 practices of grazing, mowing and fertilisation intensities). The additional C storage was calculated as the difference between the current management practices (i.e. the baseline) and soil C stock changes under mitigation practices. Costs to implement mitigation options were assessed for each C storing practice using an economic model (BANCO), and by considering their potential applicability (i.e. area and feasibility) and related technical costs.

Results and discussion

Literature analyses show that C storage potential depends on land cover history, initial C stock status, soil climate, age and botanical composition of grassland, and agricultural practices. Likewise, the reported magnitude of C storage potential was related to measurement method; studies based on gas exchange

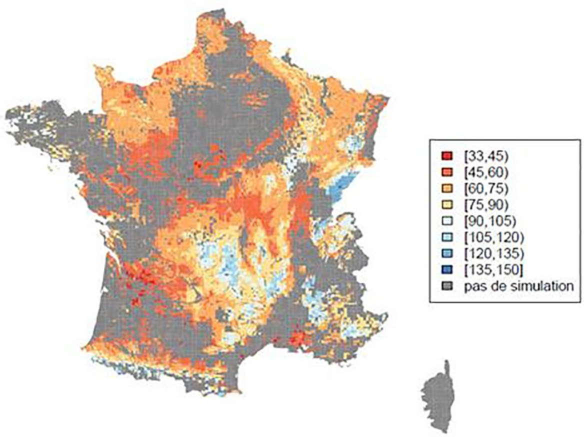

Figure 1. Soil C stock of French permanent grassland (Mg C ha^{-1}) of the 0-30 cm soil depth. Numbers in legend indicate C stock ranges per colour.

measurements (i.e. eddy covariance method) tend to have higher values than studies analysing soil samples. This difference can be explained by the fact that net changes in soil C pools (i.e. changes in C stock of fine soil) are relatively small compared to C storage of the whole ecosystem (including root biomass, litter) as determined by gas exchange measurements, especially when measured over short time periods (<5 year) (Smith *et al.,* 2020).

Even so, values reported by the literature worldwide are extremely variable (from -3.3 to +4 Mg C ha^{-1} yr^{-1} for the 0-30 cm soil depth). The highest C storage values were observed for high C input systems (organic fertilisation), and when meadows are planted after cultivation. The supply of mineral or organic fertilisers generally has a positive effect on C storage (in the order of 0 to +0.3 Mg C ha^{-1} yr^{-1} compared to an unfertilised control). Also grazing has an advantage over mowing for moderate to intensively used grassland systems. According to literature review three carbon storing practices were identified for temporary and permanent grasslands: expansion of temporary grasslands (instead of silage maize); moderate intensification of low productive grasslands (+50 kg N ha^{-1}); replacement of mowing events by grazing.

French C storage mapping by modelling (i.e. 30-year variations in C stocks under current practices) showed a mean baseline value of +0.26 and +0.21 Mg C ha^{-1} yr^{-1} for temporary and permanent grasslands, respectively. Nonetheless, there were large differences in C storage potential due to climate, soil properties, initial soil C stocks (Figure 1) and management.

Applying mitigation options resulted in additional +0.16 Mg C ha^{-1} yr^{-1} when N was added to low productive grasslands (56% of permanent grassland area), and +0.47 Mg C ha^{-1} yr^{-1} when maize silage was partly replaced by sown grasslands (38% of arable land). Related costs to implement these mitigations were 43 €/ Mg CO_2 (i.e. 28 € ha^{-1}) and 217 €/ Mg CO_2e (i.e. 91 € ha^{-1}) for improvements of permanent grassland and temporary grasslands.

Conclusions

Grasslands contribute to the French C storage potential through already existing good management practices. These practices should be maintained to avoid future carbon losses. For areas and practices with low C storage potential, improved agricultural practices (i.e. insert grass-ley in crop rotation, replace mowing by grazing) were identified to mitigate climate and offset GHG emissions.

References

Abdalla M., Hastings A., Chadwick D.R., Jones D.L., Evans C.D., Jones M.B., Rees R.M. and Smith P. (2018) Critical review of the impacts of grazing intensity on soil organic carbon storage and other soil quality indicators in extensively managed grasslands. *Agriculture Ecosystems & Environment* 253: 62-81.

Byrnes R.C., Eastburn D.J., Tate K.W. and Roche L.M. (2018). A global meta-analysis of grazing impacts on soil health indicators. *Journal of Environmental Quality* 47(4): 758-765.

Conant R.T., Cerri C.E.P., Osborne B.B. and Paustian K. (2017) Grassland management impacts on soil carbon stocks: a new synthesis: A. *Ecological Applications* 27(2), 662-668.

Ma S., Lardy R., Graux A.I., Ben Touhami H., Klumpp K., Martin R. and Bellocchi G. (2014) Regional-scale analysis of carbon and water cycles on managed grassland systems. *Environmental Modelling and Software* 72, 356-371.

Meersmann *et al.* (2011) Spatial analysis of soil organic carbon evolution in Belgian croplands and grasslands, 1960-2006. *Global Change Biology* 17, 466-479.

Pellerin *et al.* (2019) Stocker du carbone dans les sols français, Quel potentiel au regard de l'objectif 4 pour 1000 et à quel coût ? Synthèse du rapport d'étude. INRA, France, 114 p.

Soussana J.F., Tallec T. and Blanfort V. (2010) Mitigating the greenhouse gas balance of ruminant production systems through carbon sequestration in grasslands. *Animal* 4(3): 334-350.

Smith P. *et al.* (2020) How to measure, report and verify soil carbon change to realise the potential of soil carbon sequestration for atmospheric greenhouse gas removal. *Global Change Biology* (in press)

Trade-off between forage quality and yield by adapting the harvesting regime to promote flowering in ley grassland

Komainda M.[1], Muto P.[2] and Isselstein J.[1,3]

[1]Georg-August University of Göttingen, Department of Crop Sciences, Grassland Science, Von-Siebold-Str. 8, 37075 Göttingen, Germany; [2]Natural England, Lancaster House, Hampshire Court, Newcastle upon Tyne, NE4 7YH, United Kingdom; [3]Centre of Biodiversity & Sustainable Land Use, Grisebachstr. 6, 37077 Göttingen, Germany

Abstract

Biodiverse, multi-purpose leys have received much attention as a means of providing quality forage, nectar and pollen resource and soil improvement in arable rotations. Optimizing the delivery of these various outcomes depends largely on the timing of cutting or grazing, which, in-turn, influences the species composition of the mixture. There have been few studies that have investigated the influence of cutting regime of different mixtures on forage productivity, quality and floral provision. Therefore, a field experiment with varying cutting regimes (plot size = 4.5 m^2, n=4) was conducted in Göttingen comparing a simple and a complex mixture. Three cutting regimes were compared: (1) a standard cutting regime; and two alternatives: (2) delayed cut in June for early flowering or (3) delayed cut in August for late flowering. The cutting regime and mixture affected forage biomass production, forage quality and floral resources during the summer months. We found a trade-off between forage quality and biomass production: a delayed harvest in June increases the yield at the cost of quality, whereas a low loss of quality was found in August, however, at the cost of biomass production.

Keywords: multispecies mixtures, insects, legume mixtures

Introduction

Diverse herb-rich leys containing productive legumes, dicotyledonous forbs and improved grass cultivars represent a viable alternative to nutrient-demanding ryegrass-dominated leys with respect to forage yield and quality (Grace *et al.*, 2018). The functionality of such mixtures was proven successful under varying climatic conditions such as drought (Finn *et al.*, 2018) or as option to reduce greenhouse gas emissions (Cong *et al.*, 2017). Complex mixtures also increase phytodiversity in agricultural systems (Sanderson, 2010) and provide a pollen and nectar resource for invertebrates (Hatt *et al.*, 2019). To promote invertebrates and to optimise the yield and quality of forage, the management of cutting dates is essential (Clark *et al.*, 2019). The present study compares different cutting regimes to allow early, mid- or late-season flowering and demonstrates the impact on forage quality and biomass production and trade-offs between them when an aim is the provision of floral resources for pollinators.

Materials and methods

The present study is based on a field trial established in Central Germany (Göttingen, 9.3 °C, 645 mm) in spring 2017 as a two-factorial field experiment in a randomized block design with four replicates and a plot size of 4.5 m^2. Treatments were seed mixture (diverse vs grass) and the harvest regime (standard, early and late*)*. The seed mixtures were (1) a standard grass mixture containing *Lolium perenne, Poa pratensis, Festuca pratensis, Phleum pratense* and *Trifolium repens* (95% grasses in the mixture) and (2) a diverse herbal ley mixture containing 43% grasses, 35% legumes and 22% dicotyledonous non-leguminous forbs, (16 species). The harvesting regimes refer to a standard common for grass-dominated leys under the present climate and two alternatives (Table 1). The alternatives varied with respect to timing of flowering: early flowering in June or late flowering in August (to allow for flowering in order to benefit pollinators). Therefore, early flowering refers to a delayed second cut in June, while late flowering refers to a delayed

Table 1. The harvest regimes during the experimental years.[1]

Regime	Cut 1	Cut 2	Cut 3	Cut 4
Standard	10 May	20 Jun	10 Aug	15 Oct
Early flowering	01 May	30 Jun	15 Aug	15 Oct
Late flowering	01 May	01 Jun	30 Aug	15 Oct

[1] The standard harvest regime refers to an intensive cutting system for dairy cows as common under the present climate. The early and late flowering followed a delayed harvest during the cut 2 (June) and cut 3 (August), respectively.

third cut in August (Table 1) within each mixture treatment. Regular harvesting was conducted four times annually using a plot harvester during two full production years (2018 and 2019). At each harvest, the biomass dry matter yield (DMY) was determined after drying, and forage quality (crude protein (CP) and neutral detergent fibre (NDF)) was determined using NIRS. Linear mixed effects models were calculated for each target variable with the harvest regime, mixture and cut as well as their interactions as fixed factors and plot nested in block as random factor using the package 'nlme' in R (3.5.1, 2018). Minimum adequate models were determined by the Akaike's Information Criterion and Tukey tests were followed using 'emmeans' ($P \leq 0.05$). We expected pronounced forage differences between treatments in cut 2 and cut 3. Our hypothesis was that there are interactions between harvest regime and mixture for each harvest date and that a diverse mixture has a higher yield and better forage quality than a grass-dominated sward.

Results and discussion

During June and August, the diverse sward (predominantly *Medicago sativa*) produced flowers attracting pollinators (not shown). There was a significant interaction between mixture × harvest regime for DMY ($P \leq 0.001$) and CP content ($P \leq 0.01$) but the interaction with cutting date was only significant either for mixture ($P \leq 0.001$) or harvest regime ($P \leq 0.001$). The application of a modified harvesting regime for a standard grass-mixture had no effect on the average DMY per cut (1,037 kg ha^{-1}). Growing a diverse compared to a grass-dominated mixture, however, increased the DMY significantly by 82% (average for early flowering and or standard harvesting regime) and 76% (late flowering). Comparisons of means for the three-fold interaction between harvest regime × mixture × cut revealed significant differences at the modified cutting dates (cut 2 and cut 3) (Table 2). The diverse mixture in the early flowering regime outyielded all other mixtures (6,277 kg ha^{-1}). In the late flowering regime, the third cut was enhanced by the diverse mixture but without DMY advantage of the late flowering regime within the diverse mixture (Table 2). On average, cut 2 and cut 3 produced 44 and 22%, respectively of the annual DMY. The harvest regime had a significant effect on NDF in interaction with cut ($P \leq 0.001$) but no interaction of harvest regime with mixture was found. However, the interaction between regime × mixture was significant for NDF ($P \leq 0.001$). At cut 3 in August no effects of mixture or harvest regime were detected (Table 2), whereas the early flowering regime resulted in more NDF as consequence of delayed harvest at cut 2 irrespective of mixture. The diverse mixture had on average a CP content of 206 g kg^{-1} dry matter (DM) while the grass-dominated sward had significantly less (173 g kg^{-1} DM). At cut 2, the diverse mixture had higher CP contents than the grass-dominated sward, but at the third cut the CP contents were modified by harvesting regime and not by mixture. Our hypothesis is therefore confirmed.

Table 2. Dry matter yields (DMY, kg $ha^{-1} \pm$ standard error), crude protein (CP) and neutral detergent fibre (NDF) contents (g kg^{-1} dry matter $\pm$ standard error) for the cuts in June and August for each mixture and harvesting regime.[1]

Cut	Regime	Mixture	DMY		NDF		CP	
Cut 2 June	standard	grass	2,367±197	ab	457±12.9	c	138.±6.7	b
	standard	diverse	4,956±398	d	435±5.5	bc	198±6.7	c
	early	grass	3,844±197	cd	472±12.9	c	104±6.7	a
	early	diverse	6,277±398	e	449±5.5	bc	161±6.7	b
	late	grass	1,505±197	a	428±12.9	ab	146±6.7	b
	late	diverse	2,780±398	bc	406±5.5	a	223±6.7	c
Cut 3 August	standard	grass	308±152	a	446±15.1	a	188±5.2	b
	standard	diverse	6,091±459	c	449±13	a	182±3.6	b
	early	grass	35±174	a	432±16.2	a	194±5.8	b
	early	diverse	5,662±461	bc	434±13.2	a	186±3.6	b
	late	grass	606±152	a	483±15.2	a	144±5.2	a
	late	diverse	5,075±459	b	485±13	a	156±3.6	a

[1] Lowercase letters indicate differences between harvest regimes × mixtures for each harvest date ($P \leq 0.05$).

Conclusions

Diverse leys are a source of flowers for pollinators and produce more forage of better quality than a standard mixture. We found a trade-off between forage quality and biomass production by modifying the harvesting regime: a delayed harvest in June increases biomass production at the cost of quality, whereas the loss of quality is less in August, however, at the cost of biomass production.

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References

Brink G.E., Sanderson M.A. and Casler M.D. (2015) Grass and legume effects on nutritive value of complex forage mixtures. *Crop Science* 55, 1329-1337.

Clark S.G., Mitchell M.L., Nie Z., Burnett V.F., Butler K.L., Zollinger R.P. and Seymour G.R. (2019) Maximising lucerne (Medicago sativa L.) production with fixed-length recovery intervals after defoliation in mild winter environments. *Journal of Agronomy and Crop Science* 205, 88-98.

Cong W.-F., Jing J., Rasmussen J., Soegaard K. and Eriksen J. (2017) Forbs enhance productivity of unfertilised grass-clover leys and support low-carbon bioenergy. *Scientific Reports* 7, 1422, 1-10.

Finn J.A., Suter M., Haughey E., Hofer D. and Lüscher A. (2018) Greater gains in annual yields from increased plant diversity than losses from experimental drought in two temperate grasslands. *Agriculture Ecosystems and Environment* 258, 149-153.

Grace C., Boland T.M., Sheridan H., Lott S., Brennan E., Fritch R. and Lynch M.B. (2018) The effect of increasing pasture species on herbage production, chemical composition and utilization under intensive sheep grazing. *Grass and Forage Science* 73, 852-864.

Hatt S., Uytenbroeck R., Lopes T., Mouchon P., Osawa N., Piqueray J., Monty A. and Francis F. (2019) Identification of flower functional traits affecting abundance of generalist predators in perennial multiple species wildflower strips. *Arthropod-Plant Interactions* 13, 127-137.

Sanderson M. (2010) Stability and plant species diversity in managed grasslands: a retrospective study. *Basic and Applied Ecology* 11, 216-224.

Grass-feeding dairy cows increases the land use efficiency and the supply of ecosystem services

Koppelmäki K.[1,2], Lamminen M.[1], Helenius J.[1] and Schulte R.P.O.[2]
[1] *University of Helsinki, Department of Agricultural Sciences, P.O. Box 27, 00014 University of Helsinki, Finland;* [2] *Wageningen University & Research, The Farming Systems Ecology, P.O. Box 430, 6700 AK Wageningen, the Netherlands*

Abstract

Spatial separation of crop and livestock production has resulted in regional imbalances in nutrient flows, reliance on external inputs, and carbon losses from soil. It has also created a situation with very little demand for grass in crop production areas. Simultaneously, the intensification of production has decreased the share of forage in ruminant diets. In this scenario study we modelled how a change in forage:concentrate proportion (of 25:75, 50:50, 75:25 or 100:0) in dairy cow diets is linked to delivery of ecosystem services. Higher forage use increased the milk production per hectare and nutrient cycling and carbon input into the soil were increased and methane emissions varied.

Keywords: carbon, nitrogen, phosphorus, ruminants, silage

Introduction

Intensification of agricultural production has resulted in the spatial separation of crop and livestock production. This has caused changes in agricultural land use. In crop production areas, the cultivation of annual crops has increased while grassland production has become concentrated in regions with high livestock densities. This has led to imbalances in nutrient flows, because on one hand crop farms rely on mineral fertilisers, and on the other hand nutrients are concentrated in areas with high livestock density, and there are carbon losses from the soils on crop farms (Heikkinen *et al.*, 2013; Uusitalo *et al.*, 2007).

In Southern Finland, where the livestock density is low, farms are producing mainly annual crops. However, there is a demand for grassland from the environmental point of view as perennial grasses provide multiple ecosystem services such as erosion control and carbon sequestration (Karhu *et al.*, 2012). The use of forage has also decreased in dairy production as the intensification of production has meant changes in cow diets. Simultaneously, the proportion of concentrates has increased, which has resulted in higher milk yields per cow. Questions remain as to how the proportion of forage in dairy cow diets is linked to agricultural land use and to the delivery of ecosystem services. In this study, we tested how a change in the proportion of forage in cow diets impacts the supply of ecosystem services (primary production, water purification, provision of nutrient cycling, carbon sequestration) in a crop production region with a low livestock density in Southern Finland.

Materials and methods

We conducted this modelling study in the region of Uusimaa in Southern Finland. Uusimaa has a low livestock density (0.1 animal units per ha) and cultivation of annual crops is the dominant agricultural land use. To study the impact of changes in ruminant diets on land use and the supply of ecosystem services, we built four scenarios with forage:concentrate ratios of 25:75, 50:50, 75:25 or 100:0 (Table 1). The Lypsikki model (Huhtanen and Nousiainen, 2011) was used to predict dry matter intake (DMI) and energy corrected milk (ECM) yield responses for formulated diets. Diets of dry cows and replacement stock were similar in all treatments. The dry cow diet had a forage:concentrate ratio of 92:8; it consisted of grass silage, straw, barley, faba beans and mineral premix, and DMI)was set to 8.5 kg d^{-1}. The feed consumption of replacement stock was based on production cost estimates of ProAgria rural advisory

Table 1. Cow diets (kg dry matter) and energy corrected milk yield. Reference diet (50:50) shaded.

Forage:concentrate ratio	25:75	50:50	75:25	100:0
Silage	2,036	3,931	5,230	5,616
Grain (barley)	4,668	2,878	1,106	0
Broad bean	720	509	316	0
Rapeseed meal	569	404	249	0
Minerals, vitamins	105	105	105	105
Total	8,098	7,827	7,006	5,721
Energy corrected milk yield kg cow^{-1} yr^{-1}	10,004	9,910	8,886	6,734

services. The composition of feeds was based on the Finnish feed table values. A reference scenario with a 50:50 ratio represented the current feeding practices for milking cows.

To assess the impacts on land use and the supply of ecosystem services, we applied a multi-criterial assessment with following indicators: proportion (%) of silage in the feed production area, energy corrected milk production (kg ECM ha^{-1}), proportion (%) of recycled N and P, methane emissions from cows (CH_4 ECM (g kg^{-1}), total CH_4 emission from cows and carbon input to the soils (kg ha^{-1}). To calculate the land area needed to produce feed, we used the average yields in the region with the exception of silage yields where because of lack of data for average yields we used our own estimation, 6,400 kg dry matter yr^{-1}. Methane emissions were based on the Lypsikki-model, and manure nutrient data were derived from the literature. The land area needed to produce feed in the 50:50 scenario was used as the available land area for milk production.

Results and discussion

Increasing the proportion of silage in cow diets increased milk production from the agricultural land area used for milk production (Figure 1). Even though higher forage proportion in cow diets decreased milk yield at the animal level, re-allocating of land from concentrate grain feed production to grass forage enabled an increased animal number, which resulted in increased total milk production from the same land area. This is because grass production requires less land area than barley and broad bean production due to the relatively low yields of barley and broad bean (3,390 and 1,887 kg ha^{-1}, respectively) in Finnish conditions. The current cultivated grass area in Uusimaa region is 35,300 ha, and the area needed for grass production would increase by 2,242 ha and 5,438 ha in the 75:25 and 100:0 scenarios, respectively.

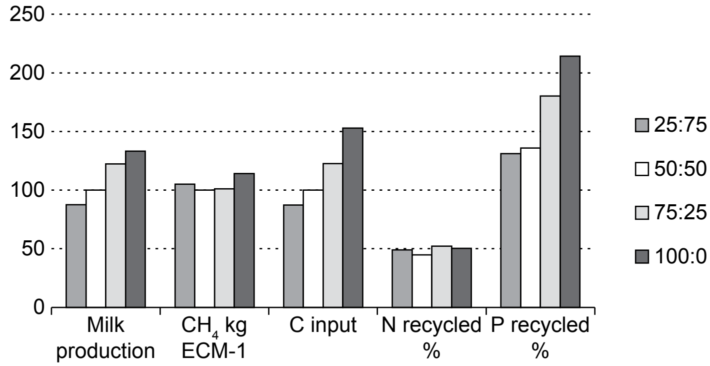

Figure 1. Milk production, methane (CH_4) emissions (kg energy corrected milk^{-1} and carbon (C) input are relative to reference diet (50:50) which is set to 100%. Share of recycled nitrogen (N) and phosphorus (P) is an absolute value in different scenarios.

Feeding ratios of 25:75 and 100:0 increased methane emissions per kilogram of produced milk (g kg^{-1}) but the 75:25 feeding ratio did not differ from 50:50 ratio (Figure 1). Absolute methane emissions from milking cows (kg yr^{-1}) were lower in the 25:75 feeding ratio but higher in the 75:25 and 100:0 than in the reference diet. This was because of the lower number of cows in the 25:75 and higher in the 75:25 and 100:0. The impact of higher absolute methane emissions in the 75:25 on environmental footprint of dairy production depends on whether the scenarios result in an increased total number of cows or if increased production replaces production from other regions. The proportion of recycled N increased in each scenario compared to the reference diet (Figure 1). P was extracted in the manure at 131-241% of the amount harvested in crops and silage. In practice, most dairy farms use industrial concentrate products and buy part of the cereals from other farms. Thus, in order to enhance nutrient cycling it would be important to increase co-operation with crop farms.

The impacts on land use and nutrient cycling are substantial when considering only the area used for milk production, but relatively small if considering the whole agricultural land use of the region. This is because the number of cows is low in the region.

Conclusions

Increasing forage into the diet of cows improves milk yield, carbon sequestration and cycling of nutrients from the area used for milk production. The same area of land used for feed production currently can produce more milk if the cow diets are based more on grass-feed. Instead of only measuring the efficiency of milk production by amount of milk per cow, land use should also be considered. This modelling study demonstrated the environmental benefits of silage in dairy cow diets and its role in supplying other ecosystem services.

References

Heikkinen, J., Ketoja, E., Nuutinen, V. and Regina, K. (2013) Declining trend of carbon in Finnish cropland soils in 1974-2009. *Global Change Biology* 19, 1456-1469.

Huhtanen, P. and Nousiainen, J. (2011) LYPSIKKI – a novel approach for the formulation of dairy cow rations. In: *Proceedings 2nd Nordic Feed Science Conference*, Uppsala, Sweden. pp. 62-67.

Karhu, K., Gärdenäs, A.I., Heikkinen, J., Vanhala, P., Tuomi, M. and Liski, J. (2012) Impacts of organic amendments on carbon stocks of an agricultural soil – comparison of model-simulations to measurements. *Geoderma* 189-190, 606-616.

Uusitalo, R., Turtola, E., Grönroos, J., Kivistö, J., Mäntylahti, V., Turtola, A., Lemola, R. and Salo, T.(2007) Finnish trends in phosphorus balances and soil test phosphorus. *Agricultural and Food Science* 16, 301-316.

Establishment of herbs in species-poor grassland

Krautzer B., Gaier L., Weber J., Graiss W. and Klingler A.
Agricultural Research and Education Centre Raumberg-Gumpenstein, 8952 Irdning, Austria

Abstract

Changes in land use have led to increasing loss of areas with flowering plants for pollinating insects. A field trial was conducted at Raumberg-Gumpenstein, in which six different methods of soil disturbance with over seeding, at two different sowing dates, were compared to determine whether it is possible to improve the biodiversity of species-poor extensive plant stands by seeding of a locally suitable perennial herb mixture. Floristic enrichment of species-poor grassland stands was found to be successful with the different techniques. No statistically significant difference could be detected between spring and late summer sowing.

Keywords: reseeding, plant diversity, species richness, pollinators

Introduction

There has been a massive decline of species-rich semi-natural grassland throughout Europe. In Austria, the loss of such habitats during the last 60 years has reached 840,000 ha, or nearly 50%, and this has resulted in a tremendous decline in plant biodiversity with corresponding effects on insect-biomass, leading to a loss of predators such as farmland birds. This extreme decline of diversity increasingly poses existential problems for bees, wild bees and other flower-pollinating insects (Seibold *et al.*, 2019). It is therefore necessary not only to protect such valuable habitats, but also to re-establish them into our cultural landscape. Due to changes in the farming systems, grasslands that were previously under intensive management have often been reduced to two-cut management, or are used as extensive pasture. After this extensification the vegetation often remains dominated by grasses, and contains only a small amount of herbs (dicots). The soil seed bank of grassland formerly under intensive management generally lacks seeds of most herb species, and so the change to more extensive management, by itself, seldom allows an increase in plant biodiversity. Many possibilities have been shown for promoting biodiversity by technical interventions in landscaping and public green areas (Scotton *et al.*, 2012), and there is also a great potential for improving plant biodiversity in species-poor agricultural grassland, managed with two to three cuts per year. A field experiment was therefore conducted to determine which methods can be used successfully to re-establish herbs into species-poor grassland by means of seed introduction.

Materials and methods

Seven different variants for over-seeding with herbs were tested at two sites with species-poor meadows, designated site A and B, situated 200 m apart, and located at the research institute Gumpenstein. Site characteristics were as follows: mean soil pH of 5.5; elevation 700 m a.s.l.; annual precipitation 1,030 mm, and mean temperature 8.2 °C. The management prior to the experiment was extensive grazing. Established took place at the beginning of August 2017 and April 2018. In each case there were seven variants: (1) no seeding (control), (2) seeding without soil opening, (3) iron rake, (4) scarifier, (5) strong harrow, (6) rotary strip seeder, and (7) reversible rotor harrow (Stoneburier). The treatments were established in rectangular plots of 2×4 m, each replicated 4 times. After these technical interventions, each plot was over-seeded with 2 g m^{-2} of a seed mixture containing 42 different grassland herbs. After seeding the plots were then consolidated with a prism roller.

There was a slow development of the introduced species during the first growing season in 2018, and so the vegetation survey took place at the beginning of June 2019. Vegetation cover of each species and species number of all established species were recorded eight times per variant within an area of 1×1 m. Statistical analyses were performed using R (R Core Team 2019). For evaluation, a linear model was developed and as Post Hoc Test a Tukey-Test with a significance level of 0.05 was performed.

Results and discussion

The over-seeding of the herb mixture was successful with all technical variants of soil disturbance/ surface cultivation. The observed values of the late summer (2017) trial were generally higher than those of the spring (2018) trial (Figure 1). This can be explained by the earlier seeding date and the resulting longer growing period. A comparison of the techniques with sward disturbance and sowing, relative to the control variant with no seeding, showed that the rotary strip seeder resulted in significantly better establishment. This is in accordance with results of Gornish *et al.* (2019) that the strip sowing technique has great potential, since the increased heterogeneity of the site leads to a significant increase in biodiversity. The reversible rotor harrow showed by far the best vegetation cover of the sown herbs. In the late summer seeding trial, in addition to the strip seeder, it was found that the scarifier and the strong harrow also showed significant differences relative to the control. The reversible rotor harrow again showed the best results.

A comparison of the number of established species from the seed mixture shows a very similar picture with regard to differences between the technical variants (Figure 2). With regard to the two sowing times, differences between technical variants as well as the average number of established species are comparable. The establishment success, however, increases with the degree of soil disturbance/opening, although the differences between the techniques are not so pronounced in the spring trial. The reversible rotor harrow

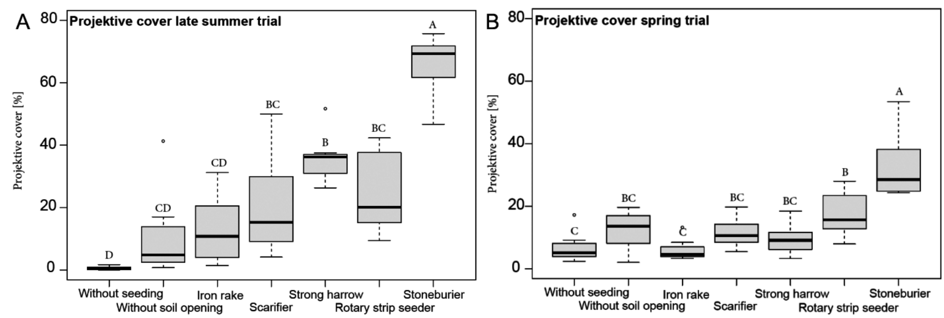

Figure 1. Comparison of the projective coverage of sown herbs after soil opening/ disturbance with different techniques, at two different sowing dates.

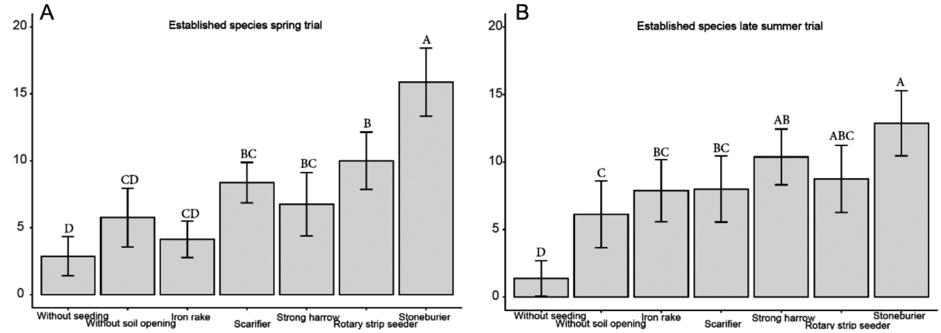

Figure 2. Number of established species of a sown herb mixture after soil opening with different techniques at two different sowing dates.

shows the highest number of established species. Experiments by Pywell *et al.* (2007), where the effect of complementary seeding of forbs after soil opening by harrowing was compared with slot-seeding, also confirm that the disturbance intensity of the soil has great effects on species richness and abundance. A comparison of the establishment success of the sown herbs shows clear differences. A total of 31 of the 42 species sown could be observed in the plots. Their establishment capacity has been ranked with regard to the number of observations as well as the projective cover achieved (Table 1). Species with good establishment success were observed in almost all treatments, while species with low establishment capacity were found only in treatments with the reversible rotor harrow.

Table 1. Establishment capacity of herbs.[1]

Good	Medium	Low
*Achillea millefolium**	*Silene vulgaris*	*Prunella grandiflora****
*Plantago lanceolata**	*Anthyllis vulneraria***	*Cichorium intybus****
Galium album	*Sanguisorba minor*	*Crepis biennis*
Leucanthemum vulgare	*Knautia arvensis*	*Lychnis flos-cuculi*
Stellaria graminea	*Leontodon hispidus*	*Verbascum nigrum*
Lotus corniculatus	*Dianthus carthusianorum*	*Dianthus superbus*
Trifolium pratense	*Centaurea jacea*	*Prunella vulgaris****
*Campanula patula**	*Daucus carota*	*Carum carvi*
*Rumex acetosa***	*Salvia pratensis*	*Galium verum****
*Hypericum perforatum***		*Betonica officinalis****
		Hypericum maculatum
		Silene dioica

[1] * = low share included in the original plant stands; ** = only established in the spring trial; *** = only established in the late summer trial.

Conclusions

Floristic enrichment of species-poor grassland with different over-sowing techniques was shown to be successful. The more the soil is opened by the technique applied before oversowing, the better the establishment success. The persistence of these species as a function of cutting frequency can only be assessed after several years of observation.

References

Gornish E.S., Shaw J. and Gillespie B.M. (2019) Using strip seeding to test how restoration design affects randomness of community assembly. *Restoration Ecology* 27, 1199-1205. https://doi.org/10.1111/rec.12988

Pywell R.F., Warman E.A., Walker K.J. and Bullock J.M. (2007) Enhancing grassland diversity using slot-seeding and harrowing. In: Britt C., Cherrill A., Duc M.L., Marrs R., Pywell R.F., Sparks T. and Willoughby I. (eds.) *Vegetation Management*. Wellesbourne: Association of Applied Biologists, pp. 191-198.

Scotton, M., Kirmer, A. and Krautzer, B. (eds.) (2012) *Practical handbook for seed harvest and ecological restoration of species-rich grasslands.* Padova, ISBN 978 88 6129 800 2, 124 pp.

Seibold, S., Gossner, M.M., Simons, N.K., Blüthgen, N., Müller, J., Ambarlı, D., Ammer, C., Bauhus, J., Fischer, M., Habel, J.C., Linsenmair, K.E., Nauss, T., Penone, C., Prati, D., Schall, P., Schulze, E.D., Vogt, J., Wöllauer, S. and Weisser, W.W. (2019) Arthropod decline in grasslands and forests is associated with landscape-level drivers. *Nature* 574, 671-674. https://doi.org/10.1038/s41586-019-1684-3

The effect of drought on the extractability of proanthocyanidins in sainfoin (*Onobrychis viciifolia*)

Malisch C.S.[1], Lewandowski L.[1], Salminen J.-P.[2], Taube F.[1] and Lüscher A.[3]
[1]Grass and Forage Science / Organic Agriculture, Institute of Crop Science and Plant Breeding, Christian-Albrechts-Universität zu Kiel, Hermann-Rodewald Str. 9, 24118 Kiel, Germany; [2]Natural Chemistry Research Group, Department of Chemistry, University of Turku, Vatselankatu 2, 20500 Turku, Finland; [3]Forage Production and Grassland Systems, Agroscope, Reckenholzstrasse 191, 8046 Zurich, Switzerland

Abstract

Proanthocyanidins (PAs) in forage legumes may be present in either extractable or non-extractable form. Despite potential benefits of the non-extractable fractions in ruminant nutrition, few studies have analysed the composition of PA fractions in forages and a potential genotype × environment interactions has generally been disregarded, thus far. Consequently, this study examined the impact of drought on the composition of PA fractions across five sainfoin (*Onobrychis viciifolia*) accessions and based on their ontogenetic stage. Generally, drought stressed plants showed a 44% higher (P<0.01) extractable PA concentration than rainfed plants. However, the increase was dependent on the ontogenetic stage, with generative plants showing a 27% increment (P<0.05), compared to 59% (P<0.001) in vegetative plants. Protein- and fibre-bound PAs were unaffected by drought independent of the ontogenetic stage and only accounted 7-13 and 2-4% of PAs. As the effectiveness of non-extractable PAs has not yet been established, no conclusions can be drawn with regards to the effect of these minor fractions on environmental and animal health effects. However, the low concentration combined with the absence of changes under drought indicates both a low effect size as well as a high predictability, both of which are beneficial for future analyses.

Keywords: *Onobrychis viciifolia*, non-extractable proanthocyanidins, condensed tannins, water stress

Introduction

Proanthocyanidins (PA, syn. condensed tannins) are oligomeric or polymeric plant secondary metabolites that have been shown to improve animal health and environmental performances in ruminant agriculture by acting anthelmintic and reducing methane emissions (Mueller-Harvey *et al.*, 2019).

In previous determinations of PA concentrations, the analysis was limited mainly to the acetone/water-soluble PA fraction. In addition to the fraction that is soluble in organic solvents, there are also insoluble PA fractions which are bound either to protein or fibre. There are, however, indications that these bonds can dissipate after the rumen, as a higher antiparasitic effect was detected in the abomasum compared to the rumen due to higher PA concentrations (Desrues *et al.*, 2017). Therefore, we have assessed not only the soluble PA fraction but also the proportions of protein- and fibre-bound fractions of the PA-containing fodder plants of sainfoin in order to estimate their bioactive value. Additionally, the effect of drought on the plants was determined to detect how environmental impacts might result in shifts among the PA fractions.

Materials and methods

Thirty sainfoin accessions were cultivated in a field experiment located in the North of Zurich, Switzerland (47°44' N 8°53' E, 482 m a.s.l). The experimental setup was described in Malisch *et al.* (2016). Briefly, of the thirty accessions, a subset of five accessions was used for PA analysis. Each treatment was replicated twice and contained nine individual plants per replicate, with a 0.25×0.5 m distance to each other (within and in between rows). Drought was induced using rainout shelters. These were designed to maintain environmental conditions (temperature, irradiance) which resembled the unsheltered control. Drought lasted for 127 days, from 12 June to 17 October 2013 and reached a soil water potential of below -1.5 MPa. Half of the plants were cut at week 7 of the drought period, shifting their ontogenetic stage back to vegetative, while the uncut plants continued generative growth. The experiment was carried out as a split-plot design with the four treatment combinations being the main plot and the accessions randomly distributed within, as subplots. The effect of the treatments and their interactions on the dependent variables were determined by using a linear mixed regression model, where the treatments and accessions were fixed factors, while the variances due to the block, the main plot, the subplot and the plant were assigned as random variable. Except mentioned otherwise, all results presented here are the mean values taken from two sampling events at peak drought, sampled at weeks 10 and 14 of the drought period. PA fractions were determined according to Terrill *et al.* (1992) using the HCl-Butanol assay.

Results and discussion

In the first 6 weeks, drought stress had no effect on the concentration of soluble, protein-bound or fibre-bound PAs (results not shown). Across week 10 and 14, the soluble PA (S-PA) concentration was significantly (P<0.05) higher in the drought stressed plants in the vegetative stage, while protein (P-PA) and fibre bound PA (F-PA) were not affected by environmental conditions (Table 1). Soluble PA generally accounted for 83-90% of the total PA and thus was by far the largest fraction, with P-PA ranging from 7 to 13% and F-PA from 2-4%.

The PA compositions averaged across environments were uniform across accessions with S-PA generally accounting for 83-87%, while P-PA and F-PA were in the range of 9-12 and 3-5%, respectively (Table 1). Also. PA compositions among accessions were stable across environments with no significant accession × treatment (P=0.1) or accession × treatment × sampling event (P=0.22) interaction.

The general PA composition for sainfoin without drought impact is in line with previous findings (Girard *et al.*, 2018). Regarding the impact of drought, the effect of increasing S-PA concentrations under drought has been discussed before (Malisch *et al*, 2016).The lack of differences in the protein and fibre bound PAs in sainfoin across environments and plant age was surprising, however, as plant age increases cell wall contents, whereas protein and cell content decrease, thus an increase in F-PA in generative plants was anticipated. At the same time, drought has been shown to reduce neutral and acid detergent fibre

Table 1. Concentration of soluble (S-PA), protein-bound (P-PA), fibre-bound (F-PA) and total PAs dependent on treatments averaged over harvests after 10 and 14 weeks, for plants with (cut+) or without (cut-) additional cutting, and with (drt) or without (ctr) rain exclusion (top), or averaged across all treatments for each of the five accessions (bottom).

		PA (mg g^{-1} DM)			
		S-PA	P-PA	F-PA	Total
Treatment	ctr/cut-	13.13 a	2.07 a	0.69 a	15.89 a
	ctr/cut+	15.18 a	1.76 a	0.54 a	17.48 a
	drt/cut-	16.74 a	1.34 a	0.63 a	18.71 a
	drt/cut+	24.14 b	1.97 a	0.61 a	26.72 b
	SE	0.73	0.09	0.03	0.75
Accession	CPI 63750	15.38 a	1.67 a	0.57 a	17.65 a
	Esparsette	15.54 a	1.67 a	0.62 ab	17.83 a
	Perly	11.57 b	1.67 a	0.67 b	13.92 b
	Taja	15.76 a	1.84 a	0.62 ab	18.28 a
	Visnovsky	12.64 b	1.85 a	0.56 a	15.07 b
	SE	0.60	0.06	0.03	0.60

(NDF and ADF), as well as lignin in forages, hence control treatments should generally have higher F-PA concentrations (Peterson *et al.*, 1992). Generally, the P-PA and F-PA shares can be considered low in comparison with other plant species, as for example in birdsfoot trefoil (*Lotus corniculatus*) cv. Polom, S-PAs accounted for 38% and F-PA for 11% (Girard *et al.*, 2018).

Conclusions

The results of this study show that the proportions of bound proanthocyanidins in sainfoin are likely to be of lesser significance compared to soluble PAs due to their much lower concentrations. This increases the likelihood that the analysis of S-PA is most important in sainfoin. Together with the fact that only S-PA were affected by the environment, and that this behaviour was uniform across all accessions, this can be considered very promising as this facilitates the predictability of future studies with sainfoin.

Acknowledgements

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References

Desrues O., Mueller-Harvey I., Pellikaan W., Enemark H.L. and Thamsborg S.M. (2017) Condensed tannins in the gastrointestinal tract of cattle after sainfoin (*Onobrychis viciifolia*) intake and their possible relationship with anthelmintic effects. *Journal of Agricultural and Food Chemistry* 65, 1420-1427.

Girard M., Dohme-Meier F., Kragten S., Große-Brinkhaus A., Arrigo Y., Wyss U. and Bee G. (2018) Modification of the proportion of extractable and bound condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) and sainfoin (*Onobrychis viciifolia*) during wilting, ensiling and pelleting processes. *Biotechnology in Animal Husbandry* 34, 1-19.

Malisch C.S., Salminen J.-P., Kölliker R., Engström M.T., Suter D., Studer B. and Lüscher A. (2016) Drought effects on proanthocyanidins in sainfoin (*Onobrychis viciifolia* Scop.) are dependent on the plant's ontogenetic stage. *Journal of Agricultural and Food Chemistry* 64, 9307-9316.

Mueller-Harvey I., Bee G., Dohme-Meier F., Hoste H., Karonen M., Kölliker R., Lüscher A., Niderkorn V., Pellikaan W.F., Salminen J.-P., Skøt L., Smith L.M.J., Thamsborg S.M., Totterdell P., Wilkinson I., Williams A.R., Azuhnwi B.N., Baert N., Brinkhaus A.G., Copani G., Desrues O., Drake C., Engström M., Fryganas C., Girard M., Huyen N.T., Kempf K., Malisch C., Mora-Ortiz M., Quijada J., Ramsay A., Ropiak H.M. and Waghorn G.C. (2019) Benefits of condensed tannins in forage legumes fed to ruminants: importance of structure, concentration, and diet composition. *Crop Science* 59, 861-885.

Peterson P.R., Sheaffer C.C. and Hall M.H. (1992) Drought effects on perennial forage legume yield and quality. *Agronomy Journal* 84, 774-779.

Terrill T.H., Rowan A.M., Douglas G.B. and Barry T.N. (1992) Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of the Science of Food and Agriculture* 58, 321-329.

Trade-offs between yields, forage quality and botanical diversity in permanent grasslands of the Vosges Mountains in France

Mesbahi G.[1,2], Bayeur C.[2] and Plantureux S.[1]

[1] Université de Lorraine, Inrae, LAE, 54000 Nancy, France; [2] Parc Naturel Régional des Vosges du Nord, 67290 La Petite Pierre, France

Abstract

Assessing trade-offs between forage production and ecological characteristics delivered by grasslands is a growing concern for stakeholders and scientists. We sampled 50 grasslands from the Vosges Mountains (north-eastern France), and measured the agronomic (forage yield and quality) and biodiversity characteristics of each grassland. We assessed yield through dry matter production; fodder quality through organic matter digestibility, protein, energy and mineral content; and biodiversity through total and oligotrophic plant species richness and ecological indices. Using a Hierarchical Clustering on Principal Components, our results show that grasslands can be classified into three classes. The first class is made of grasslands associated with high quality forage but poor ecological value, the second of diverse and productive grasslands associated with poor forage quality, and the last one of grasslands and moors of high ecological value but poor forage yield and quality. These classes are mainly determined by agricultural practices and soil properties. Our study highlights the trade-offs between the agronomic and ecological characteristics of grasslands: grasslands cannot produce high yields, qualitative forage and protect biodiversity at the same time. We argue that agronomists and naturalists must work together at both farm- and landscape-scales to produce forage in sufficient quantity and quality while protecting biodiversity.

Keywords: yield, forage quality, species diversity, agroecological characteristics, soil

Introduction

Permanent grasslands provide diverse agroecological characteristics of global importance, such as forage production and species habitat. It is generally accepted that grasslands can either produce high quantity of forage or support biodiversity. Indeed, grasslands are mainly fertilised to increase yield, which decreases botanical diversity as side-effect. Conversely, the effects of biodiversity on yield is still debated: no agreement has been found with regard to permanent grasslands (Li *et al.*, 2019). Forage from unfertilised grasslands (Aydin and Uzun, 2005) and species-rich grasslands (French, 2017) could have higher quality due to the presence of legumes. So far, few studies have considered forage yield, forage quality and biodiversity simultaneously in order to study trade-offs and synergies. Moreover, biodiversity is often only studied in terms of species richness, without interest in the ecological value of different species. We hypothesized that yield is negatively correlated to forage quality and diversity of vascular plants.

Materials and methods

We studied botanical composition of 50 grasslands (complete list of species of the main vegetation community, contribution to biomass of species in 6 plots 70×70 cm) a few days before their first utilisation, in the Vosges Mountains (north-eastern France). Climate and geology vary through the influence of latitudinal, longitudinal and altitudinal gradients.

The first aim of this study was to analyse trade-offs between grassland characteristics. We assessed yield through dry matter production; fodder quality through organic matter digestibility, protein, energy and mineral content; and biodiversity through total plant species richness, diversity indices and oligotrophic species richness as proxy of preservation of patrimonial habitats. We used a Hierarchical Clustering on Principal Components (HCPC) to observe trade-offs among characteristics. The second aim was to identify the determinants of the trade-offs, so we added determinants related to environment, management and vegetation to the HCPC as supplementary variables.

Results and discussion

The five first components explained 85.3% of the variance, and the classification produced three classes of grasslands. The first class regrouped 15 grasslands, the second class 27 grasslands and the third class 8 grasslands. Characteristics and determinants related to each class are described in Table 1.

The second class highlighted a positive correlation between biodiversity and yield, which is still largely debated: yield could be more influenced by key species or traits than biodiversity (Mahaut *et al.*, 2020). However, the second class confirmed the negative correlation between diversity and digestibility (Hofmann and Isselstein, 2005), but contrasted with studies demonstrating synergy between diversity and protein content (Aydin and Uzun, 2005; French, 2017). This class also confirmed that mowing and late use improve botanical diversity (Fischer and Wipf, 2002).

The third class showed trade-offs between total botanical diversity and oligotrophic species richness, highlighting that species richness is not necessarily a useful indicator of ecological value (Pykälä *et al.*, 2005). This class also confirmed that oligotrophic species are favoured by poorly fertilised grasslands (Garnier *et al.*, 2018), but contrasted with previous studies assuming that legumes are favoured by weakly fertile soils (Suding *et al.*, 2005).

Table 1. Results of grassland classification (HCPC).[1]

			Class 1	Class 2	Class 3	Overall mean
Characteristics	Quantity	Yield (Mg ha^{-1})	-	**2.1**	1.0	1.8
	Quality	Digestibility (%)	**74**	64	-	67
		Protein	**63.0**	55.7	54.0	57.6
		Energy	**0.98**	0.81	-	0.86
		Ca	**0.74**	-	0.36	0.61
		K	**2.54**	1.57	1.36	1.82
		Mg	**0.29**	-	0.17	0.23
		P	**0.34**	0.20	-	0.24
	Plant diversity	Total richness	-	**32.3**	23.1	30.5
		Shannon index	-	**3.56**	2.66	3.36
		Simpson index	-	**0.88**	0.76	0.85
		Oligotrophic richness	5.6	-	**9.7**	7.1
Determinants	Climate	Mean temperature (°C)	-	-	8.5	9.3
	Soil	Clay (%)	-	**20.8**	-	17.7
		Silt (%)	-	**34.8**	-	30.8
		Sand (%)	-	44.6	**64.1**	51.6
		pH	-	**5.9**	4.8	5.6
	Management	Date of 1st use (heat unit)	605	**1,035**	-	910
		Cut/grazing	-	**0.80**	-	0.63
	Soil + management	C/N	-	-	**12.8**	11.7
	Vegetation	Legumes (%)	-	-	4.0	9.9

[1] Characteristics and determinants significantly related to each class ($P<0.05$) are shown. In bold, values that are above overall mean.

First and third classes underlined trade-offs between oligotrophic species richness and forage yield or quality, which could cause difficulties to conserve these species already threatened by intensification (Garnier *et al.*, 2018). Finally, first and second classes confirmed that early use of grasslands increases forage quality (Bruinenberg *et al.*, 2002). In the present study, forage quality did not include the quality of products like milk, cheese and meat, nor animal health. These characteristics could affect the trade-offs between botanical diversity and forage quality, as they are improved by diversity (Martin *et al.*, 2005; Poutaraud *et al.*, 2017).

Conclusions

Our study highlighted the trade-offs between agronomic and ecological value of grasslands. Grasslands could not produce high yields, quality forage and protect botanical diversity at the same time. Soil and management mainly determined these trade-offs. Agronomists and conservation scientists must work together at both farm- and landscape-scales to find solutions for producing forage of sufficient quantity and quality while protecting biodiversity.

Acknowledgements

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References

Aydin I. and Uzun F. (2005) Nitrogen and phosphorus fertilization of rangelands affects yield, forage quality and the botanical composition. *European Journal of Agronomy* 23, 8-4.

Bruinenberg M.H., Valk H., Korevaar H. and Struik P.C. (2002) Factors affecting digestibility of temperate forages from seminatural grasslands: a review. *Grass and Forage Science* 57, 292-301.

Fischer M. and Wipf S. (2002) Effect of low-intensity grazing on the species-rich vegetation of traditionally mown subalpine meadows. *Biological Conservation* 104, 1-11.

French K.E. (2017) Species composition determines forage quality and medicinal value of high diversity grasslands in lowland England. *Agriculture, Ecosystems & Environment* 241, 193-204.

Garnier E., Fayolle A., Navas M.-L., Damgaard C., Cruz P., Hubert D., Richarte J., Autran P., Leurent C. and Violle C. (2018) Plant demographic and functional responses to management intensification: A long-term study in a Mediterranean rangeland. *Journal of Ecology* 106, 1363-1376.

Hofmann M. and Isselstein J. (2005) Species enrichment in an agriculturally improved grassland and its effects on botanical composition, yield and forage quality. *Grass and Forage Science* 60, 136-145.

Mahaut L., Fort F., Violle C. and Freschet G.T. (2020) Multiple facets of diversity effects on plant productivity: species richness, functional diversity, species identity and intraspecific competition. *Functional Ecology* 34 (1), 287-298.

Martin B., Verdier-Metz I., Buchin S., Hurtaud C.and Coulon J.-B. (2005) How do the nature of forages and pasture diversity influence the sensory quality of dairy livestock products? *Animal Science* 81.

Poutaraud A., Michelot-Antalik A. and Plantureux S. (2017) Grasslands: A source of secondary metabolites for livestock health. *Journal of Agricultural and Food Chemistry* 65, 6535-6553.

Pykälä J., Luoto M., Heikkinen R.K. and Kontula T. (2005) Plant species richness and persistence of rare plants in abandoned semi-natural grasslands in northern Europe. *Basic and Applied Ecology* 6, 25-33.

Suding K.N., Collins S.L., Gough L., Clark C., Cleland E.E., Gross K.L., Milchunas D.G. and Pennings S. (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences of the USA* 102, 4387-4392.

Changes in botanical composition of a pasture during six years after reintroduction of cattle grazing

Mrázková M.[1], Kolářová M.[2], Holec J.[2], Bilošová H.[1], Hadrová S.[1] and Tyšer L.[2]
[1]Agrovýzkum Rapotín Ltd., Výzkumníků 267, 788 13 Vikýřovice, Czech Republic; [2]Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague, Czech Republic

Abstract

The aim of this study was to evaluate changes in the floristic composition near Švýcárna lodge (the Czech Republic, 1,304 m a.s.l.), where cattle grazing was introduced on a pasture area of 3.6 ha in 2012 following long-term management cessation. The pasture was divided into two grazing sub-localities: P1 (Nar) with a dominance of *Nardus stricta* and *Avenella flexuosa* and P2 (Des) with a dominance of *Deschampsia cespitosa*. For grazing, Highland cattle were used with a stocking rate up to 1 livestock unit per ha and year. The floristic compositions of the plots were evaluated and changes in the species richness analysed by regression. After six years of restored grazing it was found that the overall species richness was enhanced.

Keywords: suckler cows, renewed grazing, species diversity, nature conservation, cattle

Introduction

Grazing is considered to be a suitable tool for maintaining the biodiversity of grasslands, as reviewed by Metera *et al.* (2010). The same was also proven for the territory of the Czech Republic (Mládek *et al.,* 2006; Pavlů *et al.,* 2007). For this reason, grazing is renewed in the Czech Republic in the protected areas where it was carried out in the past (e.g. the White Carpathian Mountains (Mts.), the Giant Mts., the Beskydy Mts.). In 2012, grazing was restored in the Hrubý Jeseník Mts. (the Praděd National Natural Reserve) in the Švýcárna lodge surroundings. The main aim of cattle grazing reintroduction was to reinforce non-productive functions of alpine grasslands, mainly floristic diversity. The aim of the study was to evaluate changes in floristic composition in the locality of Švýcárna lodge (1,304 m a.s.l., the Praděd National Natural Reserve), where cattle grazing was introduced in 2012 after a long-term (70 years) management cessation.

Materials and methods

The research was conducted in the mountain area near Švýcárna lodge situated in the Hrubý Jeseník Mts. (the Praděd National Nature Reserve; 1,304 m a.s.l.). An average annual temperature is 0.9 °C and annual precipitation is 1,231 mm, in Praděd (2.5 km distant from the Švýcárna lodge). The rotational grazing system (three grazing cycles per year) was conducted on the site, which was divided by the road into two grazing sub-localities differing in dominant grass species. The sub-locality P1 (Nar) with a dominance of *Nardus stricta* and *Avenella flexuosa* was situated above the lodge, while the sub-locality P2 (Des) with a dominance of *Deschampsia cespitosa* was situated below the lodge. The whole plot area was 3.6 ha. The stocking rate was up to 1 livestock unit per ha and year.

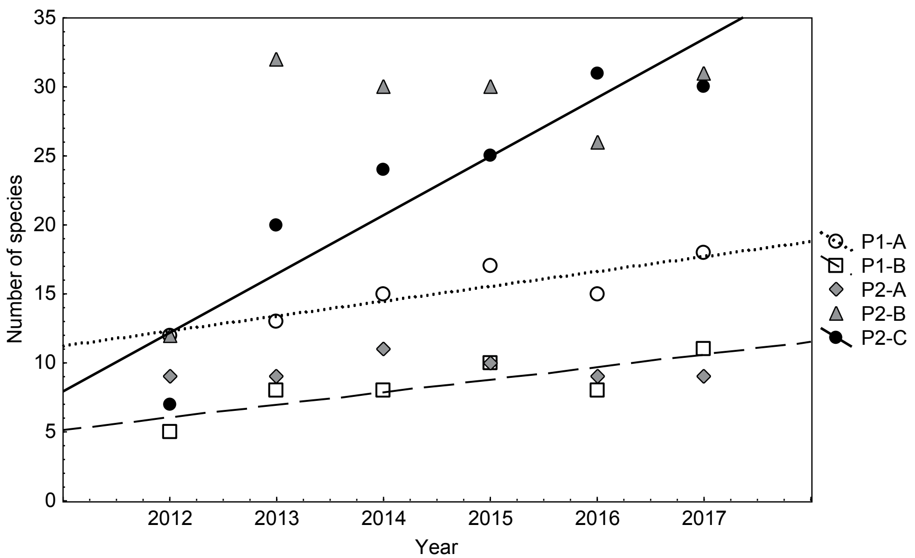
To monitor changes in floristic composition, five permanent plots (one plot area: 5×5 m) were established in 2012 at different sites on the grazed area (two in the sub-locality P1: P1-A, P1-B; and three in the sub-locality P2: P2-A, P2-B, P2-C). The floristic composition was determined each year at the beginning of July. We evaluated recorded phytosociological relevés (Moravec *et al.,* 1994) on the permanent plots. The number of species for each plot in relation to the year was analysed by linear regression model in the Statistica (version 13.2).

Results and discussion

In total, 67 plant species were found in the experimental area over the 6 years. Of the total number of plant species found, 15 were in the Red List of Vascular Plants of the Czech Republic (Grulich, 2017). On all experimental plots, the species richness increased from 2012 to 2017 (Figure 1), and one and three the change was statistically significant. The strongest increase and the highest mean number of species were recorded on plots with high soil moisture (P2-B: 32 species; P2-C: 31 species). This was probably a consequence of mechanical soil disturbance caused by cattle movement and thus creation of small open habitats free of vegetation with the possibility for establishing new species. Soil moisture, however, was evaluated only visually.

Almost independently of the vegetation type, cessation of grassland management leads to a successional change and loss of plant species diversity (Isselstein *et al.*, 2005). Therefore, positive changes in localities where renewed grazing was introduced after a long-term of grassland management cessation can be expected regarding floristic composition and plant species diversity. Animal browsing and trampling causes the creation of open habitats that enable many plant species to germinate and survive. Grazing also affects the return of nutrients into the soil, which changes chemical properties of the soil (Krahulec *et al.*, 1996).

So far, ecologists have not been able to establish one general, unifying theory for successionally induced changes in species composition following abandonment (Kahmen and Poschlod, 2004). The course of succession seems to be unique for each site and year. As Tasser and Tappeiner (2002) explained, succession starts immediately after abandonment. Depending on altitude, succession proceeds at variable speeds and with different numbers of stages. According to Hejcman *et al.* (2002) the typical pasture sward does not



P1-A: y = -2,172.1714 + 1.0857*x; r = 0.8907; *P*=0.0173
P1-B: y = -1,833.4952 + 0.9143*x; r = 0.8281; *P*=0.0418
P2-C: y = -8,553.181 + 4.2571*x; r = 0.9102; *P*=0.0117
P2-A: r = -0.0639; *P*=0.9043
P2-B: r = 0.5453; *P*=0.2631

Figure 1. Scatterplots and linear regression lines of the species number in the experimental plots against the year; regression equations and correlation coefficients (*r*).

recreate itself earlier than 40 years after the renewed grazing introduction. Nevertheless, some positive results can be found in short-term periods as some current studies have indicated.

Our study showed that renewed cattle grazing results in an increase in plant species richness. Another example of the successful introduction of renewed grazing of small ruminants is the experimental pasture of sheep in the National Nature Reserve Mohelno Serpentine Steppe (Veselý and Řepka, 2005). A similar approach with positive results was also applied in the Protected Landscape Area Beskydy and in the Protected Landscape Area Bílé Karpaty (Piro and Wolfová, 2008). Traditional ways of grassland management via sheep and cattle grazing are used in the Krkonoše National Park and in the Louny Central Uplands in the Czech Republic.

Conclusions

This study, conducted in the Protected Landscape Area Jeseníky, shows that renewed cattle grazing in the surroundings of Švýcárna lodge is both a symbolic revival of the traditional way of grassland management and primarily a tool for suppressing ecological succession (which leads to the overgrowing of biologically valuable habitats) and to enhance species diversity. After six years of restored grazing, the overall species richness was enhanced. This was most likely due to cattle trampling which has enabled the creation of small open habitats for new species germination and survival.

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References

Grulich V. (2017) The red list of vascular plants of the Czech Republic. *Příroda*, 35, 75-132. (in Czech)

Hejcman M., Pavlů V. and Krahulec F. (2002) Livestock grazing and its use in conservation practice. *Zprávy České botanické společnosti*, 37, 203-216. (in Czech)

Isselstein J., Jeangros B. and Pavlů V. (2005) Agronomic aspects of biodiversity targeted management of temperate grasslands in Europe – a review. *Agronomy Research*, 3, 139-151.

Kahmen S. and Poschlod P. (2004) Plant functional trait responses to grassland succession over 25 years. *Journal of Vegetation Science*, 15, 21-32.

Krahulec F., Blažková D., Balátová-Tuláčková E., Štursa J., Pecháčková S. and Fabščičová M. (1996) Giant Mountains meadows: plant communities and their dynamics. *Opera Corcontica*, 33, 3-250. (in Czech)

Metera E., Sakowski T., Słoniewski K. and Romanowicz B. (2010) Grazing as a tool to maintain biodiversity of grassland – a review. *Animal Science Papers and Reports*, 28, 315-334.

Mládek J., Pavlů V., Hejcman M. and Gaisler J. (2006) *Grazing as a means of maintaining permanent grassland in protected areas*. VÚRV, Praha. (in Czech)

Moravec J. (ed.) (1994) *Phytocoenology*. Academia, Praha. (in Czech)

Pavlů V., Hejcman M., Pavlů L. and Gaisler J. (2007) Restoration of grazing management and its effect on vegetation in an upland grassland. *Applied Vegetation Science* 10, 375-382.

Piro Z. and Wolfová J. (eds.) (2008) *Preserving biodiversity of the Carpathian meadows*. FOA – Nadační fond pro ekologické zemědělství, Praha. (in Czech)

Tasser E. and Tappeiner U. (2002) Impact of land use changes on mountain vegetation. *Applied Vegetation Science* 5, 173-184. https://doi.org/10.1111/j.1654-109X.2002.tb00547.x

Veselý P. and Řepka R. (2005) Specification of vegetation evaluation after 5 years sheep grazing in NNR Mohelno Serpentine Steppe. In: *Proc. VIth Kábrt's Dietetic Days*, University of Veterinary and Pharmaceutical Sciences in Brno, pp. 58-61. (in Czech)

Grazing absence after pastoral fires leads to a plant diversity loss in high-valuable Pyrenean grasslands

Múgica L.[1,2], Canals R.M.[1,2], Peralta J.[3] and San Emeterio L.[1,2]

[1]Dpto. de Agronomía, Biotecnología y Alimentación, Universidad Pública de Navarra, Campus Arrosadia s/n, 31006 Pamplona, Spain; [2]ISFOOD – Institute for Innovation & Sustainable Development in Food Chain, Universidad Pública de Navarra, Campus Arrosadia s/n, 31006 Pamplona, Spain; [3]Dpto. Ciencias, Universidad Pública de Navarra, Campus Arrosadia s/n, 31006 Pamplona, Spain

Abstract

Global change is decoupling traditional land uses, reducing grazing and changing the patterns of controlled fires in Europe, thus threatening landscape diversity. This study aimed at analysing the efficacy of the current herbivorism pressure to control shrub sprout after traditional burnings in temperate grasslands of SW Pyrenees. Three experimental sites were established, comprising the combination of pastoral burning (done/undone) and grazing (current/no grazing). Floristic inventories and height measurements (the shrub *Ulex gallii* and the perennial grass *Brachypodium rupestre*) were done in summer during seven consecutive years after burnings. Current grazing reduced *U. gallii* and *B. rupestre* dominance in the long-term compared with the non-grazing situation. When the cover of *B. rupestre* was higher than 60%, *U. gallii* declined sharply, suggesting competition for habitat dominance between these two species. Grazing notably enhanced plant diversity, but site characteristics were important too. Our results highlight how land-use changes affect mountain grasslands dynamics and the importance of site-specific features.

Keywords: pyric herbivory, grazing reduction, gorse-encroachment, *Brachypodium rupestre*, plant diversity

Introduction

Inherited natural grasslands in Europe are the result of the combination of traditional pastoral burnings and grazing practices occurring during millennia. In recent decades, global change has been reducing livestock grazing pressure on grasslands and in some areas the use of prescribed fires to control shrub encroachment has increased. Though different scenarios arise due to land use changes, most of the landscape is becoming dominated by shrub and forest encroachment, threatening the diversity of highly valuable grasslands and increasing the risk of wildfires (Canals, 2019). Knowledge is scarce on the structural, floristic and diversity changes in plant communities due to the alteration of the traditional fire and herbivory regimes, as well as on its consequences on the evolution of diverse grasslands and mosaic landscapes. This long-term monitoring experiment aimed to quantify the efficacy of current livestock grazing (lower stocking rates than previously) to control shrub regrowth after traditional bush-to-bush pastoral fires in lightly gorse-encroached grasslands of SW Pyrenees.

Materials and methods

The research area, Aezkoa Valley in South-Western Pyrenees (43°0'N 1°10'W), has a rainy and misty temperate climate (Temp = 9.3 °C; rainfall 1,990 mm yr^{-1}). Soils are organic and acidic, with clay-loamy textural classes and moderate nutrient contents. The landscape is formed by beech forests, gorselands of *Ulex gallii* Planch. and diverse perennial grasslands. The area supports a traditional extensive mixed grazing from May to October and periodic bush-to-bush winter burnings of small and dispersed areas (San Emeterio *et al.*, 2016). In early spring 2012 we selected three sites (Ezkanda, Azalegi and Aritzelate) in a lightly gorse-encroached area (1,100 m a.s.l., slopes >15°). The experimental design comprised the combination of traditional burnings and grazing in the three sites: burned-grazed, burned-ungrazed, unburned-grazed and unburned-ungrazed. We surveyed the vegetation every summer from 2012 to 2018:

(1) quadrat cover method for gorse regrowth and diversity in burned patches (overall 144, 18 per date), and (2) point quadrat method for floristic composition evolution in the four subplots (overall 84, 12 per date). Besides, *U. gallii* (burned patches, 126 per date, 2017 and 2018) and *Brachypodium rupestre* (Host) Roem. & Schult. (10 plants per subplot, 2012-2018) heights were measured. Generalized additive mixed models (GAMMs) in R v.3.5.2. (R Core Team, 2018) were used to evaluate plant cover, biodiversity indexes and *B. rupestre* height, linear mixed-effect models for *U. gallii* height and multivariate ordination approach for floristic composition evolution in the four subplots.

Results and discussion

Mid-term plant communities evolution after the burnings depended strongly on the occurrence of herbivory. Despite that grazing exclusion generally allowed gorse regrowth and decreased herbaceous cover (F=11.18, P=0.001), the final outcomes were site-specific (Table 1, Figure 1). The cover of *B. rupestre* was not affected by grazing (Table 1); however, herbivory affected significantly the height attained by the grass (F=39.42, P<0.001) and by *U. gallii* (LR=17.432, P<0.001). The results suggested competition for grassland dominance between these two species, the taxa most benefited by the post-burning conditions (Reyes *et al.*, 2009; San Emeterio *et al.*, 2016), due to shading. At the Ezkanda site, when *B. rupestre* cover was higher than 60%, *U. gallii* had lower cover than 35% (Table 1). Although floristic composition in the first year was similar among subplots, they diverged greatly over time at the three sites. We reported two gradients in excluded subplots depending on the site: from grazed-tolerant grass-dominated communities (lower-right side) to (1) *B. rupestre*-dominated communities (lower-left side, Ezkanda) and to (2) *U. gallii*-expanded communities (upper-left side, Azalegi and Aritzelate) (Figure 1). Grazing pressure significantly enhanced grassland diversity, species richness and Shannon diversity index, but the outcomes varied between sites (Table 1). Animals foraging and trampling create small areas of bare soil (F=25.94, P<0.001) and vegetation gaps, providing ecological niches for new species and adding heterogeneity and complexity to the post-fire dynamics (Canals and Sebastià, 2000).

Table 1. *Ulex gallii* and. *Brachypodium rupestre* cover and diversity indexes for grazed (GD) and ungrazed (UG) burned patches in the first and the last year of study.[1]

Variable	Year	*Ezkanda*		*Azalegi*		*Aritzelate*		Parametric terms		
		GD	UG	GD	UG	GD	UG	*df*	*F*	*P*-value
U. gallii	2012	8.2	16.4	6.6	14.8	16.3	24.5	*S* 2	15.73	<0.001
		(-2.0, 18.5)	(5.7, 27.2)	(-4.5, 17.6)	(3.7, 25.8)	(5.3, 27.3)	(13.5, 35.5)	*G* 1	10.7	0.001
	2018	8.2	34.6	39.9	66.3	68.8	95.2			
		(-2.0, 18.5)	(14.0, 55.2)	(16.8, 63.1)	(43.1, 89.6)	(45.6, 92.0)	(71.9,118.5)			
B. rupestre	2012	43.1		20.6		24.7		*S* 2	15.21	<0.001
		(29.2, 57.1)		(11.7, 29.6)		(14.0, 35.3)				
	2018	70.9		20.6		24.7				
		(56.3, 85.6)		(11.7, 29.6)		(14.0, 35.3)				
Species richness	2012	10.6	10.1	12.5	12.1	12.7	12.3	*S* 2	4.58	0.012
		(7.8, 11.5)	(8.7, 11.6)	(10.7, 14.3)	(10.3, 13.8)	(11.1, 14.3)	(10.7, 13.9)	*G* 1	54.3	<0.001
	2018	16.7	7.6	18.6	9.5	18.9	9.7			
		(15.0, 18.5)	(5.8, 9.3)	(16.7,20.6)	(7.5, 11.4)	(17.0, 20.8)	(7.8, 11.5)			
Shannon index	2012	1.07	1.29	1.52	1.74	1.49	1.71	*S* 2	7.05	0.001
		(0.86, 1.27)	(1.08, 1.49)	(1.31, 1.72)	(1.54, 1.94)	(1.27, 1.70)	(1.50, 1.92)	*G* 1	13.25	<0.001
	2018	1.63	0.94	1.94	1.26	1.56	0.88			
		(1.39, 1.87)	(0.70, 1.19)	(1.71, 2.19)	(1.03, 1.50)	(1.31, 1.81)	(0.63, 1,13)			

[1] Predicted values, 95% confidence interval, and significances of parametric terms from GAMM models (*S*, site; *G*, grazing).

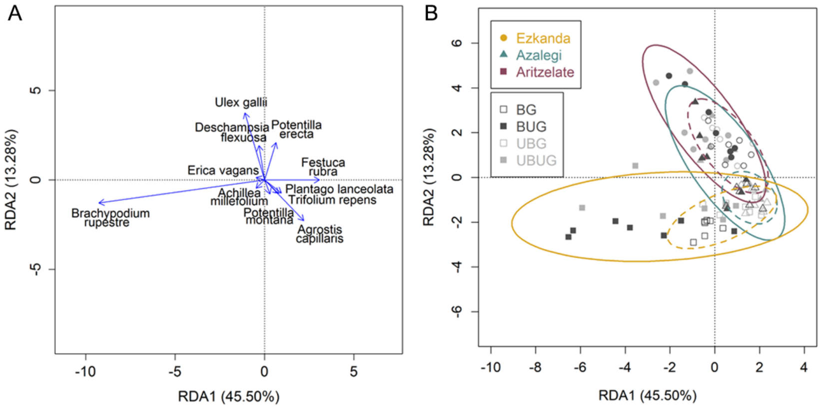

Figure 1. (A) Correlation among most abundant species vectors. (B) Ordination diagram of inventories; confidence ellipses (95%) for site-grazing interaction; grazed (G, dotted lines) and ungrazed (UG; solid lines); burned (B, dark grey) and unburned (UB, light grey).

Conclusions

The current grazing pressure in temperate grasslands of SW Pyrenees, despite its decline, maintains diverse grasslands after traditional fires applied for gorse expansion control. However, site-specific features are important when managing pastoral burnings and livestock grazing in mountain ecosystems, since the expansion of perennial tall grasses of difficult control, such as *B. rupestre*, may occur as a response to the changing disturbance regimes.

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References

Canals R.M. and Sebastià M.T. (2000) Analyzing Mechanisms regulating diversity in rangelands through comparative studies: a case in the Southwestern Pyrennees. *Biodiversity and Conservation* 9, 965-984.

Canals R.M. (2019) Landscape in motion: revisiting the role of key disturbances for the preservation of mountain ecosystems. *Cuadernos de Investigación Geográfica* 45(2), 515-531.

R Core Team (2018) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna.

Reyes O., Casal M. and Castro F. (2009) Resprouting ability of six Atlantic shrub species. *Folia Geobotanica* 44(1), 19-29.

San Emeterio L., Múgica L., Ugarte M.D., Goicoa T. and Canals R.M. (2016) Sustainability of traditional pastoral fires in highlands under global change: effects on soil function and nutrient cycling. *Agriculture, Ecosystems and Environment* 235, 155-163.

The influence of diverse grasslands on nitrous oxide emissions from urine and dung patches

Nyameasem J.K., Reinsch T., Malisch C., Loges R., Kluß C. and Taube F.

Department of Grassland and Forage Science/Organic Agriculture, Christian-Albrechts-Universität zu Kiel, Hermann-Rodewald-Straße 9, 24118 Kiel, Germany

Abstract

Incorporating alternative forage species into grazing systems is a potential approach to mitigate N_2O emissions, but empirical data to support this is limited. At the organic research farm Lindhof in Northern Germany, we investigated the response of grassland leys containing two to eight species in 2018, and from an irrigated binary mixture in 2019, on N_2O emissions from cow excreta patches. N_2O emissions were measured using the static chamber method starting immediately after excreta application in spring, summer and autumn and continued for 100 days. The cumulative N_2O-N emissions ranged from 0-0.08, 0.04-1.83, 0.23-2.49 kg ha^{-1} for the untreated, dung-treated and urine-treated grassland, respectively. The effect of grassland type on N_2O emission was only evident in dung-treated patches in spring. This remained true even after irrigating the plots to increase soil moisture to optimal conditions for N_2O emission. Despite increments in dung and urine N_2O-N emission factor (EF) by 19 and 55%, respectively, this was not significant. The study suggests that other confounding factors might have controlled N_2O emissions. The N_2O emission factors (EF) were generally much lower than the 2% currently utilised by the IPCC.

Keywords: emission factor, grassland mixtures, greenhouse gas, nitrogen, perennial ryegrass

Introduction

Livestock grazing is a major source of nitrous oxide (N_2O) due to the high N loads (up to 2,000 kg ha^{-1}) from urine and dung patches on pastures (Selbie *et al.*, 2015). Despite economic and ecological benefits of intensive grazing, there is high uncertainty about its greenhouse gas mitigation potential. Although several mitigating practices exist, economic and technical constraints limit their adoption, particularly in low-input and organic dairy systems. A potential approach to mitigate N_2O emission is to incorporate diverse forage species with diverse functions into grazing systems. Some plant species may mitigate N_2O emissions by producing lower urine nitrogen (N), ensuring higher N uptake due to plant morphology and biology and by influencing soil N cycling processes. High species richness of pastures might reduce N_2O emissions due to more efficient use of inorganic N (De Klein *et al.,* 2020). However, the effect of plants on N_2O emissions and underlying driving mechanisms is inconclusive. The current study tested the hypothesis that a more diversified mixed pasture sward with multiple functional groups reduces N_2O emissions from dung and urine patches.

Materials and methods

The study was located at the organic research farm Lindhof (54°27' N, 9°57' E; elevation 27 m a.s.l.) in Northern Germany. The long-term (1981-2010) mean temperature and an annual rainfall of the site are 8.9 °C and 768 mm, respectively. Soils consist of 11% clay, 29% silt, 60% sand, with a $pH_{(CaCl2)}$ of 5.7 and bulk density of 1.5 g cm^{-1}. The grassland types consisted of perennial ryegrass (*Lolium perenne)* + white clover (*Trifolium repens)* (GWC); perennial ryegrass + white and red clovers *(Trifolium pratense)* (GWRC); and perennial ryegrass + white and red clovers + forage herbs (GWRCH). The forage herbs consisted of *Lotus corniculatus, Cichorium intybus, Plantago lanceolata* and *Carum carvi*. The pastures were unfertilised and established two years before the start of the experiment. A polyvinyl chloride (PVC) collar (h=15 cm, d=60 cm) was installed to a depth of 10 cm into the soil on each plot. Homogenized and representative quantities of fresh urine (2.5 l) and dung (2.2 kg) were applied in spring, summer and autumn after collection from dairy cows, leading to N loading rates of 493±43 (mean ± standard deviation) and 397±47 kg ha^{-1} for urine and dung patches, respectively. Urine and dung application was standardised across treatments. Gas sampling was done at least once a week for 100 days around noon, using the static chamber method, with measurements starting at an increased measurement rate immediately after the excreta application. During gas sampling, collars were closed with a gas-tight chamber (h=35 cm, d=60 cm) for 60 min with gas samples taken at 20 min intervals. Gas samples were analysed using gas chromatography. N_2O emissions were interpolated according to Krol *et al.* (2016). Due to dry soil conditions in 2018, additional measurements were conducted in 2019 from an irrigated binary-species grassland (GWC*) to improve regression models. All variables were analysed as a split-split plot ANOVA with grassland type as the main plot factor, season as split-plot and excreta treatment as the split-split plot. N_2O-N emission factors were modelled by stepwise multiple regression analysis.

Results and discussion

The urine and dung patches experienced different environmental conditions after application (Table. 1). However, the effect of time (season) of excreta application on N_2O emission depended on excreta type (P<0.001) and grassland type (Table 2). Thus differences between spring and autumn N_2O emissions were significant for dung-treated GWC and GWRCH as well as urine-treated GWRC (Table 2). Krol *et al.* (2017) reported a similar trend and suggested rainfall, temperature and soil moisture deficit as explanatory factors. The effect of grassland type on N_2O-N emissions was not consistent across treatments but was only evident in the case of dung patches after spring application, where there were a 49 and 40% lower N_2O-N emissions by GWC compared to GWRC and GWRCH, respectively (Table 2).

Although there were low emissions from summer-applied urine patches in GWRCH relative to GWC, the difference was not significant. The diverse pasture did not affect N_2O emission from the dung and urine patches, although it contained species reported to inhibit nitrification and to increase the availability of C leading to N immobilisation (De Klein *et al.,* 2020). Dry soil conditions following spring and summer excreta application might have inhibited N_2O production and maybe the functional potential of the species-rich swards. It is known that WFPS and temperature are significant drivers of N_2O emission

Table 1. Environmental conditions after each excreta application.[1]

Season of excreta application	GDD/°C	Air Temp, °C	Rainfall, mm	Irrigation, mm	WFPS (%)
Spring 2018	1,718	17.2±3.7	95	-	29.6±10.9
Summer 2018	1,720	17.2±4.0	188	-	26.2±9.3
Autumn 2018	726	7.3±4.0	162	-	43.7±8.0
Spring 2019	1,591	15.9±3.7	225	379	56.3±6.4
Summer 2019	1,556	15.7±3.6	333	458	59.8±5.2

[1] WFPS = water-filled pore space; GDD = growing degree days (base temperature = 5 °C).

Table 2. Cumulative N_2O-N emissions from diverse grasslands (kg ha^{-1}).[1,2]

	Control			Urine			Dung		
	Spring	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer	Autumn
GWC	0.04^{a}	0.00^{a}	0.03^{b}	1.37^{jkab}	0.84^{kb}	0.40^{ja}	0.82^{kBb}	0.59^{jkab}	0.07^{jb}
GWRC	0.04^{a}	0.06^{a}	0.01^{a}	1.31^{kb}	0.58^{jk}	0.37^{jc}	1.62^{kAb}	1.01^{jk}	0.14^{jb}
GWRCH	0.02^{a}	0.05^{a}	0.02^{a}	1.65^{b}	0.52^{b}	0.39^{b}	1.37^{kABb}	0.65^{kb}	0.12^{jab}

[1] Non-common letters across both rows and columns for significant differences: mixture: A,B; season: j,k,l; treatment: a,b,c.

[2] GWC = perennial ryegrass + white clover; GWRC = perennial ryegrass + white and red clovers; GWRCH = perennial ryegrass + white and red clovers + forage herbs.

(Selbie *et al.,* 2015). However, even under relatively high soil moisture conditions, N_2O-N emissions were not significantly affected but there was treatment × moisture level interaction (Figure 1). This was despite increments in N_2O-N emission factor by 55 and 19% for urine and dung, respectively (Figure 2). For urine, WFPS, air temperature, and grass and herb shares explained 68% of variations in N_2O-N EF, while for dung C and N concentrations of the dung, as well as grass and herb share explained 51% of the variations in N_2O EFs. In absence of a clear impact from environmental parameters, we suspect that pasture N-uptake might have had a significant impact on N_2O-N emissions, but were unable to test this. Generally, the N_2O EFs observed for cow excreta in this study were lower than the IPCC default of 2%.

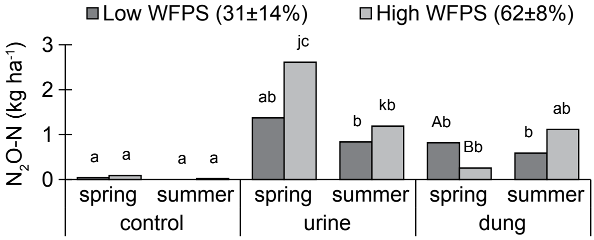

Figure 1. Cumulative N_2O-N emissions under low (2018) and high (2019) soil moisture conditions. Non-common letters for significant differences: soil moisture: A,B; season: j,k,l; treatment: a,b,c.

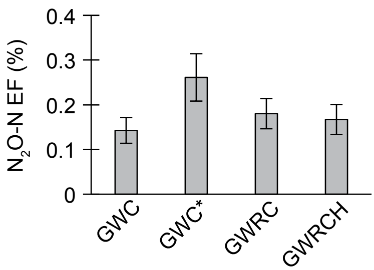

Figure 2. N_2O-N emission factors (EF) of urine and dung patches applied to diverse grasslands. * = irrigated.

Conclusions

An impact of the diverse pastures on N_2O emission was not evident in this study. It appears there were stronger factors, other than the environmental conditions, that might have influenced N_2O-N emission from the urine and dung patches. These factors should be investigated. Generally, emission factors were low compared to IPCC estimates.

Acknowledgements

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References

De Klein C.A.M., Van der Weerden T.J., Luo J., Cameron K.C. and Di H.J. (2020) A review of plant options for mitigating nitrous oxide emissions from pasture-based systems. *New Zealand Journal of Agricultural Research* 63, 29-43. https://doi.org/10.1080/00288233.2019.1614073

Krol D.J., Carolan R., Minet E., McGeough K.L., Watson C.J., Forrestal P.J., Lanigan G.J. and Richards K.G. (2016) Improving and disaggregating N_2O emission factors for ruminant excreta on temperate pasture soils. *Science of the Total Environment* 568, 327-338.

Selbie D.R., Buckthought, L.E. and Shepherd, M.A. (2015) The challenge of the urine patch for managing nitrogen in grazed pasture systems. *Advances in Agronomy* 129, 229-292. https://doi.org/10.1016/bs.agron.2014.09.004

Milk performance and feed nitrogen utilisation of grazing dairy cows supplemented with different roughage mixtures

Perdana-Decker S., Velasco E., Werner J. and Dickhoefer U.
University of Hohenheim, Animal Nutrition and Rangeland Management in Tropics and Subtropics, Fruwirthstrasse 31, 70539 Stuttgart, Germany

Abstract

Supplementing grazing animals with roughages is common in organic dairy farming, but less is known about its effect on milk performance than about concentrate supplementation. The aim of this study was to compare milk yield and nitrogen (N) utilisation of lactating dairy cows, either receiving a combined hay and clover-grass or a sole clover-grass supplementation. It was hypothesized that adding hay to clover-grass reduces dry matter intake due to its high fibre concentrations, but increases N-use efficiency due to a lower N intake. This was tested on an organic farm in southern Germany in 2019 with two groups of lactating cows (n=7 each), receiving fresh clover-grass after evening milking, with pasture grazing at night (7 h), and either hay or fresh clover-grass after morning milking. Milk yield, organic matter and N intake, and feed N utilisation did not differ between groups. These findings contradict the hypotheses; this was likely a result of the low proportion of hay in the diet. Milk urea content and urine N excretion were greater with sole clover-grass supplementation, possibly due to high concentrations of rapidly degradable protein in herbage of pasture and fresh supplement. Hence, hay supplementation of grazing dairy cows potentially decreases N surpluses without impairing milk yield.

Keywords: dairy cattle, grazing, supplementation, nitrogen utilisation

Introduction

In organic dairy systems, supplementing with on-farm produced roughages such as hay or freshly cut grasses is common (e.g. Orjales *et al.,* 2018). This strategy promotes two key principles of organic farming: sustaining the self-sufficiency of the farm, and maximising the amounts of roughage fed to ruminants. The digestive interactions between supplemental and pasture nutrient intake, however, affect pasture herbage intake, milk yield and feed nitrogen (N) utilisation (Mulligan *et al.,* 2004). Combining pasture herbage and fresh roughages such as clover-grass may result in high N surplus due to the high concentrations of rapidly degradable protein in both types of feed. The objective of the study was, thus, to test the effect of adding a feed higher in structural fibre and lower in protein, such as grass hay, to the common clover-grass supplementation in a temperate grazing system, on milk performance and feed-N utilisation of lactating dairy cows. It was hypothesised that, compared with the sole supplementation with freshly cut herbage, hay feeding decreases intake from pasture herbage due to the higher fibre concentrations, greater rumen fill and lower digestibility of hay (e.g. Adin *et al.,* 2009). As a consequence of a lower pasture herbage intake, a lower milk yield was expected. It was further hypothesised that hay addition causes a more efficient conversion of dietary N into milk N, defined as feed-N utilisation (g 100 g^{-1}), due to the lower N concentration of hay, a lower herbage and thus total N intake (Schuba *et al.,* 2017).

Materials and methods

A grazing experiment with fourteen lactating dairy cows, separated into two groups of seven animals each, was conducted on a commercial, organic farm in southwest Germany in September 2019. Groups were balanced for parity (mean 4.5; standard deviation 2) and days in milk (mean 133; standard deviation 73). All cows had joint access to grazing for 7 h during the night. After evening milking (i.e. before grazing), all animals received a mixture of freshly cut clover and grass. Following nocturnal grazing and morning milking, animals were separated into the respective groups and either supplemented with grass hay (H) or again with a mixture of freshly cut clover and grass (C). During five days of adaptation and six days of sampling, cows daily received 28 g titanium dioxide (TiO_2) in two equal dosages. Once daily, faecal spot samples were taken from all cows, alternating between morning and evening. Simultaneously, urine spot samples were collected from four cows per group. Samples of offered and refused feed were taken daily, and intake of supplemented feed measured by weighing total offered feed and refusals per herd. Pasture herbage samples were taken once per experimental period. Faecal samples were analysed for crude protein (CP), crude ash (CA), and TiO_2, and urine samples for N and creatinine to estimate daily urine volume (Valadares *et al.*, 1999) and urinary N excretion. Feed samples were analysed for dry matter, CP, neutral detergent fibre (NDF), acid detergent fibre and CA content. Milk yield was documented and sampled daily, alternating between morning or evening milking, and analysed for fat, protein, and urea content. Total organic matter intake (OMI) was calculated from daily faecal excretion determined via the external marker TiO_2 assuming a recovery in faeces of 100%. Digestibility of ingested diet organic matter was estimated from faecal CP concentration (Lukas *et al.,* 2005). The OMI on pasture was derived as difference between total and supplement intake. A mixed model of SAS (SAS Institute Inc., Cary, NC, USA, version 9.4) was applied to evaluate the effects of different supplementation on total OMI, OMI on pasture, milk performance, N intake and N partitioning.

Results and discussion

The CP concentrations were 178.4, 75.4 and 158.8 g kg^{-1} dry matter (DM) and the NDF concentrations 418, 595 and 439 g kg^{-1} DM for pasture herbage, hay and clover-grass mixture, respectively. No differences in milk yield nor OMI were observed between supplementation types (Table 1). Mean OMI from pasture was 2 kg d^{-1} lower for hay-fed than for solely clover-grass fed cows. However, these differences were not significant, possibly due to the low number of animals and because grass hay only constituted 18 g 100 g^{-1} of OMI. Total N intake also did not differ between both groups, suggesting that the lower N intake from supplemental feeds by group H was counterbalanced by the higher OMI and thus N intake from pasture. Tedeschi *et al.* (2019) also concluded that the depressing effect of supplementation on pasture herbage intake is more pronounced when supplementation occurs immediately before pasture allocation, but hay

Table 1. Daily milk production, organic matter intake (OMI) and nitrogen (N) balance of grazing lactating dairy cows supplemented with either a combination of grass hay (hay) and freshly cut clover-grass or solely freshly cut clover-grass (clover).[1]

		Supplementation types		SEM[1]	*P*-value[2]
		Hay	Clover		
Milk parameters	Milk yield, kg d^{-1}	23.3	24.0	1.58	NS
	Milk protein content, g 100 g^{-1}	3.10	3.11	0.35	NS
	Milk fat content, g 100 g^{-1}	3.77	3.80	0.32	NS
	Milk urea content, mg dl^{-1}	25.8	28.6	1.72	0.056
Intake parameters, kg animal and d^{-1}	OMI, total	18.3	17.9	17.6	NS
	OMI, hay[3]	3.27	0.00	–	–
	OMI, clover-grass mixture[3]	5.18	9.99	–	–
	OMI, concentrate[3]	0.43	0.43	–	–
	OMI, pasture herbage	9.46	7.42	0.98	NS
Nitrogen balance	N intake, g d^{-1}	508	528	31.6	NS
	Milk N secretion, g 100 g^{-1} N intake	24.4	22.5	2.18	NS
	Faecal N excretion, g d^{-1}	144	142	8.07	<0.001
	Urinary N excretion, g d^{-1}	202	264	16.9	0.017

[1] SEM = standard error of the mean.

[2] NS: not significant at $P \geq 0.05$ and no trend to differ at $P > 0.1$

[3] OMI of supplemental feed was measured on herd level, therefore no SEM and *P*-values are provided.

was offered 8 h prior to grazing in this study. The hypothesis that hay feeding would increase feed N-use efficiency was not confirmed, which is at least partly due to the minor differences in N intake between both groups (Schuba *et al.,* 2017). Also, the timing of hay feeding after access to pasture during the night might not have delivered sufficient fermentable carbohydrates to incorporate products of the rumen protein degradation into rumen microbial mass (Scharenberg *et al.,* 2009). The significantly lower urinary N excretion (P=0.017) and a trend of a lower milk urea content (P=0.056) in group H than group C, however, indicate that N was more efficiently utilised by rumen microbes and the animal.

Conclusions

The findings indicate that hay, at least in part, can be used as a replacement for fresh herbage supplementation, with no adverse effects on pasture herbage intake and milk performance. Considering the lower labour requirements for hay production compared with the daily harvest of fresh grass, further studies should test greater dietary proportions of grass hay in more animals to support the findings.

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References

Adin G., Solomon R., Nikbachat M., Zenou A., Yosef E., Brosh A., Shabtay A., Mabjeesh S.J., Halachmi I. and Miron J. (2009) Effect of feeding cows in early lactation with diets differing in roughage-neutral detergent fiber content on intake behavior, rumination, and milk production. *Journal of Dairy Science* 92, 3364-3373.

Lukas M., Südekum K.-H., Rave G., Friedel K. and Susenbeth A. (2005) Relationship between fecal crude protein concentration and diet organic matter digestibility in cattle. *Journal of Animal Science* 83, 1332-1344.

Mulligan F.J., Dillon P., Callan J.J., Rath M. and O'Mara F.P. (2004) Supplementary concentrate type affects nitrogen excretion of grazing dairy cows. *Journal of Dairy Science* 78, 3451-3460.

Orjales I., Lopez-Alonso M., Miranda M., Alaiz-Moretón H., Resch C. and López, S. (2018) Dairy cow nutrition in organic farming systems. Comparison with the conventional system. *Animal* 13(5), 1-10.

Scharenberg A., Kreuzer M. and Fohme F. (2009) Suitability of sainfoin (*Onobrychis viciifolia*) hay as a supplement to fresh grass in dairy cows. *Asian-Australian Journal of Animal Science* 22, 1005-1015.

Schuba J., Südekum K.-H., Pfeffer E. and Jayanegara A. (2017) Excretion of faecal, urinary urea and urinary non-urea nitrogen by four ruminant species as influenced by dietary nitrogen intake: a meta-analysis. *Livestock Science* 198, 82-88.

Valadares R.F.D., Broderick G.A., Valadares S.C. and Clayton, M.K. (1999) Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. *Journal of Dairy Science* 82, 2686-2696.

Can the temperate forage herb plantain (*Plantago lanceolata* L.) decrease nitrous oxide emissions from grassland on peat soils?

Pijlman J.[1], Mani D.T.C.[2], Van Groenigen J.W.[2], Erisman J.W.[1] and Van Eekeren N.[1]
[1]*Louis Bolk Institute, Bunnik, the Netherlands;* [2]*Soil Biology Group, Wageningen University, Wageningen, the Netherlands*

Abstract

Dairy grasslands on peat soils are prone to nitrous oxide (N_2O) losses as a result of relatively high soil organic matter contents, high potential N mineralization rates and shallow groundwater levels. The use of the temperate forage herb ribwort plantain (*Plantago lanceolata* L.) (RP) has been suggested as a means to reduce these emissions via the release of secondary compounds with biological nitrification inhibition (BNI) capacity. Here we report a study on the effect of varying shares of plantain (100%RP, 66%RP, 33%RP and 0%RP) sown in a mixture with perennial ryegrass (*Lolium perenne* L.) on N_2O fluxes in a dairy grassland on peat soil. Actual estimated plantain herbage shares during the measurement period were 68, 42, 22 and 0% for the treatments 100%RP, 66%RP, 33%RP and 0%RP, respectively. After calcium ammonium nitrate fertilisation, N_2O emissions were up to 26% lower (P=0.038) in the treatment 100%RP compared with the other treatments. Multiple linear regression analyses revealed a significant decrease of cumulative N_2O emissions at an increasing plantain herbage share (P=0.023). Our results suggest that plantain can reduce N_2O emissions in nutrient-rich conditions such as dairy grasslands on peat soils.

Keywords: nitrous oxide, nitrification, nitrogen, ribwort plantain, peat, grassland

Introduction

Dairy grasslands on peat soils are prone to high N_2O emissions due to the high soil organic matter content, high potential mineralization rates and shallow groundwater levels (Koops *et al.,* 1996). Biological nitrification inhibition (BNI) could be a potential means for reducing nitrification and therefore N_2O emissions. Plantain, a temperate forage herb, has been shown to release compounds with BNI capacity; allelochemicals that are released from the roots with an inhibiting effect on nitrifying organisms (Dietz *et al.,* 2013). Several studies performed on sand, silt loam and peat soils have reported that plantain decreases (potential) nitrification and N_2O emissions from the soil (Carlton *et al.*, 2019; Dietz *et al.,* 2013; Pijlman *et al.*, 2019; Simon *et al.,* 2019). However, studies on the effect of plantain on mineral N cycling in dairy grasslands on peat soils are lacking. Therefore, we measured N_2O emissions in a previously established field experiment at KTC Zegveld (the Netherlands). We hypothesised that N_2O emissions decreased with increasing shares of plantain grown in mixture with perennial ryegrass.

Materials and methods

In May 2017, twenty-four plots of 2.5×10.0 m were sown with perennial ryegrass and varying shares of ribwort plantain (100%RP, 66%RP, 33%RP and 0%RP) in a complete randomised block design with six blocks. For more details see Pijlman *et al.* (2019). In August 2019, three PVC rings (radius 10 cm, height 13 cm) per plot were inserted 10 cm into the soil at spots with a representative estimated plantain herbage share corresponding to the respective treatment. Plantain herbage share was visually estimated every other week by a standard protocol, using photographs taken from a top-down angle. Soil N_2O fluxes were measured two to three times per week during a period of 73 days, starting 5 August 2019. Build-up of N_2O concentration in the rings was measured using a photo-acoustic multi-gas monitor (Innova 1312, Innova AirTech Instruments, Ballerup, Denmark) after rings were sealed with 4.4 l polyethylene caps for a minimum of 30 min. N_2O fluxes were corrected for background air concentrations, determined every sixth measurement. Results were averaged per plot after which the cumulative soil N_2O fluxes were calculated, assuming a linear change in flux between measurements days. At days 2 and 37, calcium ammonium nitrate (CAN: 50% NH_4^+ and 50% NO_3^-) was applied to provide 50 kg N ha^{-1}. Herbage was harvested 14 days prior to the first measurement and again on day 18. At each N_2O flux measurement day, soil temperature was measured at 10 cm depth at approximately 13:00 pm and soil samples (0-30 cm) were taken for analyses of soil moisture content (drying at 105 °C for 24 hours). Data of precipitation and the average day temperatures were retrieved from weather stations Zegveld and De Bilt (KNMI, the Netherlands), respectively. Before analyses, cumulative N_2O-N flux data were log-transformed after raising values to ≥1 using a constant, in order to obtain a range of positive log-transformed values. Analysis of covariance (ANCOVA) was used to analyse for significant differences between N_2O fluxes of treatments. Additionally, a multiple linear regression analysis was done for the prediction of the soil N_2O-N flux by the estimated herbage share of plantain, in which data from 0%RP were excluded from the analysis. Block was used as covariate in both analyses.

Results and discussion

Average soil and air temperatures during the experiment were 17.2±1.9 and 15.5±3.2 °C, respectively, and cumulative precipitation was 239 mm (Figure 1). In the treatments with plantain, the estimated herbage share was lower than intended (22-68%) (Table 1). The gravimetric soil moisture content was highest for 100%RP (450 mg g^{-1}), followed by 0%RP, 33%RP and 66%RP (421-432 mg g^{-1}, Table 1). Cumulative N_2O emissions were up to 26% lower (P=0.038) at 100%RP, compared with the other treatments (Table 1, Figure 2). Multiple linear regression analyses only using data from treatments with plantain showed a significant decrease of cumulative N_2O emissions (P=0.023, r^2=0.865, n=18) at an increasing plantain herbage share.

The current study confirmed the hypothesis that the presence of plantain can lead to lower N_2O emissions from grassland on peat soils. Interestingly, the soil moisture content was the highest for 100%RP while N_2O emissions were the lowest. Soil denitrification has been shown to positively correlate with soil

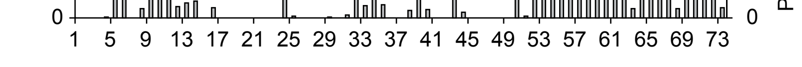

Figure 1. Average day temperature, soil temperature at 10 cm depth and daily precipitation, measured from 5 August 2019 to 16 October 2019.

Table 1. Estimated herbage share of plantain within rings, soil moisture content and cumulative N_2O-N emission per treatment.[1,2]

Treatment	Estimated plantain share (%) ± SD	Soil moisture (mg g^{-1}) ± SD	Cumulative N_2O-N emission (g m^{-2}) ± SE
0%RP	0±0	432±35	1.29^a±0.22
33%RP	22±17	427±35	1.26^a±0.22
66%RP	42±11	421±42	1.24^a±0.23
100%RP	68±10	450±49	0.95^b±0.16

[1] Values showing the same letter are not significantly different (P<0.05).

[2] RP = ribwort plantain; SD = standard deviation; SE = standard error.

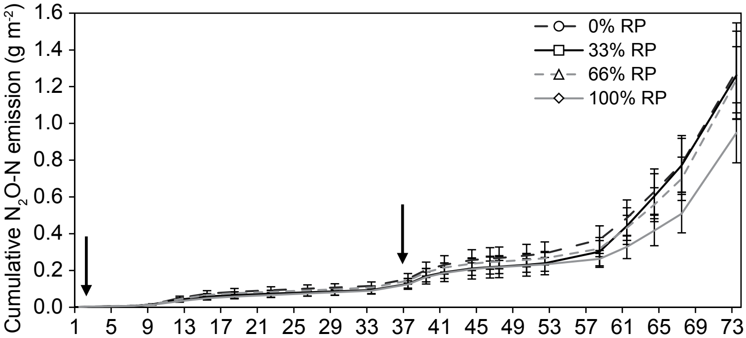

Figure 2. Cumulative N_2O-N emission per treatment, measured from 5 August 2019 to 16 October 2019. Arrows indicate calcium ammonium nitrate applications of 50 kg N ha^{-1}.

humidity (Koops *et al.*, 1996). Therefore, we expected higher N_2O emissions at higher soil humidity, for the observed soil moisture contents. This suggests other factors reduced soil N_2O emissions in the current experiment. This is in line with the suggested effect of compounds with BNI potential that are released by plantain. Previous research at the same location showed no difference in herbage N uptake between treatments, and suggested that the presence rather than the herbage share of plantain was key in decreasing N_2O emissions (Pijlman *et al.,* 2019). Current findings, after a more extended period of measurements than Pijlman *et al.* (2019) (73 vs 23 days), suggest that N_2O emissions negatively correlate with the herbage share of plantain. The observed negative correlation with the share of plantain is in accordance with research on a loamy soil (Simon *et al.*, 2019).

Conclusions

N_2O emissions after CAN fertilisation were significantly lower at increasing plantain herbage shares, when plantain was grown in a mixture with perennial ryegrass in a dairy grassland on peat soil. The use of the temperate forage herb plantain could be a means to reduce N_2O emissions from nutrient rich soils of temperate grasslands.

Acknowledgements

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References

Carlton A.J., Cameron K.C., Di. H.J., Edwards. G.R. and Clough T.J. (2019) Nitrate leaching losses are lower from ryegrass/white clover forages containing plantain than from ryegrass/white clover forages under different irrigation. *New Zealand Journal of Agricultural Research* 62, 150-172.

Dietz M., Machill S., Hoffmann H.C. and Schmidtke K. (2013) Inhibitory effects of *Plantago lanceolata* L. on soil N mineralization. *Plant and Soil* 368, 445-458.

Koops J.G., Oenema O. and Van Beusichem M.L. (1996) Denitrification in the top and sub soil of grassland on peat soils. *Plant and Soil* 184, 1-10.

Pijlman J., Berger S., Lexmond F., Bloem J., Van Groenigen J.W., Visser E., Erisman J.W. and Van Eekeren N. (2019) Can the presence of plantain (*Plantago lanceolata* L.) improve nitrogen cycling of dairy grassland systems on peat soils? *New Zealand Journal of Agricultural Research* 63, 106-122. https://doi.org/10.1080/00288233.2019.1698620

Simon P.L., De Klein C.A.M., Worth W., Rutherford A.J. and Dieckow J. (2019) The efficacy of *Plantago lanceolata* for mitigating nitrous oxide emissions from cattle urine patches. *Science of the Total Environment* 691, 430-441.

Modelling multi-species grasslands with plant-specific suitability functions

Piseddu F.[1,2], Hadj Saadi D.[1], Movedi E.[3], Picon-Cochard C.[1], Roggero P.P.[2], Confalonieri R.[3], Seddaiu G.[2] and Bellocchi G.[1]

[1]INRAE, UREP, 5 chemin de Beaulieu, 63000 Clermont-Ferrand, France; [2]University of Sassari, Via De Nicola 9, 07100 Sassari, Italy; [3]University of Milan, Via Celoria 2, 20100 Milan, Italy

Abstract

Elaborated on a 'crop suitability' concept, hierarchically arranged functions are combined to appraise the agro-environmental conditions (management, phenology, air temperature, solar radiation, water and nitrogen availability) controlling the establishment and growth of plant species in multi-species grasslands. With these functions, generic crop simulators originally developed to represent vegetation cover as an average plant evolve towards representing explicitly and dynamically the relative abundance of the different species that comprise a grassland community. Novel modelling solutions were obtained by coupling the software component CoSMo (Community Simulation Model), which implements such suitability functions, with two crop simulators of large use worldwide (CropSyst and WOFOST). Results show that these solutions are adequate in simulating the multi-year plant dynamics of Italian and French grassland sites. This occurs because the models predict changes in community plant traits from the traits of individual species (fixed model parameters) and their relative abundances as simulated at daily time steps. With an explicit representation of plant diversity, we aim to better infer the ecosystem services delivered by grassland, which depend on floristic composition. With this ongoing research, we anticipate that better estimates can be obtained of the main provisioning service, i.e. forage production.

Keywords: Community Simulation Model, grassland modelling, plant diversity, relative abundance, suitability functions

Introduction

Though major advances in modelling have been made in recent decades, the biophysical and biogeochemical simulation of grassland systems remains mostly disjointed from the dynamics of plant species composition (Van Oijen *et al.*, 2018). The development of the relationships between grassland biodiversity and ecosystem services requires a conceptual framework where ecological and biophysical/biogeochemical approaches converge in relation with agricultural practices. Modelling studies in that direction have had a strong momentum over recent years, facilitated by the INRA meta-programme ECOSERV-project MODIPRAS (2018-2019) and ongoing Italian and French research covering a variety of grassland sites in Europe. These studies rely mostly on an operational version of the dynamic model component CoSMo (COmmunity Simulation Model; Confalonieri, 2014), coupled to standalone simulators of grassland systems. Such CoSMo-based model versions simulate seasonal changes in the composition of multi-species grasslands, i.e. grassland communities with virtually any number of plant species. In this paper, we illustrate some simulation results (target variable: relative abundance of plant species) on fertilised, either mown or grazed, continental grasslands in the Massif Central of France.

Materials and methods

The generic simulation of a plant community is implemented in single-instance simulators, based on two distinct features, namely: (1) the adaptability of each species to the agro-environmental conditions defined at each integration step, and (2) the derivation of community traits from the parameters of each species and their relative abundance. This approach is derived from the 'crop suitability' concept (Confalonieri *et al.*, 2013), for which plant competition and changes in the relative abundance of plant types are simulated as a response (suitability functions) to hierarchically arranged drivers, which represent the suitability of different species to the agro-environmental conditions (management, phenology, temperature, solar radiation, water and nitrogen availability) at each time step.

Results and discussion

Overall, the simulations obtained with CoSMo at two French sites (Figure 1) indicate satisfactory estimates of relative abundances. We note bigger departures from observations with minor species (*Trisetum flavescens*, *Stellaria media*) and better simulations with CropSyst than WOFOST in the Laqueuille pasture (Figure 1, top graph).

Figure 2 presents the simulated fluctuations of two community traits. The WOFOST trait 'Max rate of assimilated CO_2' dropped in 2016, pointing to the drought event occurred in Theix in 2015. This may have impacted the community structure to the point of leading, in the following year, a drop in the rate of photosynthetically assimilated carbon. Since this is reflected in forage production minima in 2015 and

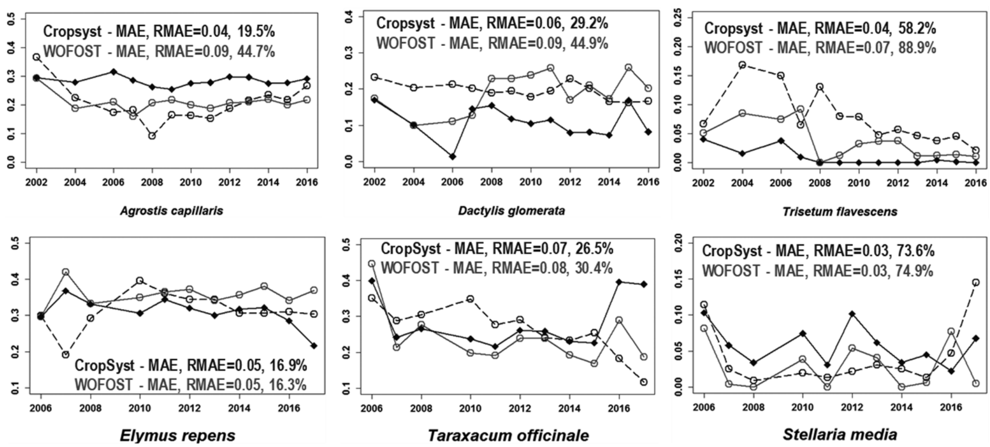

Figure 1. Inter-annual variation of observed (thick continuous line with diamonds) and simulated (CropSyst-CoSMo, hatched line with empty circles; WOFOST-CoSMo, light continuous line with empty circles) relative abundances of example species at two study sites in the Massif Central of France: Laqueuille (top graph), 45° 38′ N, 02° 44′ E, 1040 m a.s.l., fertilised, intensive grazing; Theix (bottom graph), 45° 43′ N, 03° 01′ E, 880 m a.s.l., fertilised, three cuts per year. MAE, RMAE: mean absolute error (0, optimum), percent relative mean absolute error (0, optimum).

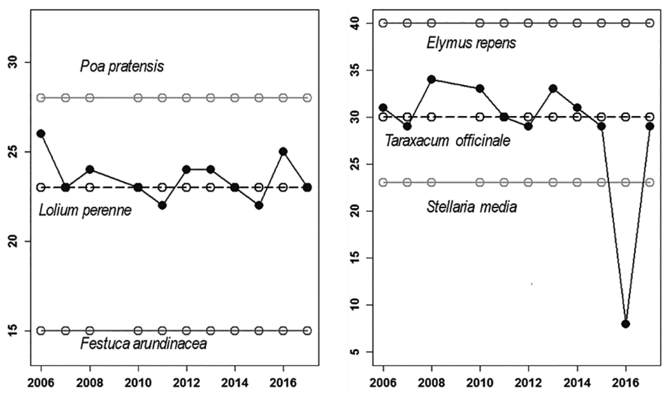

Figure 2. Derivation of two community traits in Theix (fluctuating lines) with CoSMo (specific leaf area in CropSyst; maximum rate of assimilated CO_2 in WOFOST). Input parameter values are reported for exemplary species (flat lines).

2016 (not shown), our cautious assumption is that of a 'memory effect' of the drought of one year on the next year (after Sándor *et al.*, 2018), of which this parameter would be an indicator.

Conclusions

These results confirm the potential of CoSMo suitability functions to represent plant dynamics in grassland communities (while also deriving dynamically community traits), as already highlighted by Movedi *et al.* (2019) in the Central Apennines (Italy). Particularly encouraging is the simulation of a complex system such as the Laqueuille cattle grazing. An extension of model assessment under various conditions is ongoing. An evaluation of CoSMo in abandoned grasslands of the Pannonian region would likely require an adaptation of the model to simulate the co-existence of perennial and annual species. Additional suitability functions could also be required to address specific situations, e.g. combinations of cold temperatures and water logging conditions in alpine pastures or seed back dynamics of self-reseeding annual species in Mediterranean grasslands. The coupling of CoSMo to the grassland-specific models would also expand the capabilities of the modelling framework.

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References

Confalonieri R. (2014) CoSMo: a simple approach for reproducing plant community dynamics using a single instance of generic crop simulators. *Ecological Modelling* 286, 1-10.

Confalonieri R., Francone C., Cappelli G., Stella T., Frasso N., Carpani M., Bregaglio S., Acutis M., Tubiello F.N. and Fernandes E. (2013) A multi-approach software library for estimating crop suitability to environment. *Computers and Electronics in Agriculture* 90, 170-175.

Movedi E., Bellocchi G., Argenti G., Paleari L., Vesely F., Staglianò N., Dibari C. and Confalonieri R. (2019) Development of generic crop models for simulation of multi-species plant communities in mown grasslands. *Ecological Modelling* 401, 111-128.

Sándor R., Picon-Cochard C., Martin R., Louault F., Klumpp K., Borras D. and Bellocchi G. (2018) Plant acclimation to temperature: developments in the Pasture Simulation model. *Field Crops Research* 222, 238-255.

Van Oijen M., Bellocchi G. and Höglind M. (2018) Effects of climate change on grassland biodiversity and productivity: the need for a diversity of models. *Agronomy* 8: 14.

Respiration fluxes and root decomposition in ley systems with different complexities

Poyda A., Zimmerbeutel A., Loges R. and Taube F.

Institute of Crop Science and Plant Breeding, Grass and Forage Science / Organic Agriculture, Christian-Albrechts-University Kiel, Germany

Abstract

The effect of species richness on soil carbon (C) storage has frequently been explored for permanent grasslands but knowledge gaps remain concerning this relationship for temporary grasslands. In an ongoing experiment, root biomass and respiration fluxes with and without aboveground vegetation were measured on 3-year-old grassland swards (leys) with three different levels (2, 3 and 8) of species diversity. Root biomass as well as root turnover did not differ significantly between the mixtures. However, significantly less C was released from soil after bare plot establishment at the thee-species mixture (*Lolium perenne, Trifolium repens, Trifolium pratense*) compared with the other two mixtures. This difference might be explained by a higher soil microbial C use efficiency in the rhizosphere of the 3-species mixture or lower priming on soil organic matter.

Keywords: temporary grassland, root turnover, carbon use efficiency, ley-arable systems

Introduction

Little is known about carbon (C) dynamics and sequestration potentials of ley-arable cropping systems as affected by the botanical composition and functional diversity of temporary grassland. The main benefits of a ley phase are related to the undisturbed soil structure as well as high and year-round C inputs from root production (Vertès *et al.*, 2007) making ley cropping systems one of the most promising management practices for C sequestration on arable land (Lugato *et al.*, 2015). For grassland ecosystems it has been frequently shown that higher species richness can increase soil C storage (Lange *et al.*, 2015). The main objectives of this study were, therefore, to quantify the amounts and turnover rates of belowground biomass (BGB) in temporary grassland swards differing in species richness. We hypothesise that mixtures of higher complexity produce higher amounts of BGB with lower relative turnover.

Materials and methods

From April to November 2019, measurements of the CO_2 efflux were conducted on 3-year-old ley systems in a field experiment in northern Germany (54.46 °N, 9.96 °E; 8.9 °C mean annual temperature, 778 mm mean annual precipitation) by using closed manual chambers attached to an infrared gas analyser (LI-820, LI-COR Biosciences Inc., Lincoln, NE, USA). The grassland leys differed in terms of sown species diversity on three complexity levels: *Lolium perenne* (Lp) + *Trifolium repens* (Tr) (WC), Lp + Tr + *Trifolium pratense* (Tp) (RC), Lp + Tr + Tp + *Carum carvi* (Cc) + *Cichorium intybus* (Ci) + *Lotus corniculatus* (Lc) + *Plantago lanceolata* (Pl) + *Sanguisorba minor* (Sm) (H). Prior to the first flux measurements, bare plots were established on every mixture (n=3) and constantly kept free of vegetation. Measurements were conducted twice per week during the first month after bare plot establishment, weekly until mid-August and bi-weekly for the rest of the study period. To obtain budgets of respired C over certain periods, the temperature response function of Lloyd and Taylor (1994) was fitted to plot-specific measurements and respiration was simulated by using mean daily air temperature. BGB was determined at the day of bare plot establishment (17 April) and in late summer (4 September) by taking samples from the 0-15 cm depth with a soil auger (d=8 cm), washing the roots from the soil, drying at 60 °C and correcting the biomass for ash content of samples. The C concentration in roots was measured with an elemental analyser. At every flux measurement campaign, soil samples from the 0-10 cm depth were taken and dried at 105 °C to determine the soil moisture content.

Results and discussion

Measured CO_2 efflux from bare plots was as high or higher compared to fluxes from vegetated plots for one month after bare plot establishment (Figure 1). Thus, missing autotrophic respiration was fully compensated by increased soil respiration, most likely as a result of enhanced root decomposition after removal of aboveground vegetation. From mid-May, the respiration fluxes from the two treatments separated, with higher fluxes from vegetated plots. The general pattern over the course of the study period was rather similar for both treatments, indicating similar reactions to environmental drivers. However, spatial and temporal variability was larger on vegetated plots due to a higher temperature sensitivity of the autotrophic part of respiration. As analysed by a linear mixed effect model, both treatments were significantly affected by higher air temperature during the measurements. With 0.014 g CO_2-C m^{-2} h^{-1} $°C^{-1}$ temperature sensitivity was more than three times higher on vegetated plots than on bare plots (0.003 g CO_2-C h^{-1} $°C^{-1}$). However, respiration from bare plots reacted more sensitively to changes in soil moisture (0.0075 vs 0.0026 g CO_2-C m^{-2} h^{-1} (% in weight)$^{-1}$). These relationships are valid for the main growing season, covering most of the study period, but do not consider that soil moisture contents increase while respiration fluxes decrease at the beginning of the non-growing season. Until mid-September, soil moisture and respiration followed a similar pattern, explaining these significant positive relationships.

In spring 2019, BGB was highest in the 8-species mixture (10,200 kg dry matter ha^{-1}). Due to very high spatial variability, however, differences between the mixtures were not significant (Figure 2A). When the amount of C stored in roots at the day of bare plot establishment was compared with the amount of root C on the same plots after 140 days (Figure 2B), 78 (H), 77% (RC) and 84% (WC) of initial root C was decomposed. However, differences in lost root C were not significant. In contrast, significantly less C was lost via soil respiration from bare plots of mixture RC compared with mixtures WC and H as obtained by the respiration model. As only the roots in 0-15 cm were sampled, these differences could be partly explained by effects from deeper soil layers. However, although lower rooting depths can be particularly expected under mixture WC compared to mixture H, cumulated respiration did not differ between these two mixtures. The main difference between mixture RC and the other two mixtures was the dominance of red clover. Although red clover was also a part of mixture H, its relative abundance was lower compared to RC due to higher competition by herbs, particularly Ci and Pl.

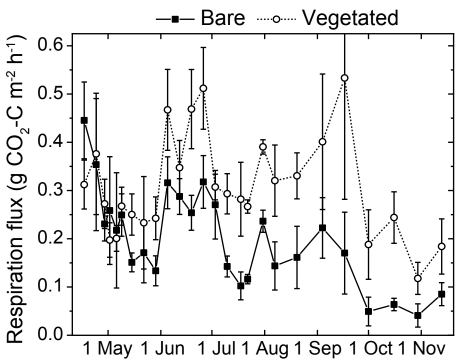

Figure 1. Measured respiration fluxes from mid-April until mid-November 2019 at the three-species mixture (RC) on vegetated plots and on plots that have been kept free of vegetation (bare). Error bars represent standard deviation.

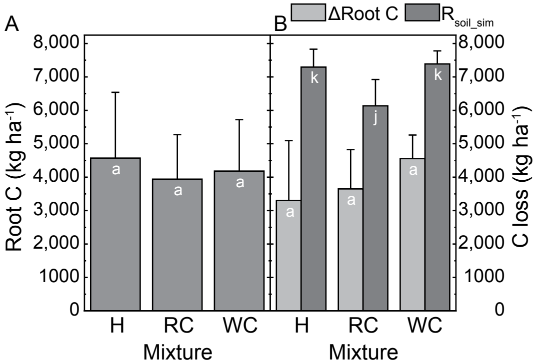

Figure 2. (A) Root carbon in the 0-15 cm soil depth at the 8- (H), 3- (RC) and 2-species mixture (WC) on 17 Apr 2019. (B) Simulated budgets of soil respiration (R_{soil_sim}) on bare plots from 17 Apr – 4 Sep 2019 and the loss of root carbon in 0-15 cm during the same period. Different lowercase letters indicate significant differences between the mixtures with $P<0.05$.

These findings might also suggest that root decomposition induced less priming under mixture RC and the decomposition of native soil organic matter was lower. Another potential explanation is that microbes utilised the root residues in mixture RC with a higher efficiency; thus, for the same amount of root C turnover less C was respired than for the other mixtures. Similar observations were made by Spohn *et al.* (2016) who found increased C use efficiencies (CUE) induced by nitrogen (N) fertilisation but no differences in turnover rates in a long-term experiment on temperate grasslands. As it can be assumed that mixture RC had highest N fixation rates due to the dominance of Tp and thus N availability in soil, the N effect on CUE might be responsible for lower respiration.

Conclusions

Our results could show that the elimination of aboveground biomass, as a typical renovation measure in intensive grassland management, increased soil respiration via root turnover, which compensated missing autotrophic respiration for several weeks. We cannot fully confirm our hypothesis of higher BGB and lower root turnover in more complex mixtures although both expectations could be confirmed numerically but not statistically. The less-complex red clover dominated grassland swards showed the lowest CO_2 release from soil respiration after bare plot establishment, possibly as a result of a higher microbial carbon use efficiency of root decomposition or lower priming on soil organic matter, which will be subject of further investigations.

References

Lange M., Eisenhauer N., Sierra C.A., Bessler H., Engels H., Griffiths R.I., Mellado-Vázquez P.G., Malik A.A., Roy J., Scheu S., Steinbeiss S., Thomson B.C., Trumbore S.E. and Gleixner G. (2015) Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications* 6:6707.

Lloyd J. and Taylor J.A. (1994) On the temperature dependence of soil respiration. *Functional Ecology* 8, 315-323.

Lugato E., Bampa F., Panagos P., Montanarella L. and Jones A. (2015) Potential carbon sequestration of European arable soils estimated by modelling a comprehensive set of management practices. *Global Change Biology* 20, 3557-3567.

Spohn M., Pötsch E.M., Eichorst S.A., Woebken D., Wanek W. and Richter A. (2016) Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. *Soil Biology & Biochemistry* 97, 168-175.

Vertès F., Hatch D., Velthof G., Taube F., Laurent F., Loiseau P. and Recous S. (2007) Short-term and cumulative effects of grassland cultivation on nitrogen and carbon cycling in ley-arable rotations. *Grassland Science in Europe* 12, 227-246.

The role of agri-environmental policy in the current trajectories of semi-natural grassland management in Latvia

Rūsiņa S.[1], Lakovskis P.[2], Elferts D.[3], Gustiņa L.[1], Dūmiņa I.[1] and Kupča L.[4]
[1]Faculty of Geography and Earth Sciences, University of Latvia, 1 Jelgavas Str., 1004 Riga, Latvia; [2]Institute of Agricultural Resources and Economics, Latvia; [3]Faculty of Biology, University of Latvia, Latvia; [4]Latvian Museum of Natural History, Latvia

Abstract

Agri-environmental schemes (AES) can be effective for improving grassland biodiversity. However, little is known about the impact of AES on the conservation of semi-natural grasslands (SNG) in post-socialist countries where the approach is comparatively new. Our intention was to clarify the response of semi-natural grassland management under AES in relation to habitat rarity, land-use intensity and agriculture potential. Firstly, we studied if the abundance of common and rare SNG habitats was related to agricultural land-use potential in Latvia. Secondly, we investigated the response of farmer uptake of grassland-related AES in relation to management intensity of agricultural land in Latvia. A cross-country analysis of distribution and management levels of eight SNG habitats was performed using regression methodology. Our results showed that agricultural land-use potential was an important factor determining the distribution of common but not rare habitats in Latvia. The share of extensive management of agricultural land was a positive driver of farmer uptake of AES for common habitats; however, it was not true for rare habitats. Our findings highlight the need for a regional and habitat-focused approach to AES designation to maximize the uptake of AES by farmers and to improve the conservation of rare habitats.

Keywords: rarity, abandonment, land-use intensity, management decision

Introduction

Semi-natural grasslands (SNG) are widely recognized for the high biodiversity, cultural heritage and landscape values they provide to human society. The Eastern European landscapes generally have lower average levels of land-use intensity than in Central and Northern Europe (Török *et al.*, 2018). However, little is known about the impact of agri-environmental schemes (AES) on the conservation status of SNG in post-socialist countries where the approach is comparatively new (Sutcliffe *et al.*, 2015). In Latvia, an action-based scheme 'Maintenance of Biodiversity in Grasslands' (AES MBG) has been running since 2004. The aim of the scheme is to foster biodiversity-friendly management of permanent grasslands across the country. Eligible areas include all SNG habitats and cultivated permanent grasslands that are important habitats for bird species. The management requirements include mowing once per season, or grazing (up to 0.9 animal units), and any improvement of grassland is forbidden. Our study aimed to fill this knowledge gap by focusing on the impacts of agricultural factors on SNG maintenance in Latvia. We hypothesized that the area of common and rare SNG habitats per grid cell is larger in regions with low agricultural land-use potential (AP) than in regions with high AP. The latter was defined as a land capability to produce agricultural products that is determined by biophysical constraints, namely, soil fertility and moisture conditions, climate, and topography. The second hypothesis was that a high share of extensive management of agricultural land per grid cell is a positive driver for farmer uptake of AES MBG, while intensive management leads to a decrease in biodiversity friendly managed SNG.

Materials and methods

Eight out of 12 SNG habitat types of the Habitat Directive (Council Directive 92/43/EEC) occurring in Latvia were analysed. Another four habitat types were excluded from analysis due to the lack of spatial data. Analysis was conducted at a country scale with a study unit of 5 km × 5 km grid cells. Data on habitat distribution and management in each grid cell was obtained from the Nature Conservation Agency and Integrated Administration and Control System of Rural Support Service. Rare and common habitats were defined as occurring in less than 50% and more than 50% of all grid cells, respectively. Rare habitats accounted for less than 10% of the total area of SNG. They included Natura 2000 habitat types 1630*, 6120*, 6210, 6230* and 6410. Common habitats included habitat types 6270*, 6510, and 6450. Together they accounted for 72% of the total area of SNG. Two sets of Tweedie compound Poisson generalized linear models (TCPGLM) were produced. Model I was related to the first hypothesis and was built to determine whether there were significant differences in abundance of common and rare SNG habitats per grid cell (response variable) in five regions of AP as defined by Boruks (2001) (explanatory variable). Model II responded to the second hypothesis and explained the effect of current management intensity of agricultural land on the uptake of rare and common habitats in AES MBG. The area of common/rare SNG habitats per grid cell managed under the AES MBG was a response variable. Two groups of explanatory variables were used: (1) extensive management variables (area of SNG, area of managed permanent grassland, area of SNG under AES, area under organic farming, area in Natura 2000 network); (2) intensive management variables (land quality, area of ploughed SNG, area of arable land). We used TCPGLM incorporating a spatial autocovariate (R package 'cpglm'), because data were spatially autocorrelated and contained a high proportion of zero values.

Results and discussion

Common habitats accounted for 82% of the SNG area, and rare habitats for 18%. Our first hypothesis was proven for common habitats: their area per grid cell was twice as high in regions with low and intermediate AP in comparison to regions with high and above intermediate AP. This finding corresponds to previous research in which Levers *et al.* (2016) showed that AP is highly correlated with intensive arable farming, while the latter is a significant driver of biodiversity loss (Allen and Hof, 2019). In contrast, the abundance of rare habitats did not differ significantly among regions, suggesting that factors other than agricultural intensity might be responsible for the current pattern of rare habitat distribution.

As we hypothesized, the second TCPGLM confirmed the significantly positive relationship between farmer uptake of common habitats in AES MBG and a share of extensive management of agricultural land (Table 1). Unexpectedly, ploughing of SNG was also a significant positive driver as the total area of common habitats under AES MBG per grid cell was significantly positively related to the total area of

Table 1. Tweedie compound Poisson GLM estimated coefficients for Model II that explain the effect of management intensity of agricultural land on the uptake of common and rare semi-natural grasslands (SNG) habitats in agri-environmental scheme (AES) MBG.

		Common habitats			Rare habitats		
		Estimate	*t* value	*P*-value[1]	Estimate	*t* value	*P*-value[1]
Intercept		0.98	2.97	0.003**	-1.19	-1.41	0.16
Factors of extensive management	Area of SNG	0.01	6.32	<0.001***	0.01	0.24	<0.001***
	Area of managed permanent grassland	0.0001	2.72	0.006**	0.0002	0.40	0.68
	Area of SNG under AES	0.04	2.35	0.02*	-0.006	-1.38	0.16
	Area under organic farming	0.0001	2.69	0.007**	0.0002	0.23	0.81
	Area in Natura 2000 network	-0.05	-0.47	0.63	0.24	1.14	0.25
Factors of intensive management	Land quality	0.10	0.74	0.46	0.08	0.24	0.81
	Area of ploughed SNG	0.006	2.05	0.04*	-0.025	-2.09	0.04*
	Area of arable land	-0.0001	-1.38	0.17	0.0002	-0.64	0.52
Spatial autocovariate		13.70	6.58	<0.001***	0.016	6.51	<0.001***

[1] Significance * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

SNG ploughed-up. One explanation for this phenomenon could be the steady increase in agricultural activity in Latvia during the last 20 years after a period of de-intensification and abandonment. This period was remarkable for simultaneous operation of two counter-directed processes, namely, industrialization and environmental awareness (Jepsen *et al.*, 2015). On the contrary, the model showed the negative impact of ploughing of SNG on the farmer uptake of rare habitats in AES MBG. At the same time, no factors of extensive management appeared to be positively related to such uptake (Table 1). This could be because rare habitats, in comparison with common habitats, are confined more to those agricultural areas that are the least suitable for modern agricultural purposes. Polarisation of the agricultural landscape with intensification of high-quality agricultural land and marginalization of low quality agricultural land has been a pronounced process in recent decades in Latvia (Jepsen *et al.,* 2015).

Conclusions

Our results indicate that under the current agri-environment policy, biodiversity-friendly management of SNG is viable only if common habitats are considered, although at the expense of some of their area. There is a strong indication that rare habitats are on the brink of extinction. A regional and habitat-specific approach to AES designation is needed urgently to stop the deterioration of rare habitats. Common habitat conservation through AES should be prioritized in regions of Latvia with low and intermediate AP. Specific result-oriented AES should be designated for rare habitats throughout the country. They are similarly rare in all regions of AP, and there is no indication of any positive influence of either intensive or extensive agriculture on rare habitat uptake in action-based AES.

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References

Allen M.A. and Hof A.R. (2019) Paying the price for the meat we eat. *Environmental Science and Policy* 97, 90-94.

Boruks A. (2001) Kadastrs un zemes novērtēšana Latvijas teritorijā [Cadaster and land evaluation in Latvia]. In: Boruks A. (ed.) *Zemes izmantošana un kadastrs Latvijā*, Latvijas Republikas Valsts zemes dienests, Rīga, pp. 139-213.

Jepsen M.R., Kuemmerle T., Müller D., Erb K., Verburg P.H., Haberl H. *et al.* (2015) Transitions in European land-management regimes between 1800 and 2010. *Land Use Policy* 49, 53-64.

Levers C., Butsic V., Verburg P.H., Muller D. and Kuemmerle T. (2016) Drivers of changes in agricultural intensity in Europe. *Land Use Policy* 58, 380-393.

Sutcliffe L.M.E., Batary P., Kormann U., Baldi A., Dicks L.V., Herzon I., Kleijn P., Tryjanowski P., Apostolova I. and Tscharntke T. (2015) Harnessing the biodiversity value of central and eastern European farmland. *Diversity and Distributions* 21, 722-730.

Török P., Janišová M., Kuzemko A., Rūsiņa S. and Dajić Stevanović Z. (2018) Grasslands, their threats and management in Eastern Europe. In: Squires V.R., Dengler J., Feng H. and Hua L. (eds.) *Grasslands of the world: diversity, management and conservation*. CRC Press, Boca Raton, pp. 64-88.

Effect of legume silage type in the diet of dairy cows on the slurry nitrogen composition

Sánchez-Vera A., Martínez-Fernández A., Elouadaf D., De La Torre-Santos S., Soldado A. and Vicente F.

Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), P.O. Box 13, 33300 Villaviciosa (Asturias), Spain

Abstract

Legumes are more susceptible to proteolysis during the silage process than grasses due to their higher protein content. However, not all legumes have the same proteolysis rate. Dairy cows feeding on legume silages excrete large amounts of nitrogen in slurry because in ruminants less than 30% of nitrogen intake is retained in milk. The objective of this work was to evaluate the amount of nitrogen excretion in dairy cows feeding two legume silages with theoretical different proteolysis rate. Six Holstein cows were divided among three different silages (grass, faba bean and pea) in a double 3×3 Latin square. Nitrogen intake, milk production and composition were not affected by treatment. The excretion of ammonia-N in faeces was not affected; however, the proportion of total-N was higher in the faba bean diet than the pea diet ($P<0.05$). The ammonia-N in the urine was twice as high with the pea diet than faba bean and control diets ($P<0.01$). The total-N excreted in the urine was also higher in cows feeding on pea silage than faba bean and grass silages ($P<0.001$). In conclusion, the pathway for the excretion of dietary nitrogen from legume silages is mainly through urine, and the metabolization of pea silage protein goes toward ammonia-N.

Keywords: legume silages, milk, nitrogen excretion, ammonia-N, urine, faeces

Introduction

There is a significant deficit in supply of high-quality protein forages for livestock in Europe. Therefore, it would be highly interesting to use home-grown protein-rich feeds, such as forage legumes (Lüscher *et al.*, 2014) to reduce imported protein feeds in dairy cow rations. Moreover, forage legumes provide a good source of proteins with positive effects on animal and environment, associated with their role in N_2-fixation and therefore a reduction in inorganic N-fertilisation. Faba bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) are legumes grown locally on the Cantabrian coast of Spain and have significant potential because the Northern area of Spain provides 60% of Spanish milk production. Legumes are more susceptible to proteolysis during the ensiling process than grasses due to their higher protein content, lower carbohydrate content and greater buffering capacity (Foster *et al.*, 2011). However, not all legumes have the same proteolysis rate (Soycan-Önenç *et al.*, 2015). In addition, protein in forage legumes is degraded rapidly within the rumen; therefore, dairy cows excrete large amounts of nitrogen because less than 30% of the nitrogen intake is retained in the milk. The objective of this work was to evaluate the amount of nitrogen excretion in dairy cows feeding on two legume silages, faba bean and field pea, with theoretically different proteolysis rates during the ensiling process.

Materials and methods

Six Holstein dairy cows with 580±16.6 kg of live weight, and 29.9±2.58 kg milk per day in 157±12 days in milk were selected and distributed within a replicated 3×3 Latin square design. Treatments consisted of isoenergic and isonitrogenous diets based on: (1) grass silage (GS: control); (2) faba bean silage (FB); and (3) pea silage (PS). The diets were formulated according to NRC (2001), with a forage:concentrate ratio of 60:40 and a crude protein level of 130 g crude protein (CP) kg^{-1} dry matter (DM), according the results in a companion paper (Elouadaf *et al.*, 2020). The crude protein contents of silages were 130, 139 and 155 g kg^{-1} DM for grass, faba bean and pea silages respectively. The GS diet included grass silage (401 g kg^{-1} DM), barley straw (193 g kg^{-1} DM), and concentrate (409 g kg^{-1} DM). The FB diet included faba bean silage (481 g kg^{-1} DM), barley straw (144 g kg^{-1} DM), and concentrate (377 g kg^{-1} DM). Finally, the PS diet included pea silage (470 g kg^{-1} DM), barley straw (131 g kg^{-1} DM), and concentrate (400 g kg^{-1} DM). The evaluation time was 42 days, divided into three periods with 10 initial days of adaptation to diet, and four sampling days. The animals were housed singly in pens and given access to drinking water and feeding *ad libitum*. The cows were milked twice daily. Individual dry matter intake, milk production, and faeces and urine excretion were recorded daily. Urine was collected on 1 l of a sulphuric acid solution (10% v/v) by means of external separators stuck on the vulva. Representative samples of feed, milk, faeces and urine were taken, pooled for each animal and period, and total N and ammonia N contents were determined. Results were contrasted by analysis of variance using a mixed model considering diet and period as fixed effects and cow as a random effect with R (R Core Team, 2018).

Results and discussion

Dry matter and nitrogen intakes, and daily quantities produced of faeces, urine and milk are shown in Table 1. There were no differences in dry matter intake among treatments. Since experimental diets were isonitrogenous (136±6.4 g CP kg^{-1} DM), the daily nitrogen intake was the same among experimental diets. The milk yield was not affected by experimental diets. The type of forage also had no significant influence on the faeces excretion. However, cows feeding on the PS diet urinated less than cows feeding on GS or FB (P<0.01). This could be due to an incomplete urine recovery in some experimental period.

The ammonia excretion by faeces was negligible (Table 2). The non-ammonia nitrogen concentration in faeces from FB treatment was greater than GS and PS treatments (P<0.05). In urine, the non-ammonia nitrogen concentration was greater for both of the legume diets, especially PS (P<0.001) than the GS diet. In addition, for the PS diet there was greater concentration of ammonia nitrogen in urine than for GS and FB diets (P<0.01). This could be indicative of a high proteolysis rate of pea silage. The protein in legume silage is subject to extensive degradation to non-protein nitrogen (NPN) in the silo. Furthermore, the NPN in legume silage is rapidly degraded to ammonia in the rumen and, if not captured as microbial protein, will end up largely as urea-N excreted in the urine. The protein content in milk was similar for all diets. However, urea content in milk tended to be greater with legume silages (298 and 281 mg l^{-1} for FB and PS, respectively) than GS (237 mg l^{-1}, P=0.075).

The average efficiency of utilisation of dietary nitrogen was 26%, with no differences among treatments. Virtually all the remaining N is excreted in the urine (30%) and faeces (44%). This nitrogen efficiency value is within the range described in previous studies (Wanapat *et al.*, 2009). The greater non-ammonia-N urine excretion with legume silages might increase emissions because all nitrogen in urine would be converted into NH_4-N within a few days.

Table 1. Nutrient intake, faeces and urine excretions and milk yield according to experimental diets based on grass silage (GS), faba bean silage (FB), or pea silage (PS).[1]

		GS	FB	PS	SD	*P*-value
Intake	Dry matter (kg d^{-1})	16.6	17.6	17.0	1.76	0.434
	Nitrogen (g d^{-1})	366	390	392	33.8	0.223
Excretion	Faeces (kg dry matter d^{-1})	6.9	6.7	7.1	0.63	0.753
	Urine (l d^{-1})	17.6^a	16.6^a	13.5^b	2.72	0.004
	Milk (l d^{-1})	20.2	20.9	20.8	2.95	0.945

[1] SD = standard deviation; different superscript letters indicate significant differences among managements.

Table 2. Nitrogen concentration in faeces, urine and milk according to experimental diets based on grass silage (GS), faba bean silage (FB), or pea silage (PS).[1]

		GS	FB	PS	SD	*P*-value
Faeces	Non-ammonia N (g kg^{-1} DM)	23.2^b	24.9^a	22.2^b	1.50	0.012
	Ammonia N (g kg^{-1} DM)	0.23	0.18	0.17	0.091	0.601
Urine	Non-ammonia N (g l^{-1})	6.3^c	7.5^b	8.9^a	1.42	<0.001
	Ammonia N (mg l^{-1})	30.8^b	26.7^b	59.0^a	20.7	0.007
Milk	Protein (g l^{-1})	31.1	32.4	31.2	2.20	0.567
	Urea (mg l^{-1})	237	298	281	49.5	0.075

[1] DM = dry matter; SD = standard deviation; different superscript letters indicate significant differences among managements.

Conclusions

The pathway for the excretion of dietary nitrogen from legume silages is mainly through urine, and the metabolisation of pea silage protein goes toward ammonia-N. Therefore, the use of slurry as fertiliser from dairy cows feeding on legume silages could increase the emissions of nitrogen to environment.

Acknowledgements

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References

Elouadaf D., Martínez-Fernández A., Soldado A. and Vicente F. (2020) Effect of protein level in the diet of dairy cows on the slurry composition to be used as fertilizer. *Grassland Science in Europe* 25, ###-###.

Foster J.L., Carter J.N., Sollenberger L.E., Blount A.R., Myer R.O., Maddox M.K., Phatak S.C. and Adesogan A.T. (2011) Nutritive value, fermentation characteristics, and *in situ* disappearance kinetics of ensiled warm-season legumes and bahiagrass. *Journal of Dairy Science* 94, 2042-2050.

Luscher A., Mueller-Harvey I., Soussana J.F., Rees R.M. and Peyraud J.L. (2014) Potential of legume-based grassland-livestock systems in Europe: a review. *Grass and Forage Science* 69, 206-228.

NRC (2001) *Nutrient requirements of dairy cattle*, 7th revised edition, National Research Council, National Academy Press, Washington, DC, USA, 381 pp.

R Core Team (2018) *R: a language and environment for statistical computing*. R Foundation for Statistical computing, Vienna, Austria.

Soycan-Önenç S., Koc F., Coşkuntuna L., Özdüven M.L. and Gümüş T. (2015) The effect of oregano and cinnamon essential oils on fermentation quality and aerobic stability of field pea silages. *Asian-Australasian Journal of Animal Sciences* 28, 1281-1287.

Wanapat M., Pilajun R. and Kongmun P. (2009) Ruminal ecology of swamp buffalo as influenced by dietary sources. *Animal Feed Science Technology* 151, 205-214.

European permanent grasslands mainly threatened by abandonment, heat and drought, and conversion to temporary grassland

Schils R.L.M.[1], Newell Price J.P.[2], Klaus V.H.[3], Tonn B.[4], Hejduk S.[5], Stypinski P.[6], Hiron M.[7], Fernández P.[8], Ravetto Enri S.[9], Lellei-Kovacs E.[10], Annett N.[11], Markovic B.[12], Lively F.[13], Ten Berge H.[1], Smith K.[2], Forster-Brown C.[2], Jones M.[2], Buchmann N.[3], Janicka M.[6], Fernandez J.[8], Rankin J.[11], McConnell D.[13], Aubry A.[13] and Korevaar H.[1]

[1]Agrosystems Research, Wageningen Plant Research, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands; [2]ADAS, United Kingdom; [3]Department of Environmental Systems Science, ETH Zürich, Switzerland; [4]Department of Crop Sciences, University of Goettingen, Germany; [5]Department of Animal Nutrition and Forage Production, Mendel University, Czech Republic; [6]Faculty of Agriculture and Biology, Warsaw University of Life Sciences, Poland; [7]Department of Ecology, Swedish University of Agricultural Sciences, Sweden; [8]Department of Forestry, ETSIAM, University of Córdoba, Spain; [9]Department of Agricultural, Forest and Food Sciences, University of Turin, Italy; [10]Institute of Ecology and Botany, MTA Centre for Ecological Research, Hungary; [11]Agrisearch, Northern Ireland; [12]Department of Livestock Science, University of Montenegro, Montenegro; [13]Agri-Food and Biosciences Institute, Northern Ireland, United Kingdom

Abstract

Permanent grassland (PG) covers around 60 Mha in the EU-28. Across Europe, PG exists in many contrasting managed or unmanaged environments where it contributes to feed supply, biodiversity, carbon sequestration, aesthetic value, recreation, clean water and prevention of soil loss. The delivery of these PG ecosystem services is under threat by land use change, climate change, and sub-optimal management. We carried out a survey among agronomists, ecologists, soil scientists, foresters and agri-environmental consultants to assess the threats for PG within their countries. Respondents described the main PG types and their areas, and assessed to what extent a particular PG type is threatened by intensification, land use change, climate change or nitrogen deposition. Threats were scored on a three-point scale: no, small or great. Replies were received from 34 experts in 11 countries (CH, CZ, DE, ES, HU, IT, ME, NL, PL, SE, UK). The dataset contained 83 PG types on a total area of 25 Mha. Abandonment, heat and drought stress, and conversion to temporary grassland were considered as the largest threats, concerning PG types covering 8 to 9 Mha. The second group of great threats comprised N deposition, conversion to arable land and intensification, causing a great threat on PG types covering 4 to 5 Mha.

Keywords: abandonment, climate, intensification, land use, N deposition, expert survey

Introduction

Permanent grassland (PG) covers around 60 Mha in the EU-28 (EUROSTAT, 2019), representing 35% of the utilised agricultural area. Throughout Europe, PG exists across different biogeographic zones in many contrasting environments, which often but not necessarily rely on regular management. PG is key to the delivery of multiple important ecosystem services (ES). It is mainly utilised to provide feed for ruminants, but also contributes to other ES such as biodiversity, pollination, carbon sequestration, provision of clean water, erosion prevention, aesthetic value and recreation (Bengtsson *et al.*, 2019). However, PG maintenance and the delivery of its ES are under threat by different drivers such as land use change, climate change, and nitrogen (N) deposition. Even though these threats are quite universal, their occurrence and impacts are regionally specific. For example, in north-west Europe, large areas of PG have been converted into arable land to cultivate maize for animal feed or for use in biogas production

(Taube *et al.*, 2014). In southern and eastern Europe, the desiccation of grasslands due to climate change is a serious threat to PG (Griffin-Nolan *et al.*, 2019). The objective of the present paper is to assess regionally specific threats to PG across Europe. The assessment will help to raise awareness and improve PG-related decision making by policy makers and farming sector businesses.

Materials and methods

We carried out an expert survey, among 34 agronomists, ecologists, soil scientists, foresters and agri-environmental consultants working in research and extension services, to assess the major threats affecting PG in Europe. For each country, a lead representative collected information from several respondents, describing the main country-specific PG types and their areas, and assessing to what extent a particular PG type is threatened by intensification, land use change (abandonment, conversion to temporary grassland, arable or forest), climate change (heat and drought stress) and N deposition. PG types were mainly based on habitat, management intensity and plant species composition. Threats were scored on a three-point scale: no, small or great threat. In this study, threat is interpreted quite broadly, either as the disappearance of PG and conversion into other land uses, or the negative effect on any of the ES. Replies were received from 11 countries or regions (CH, CZ, DE, ES-Andalusia, HU, IT, ME, NL, PL, SE, UK), representing the main biogeographic zones in Europe. Together these countries and regions cover nearly 25 Mha, representing 40% of PG in the EU-28 plus CH and ME. Overall, 83 PG types were assessed. On average, there were 8 different PG types described per country, ranging between 4 and 11 types. The 83 PG types belong to the following EUNIS categories: dry grasslands (E1; 34%), mesic grasslands (E2; 27%), (sub) alpine grasslands (E4; 10%) and (seasonally) wet grasslands (E3; 5%). The remainder comprised, among others, woodland fringes (E5), steppes (E6), wooded grasslands (E7) and heathland (F4).

Results and discussion

All assessed PG types were considered to be under some kind of threat (Figure 1). In most cases, PG types were under either a single threat or a combination of two or three threats. The area of PG types that is affected by land use change increased in the order of conversion to forest, arable and temporary grassland. Conversion to forest is a great threat on PG types covering 3 Mha in CH, CZ, ME, PL and UK, mainly in extensively used hilly and mountainous areas. Conversion to arable land (5 Mha) or temporary grassland (8 Mha) is a great threat in parts of CH, CZ, DE, ES, IT, PL, SE and UK, relatively more on (semi-) intensively used lowland areas. Land use change is considered a small threat on a larger scale, on PG types covering around 9 Mha. Intensification was considered as a great threat on 4 Mha in parts of CH, CZ, ES, HU, IT, SE and UK and a small threat on another 14 Mha. Many of the PG types where intensification is not seen as a threat are already under intensive use. At the opposite end of the intensification – extensification scale, 9 Mha in CH, CZ, DE, ES, IT, ME, PL, SE and UK were considered to be under great threat of abandonment. These areas comprise almost all areas that were identified under threat from conversion to forest, as well as many additional PG types. Heat and drought

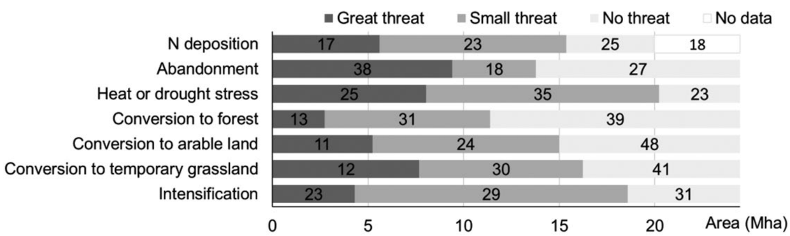

Figure 1. Permanent grassland (PG) area (bars) and number of PG types (figures inside bars) under a small threat, great threat and without a threat.

stress were considered to be a great threat on 8 Mha, not surprisingly in the warmer and drier regions of Europe (CZ, ES, HU, IT, ME and PL) and a small threat on 12 Mha, also in the cooler and wetter regions (CH, NL and UK). Finally, N deposition is a great threat on 5 Mha in CH, IT, NL, SE and UK, and a small threat on 10 Mha.

The overview of threats to PG is the outcome of an expert survey, and thus has its limitations and uncertainties. The national PG classifications used in this survey do not necessarily always match between countries or with the Eurostat classification. The expert panel was a mixture of ecologists and agronomists, with their own perception of PG threats, and inherent biases. For example, intensification may be seen as a threat to biodiversity, but may also be valued to enhance feed production. The level of detail also differed between countries. Furthermore, within PG types there may be relevant local variations that are not accounted for in this assessment. Therefore, the assignment of threats to the land areas of PG types may overestimate the actual threat as not all areas within a PG type will be affected to the same extent, or may not be affected at all.

Conclusions

All PG types were considered to be under some kind of threat. Abandonment, heat and drought stress, as well as conversion to temporary grassland were seen as the most major threats, followed by N deposition, conversion to arable land and intensification. This situation implies the need to more intensely address threats to PG by agricultural policy to ensure the delivery of the many crucial ES delivered by grassland ecosystems.

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References

Bengtsson J., Bullock J.M., Egoh B., Everson C., Everson T., O'Connor T., O'Farrell P.J., Smith H.G. and Lindborg R. (2019) Grasslands – more important for ecosystem services than you might think. *Ecosphere* 10, e02582.

EUROSTAT (2019) Utilised agricultural area by categories. Available at: https://ec.europa.eu/eurostat/web/products-datasets/-/tag00025.

Griffin-Nolan R.J., Blumenthal D.M., Collins S.L., Farkas T.E., Hoffman A.M., Mueller K.E., Ocheltree T.W., Smith M.D., Whitney K.D. and Knapp A.K. (2019) Shifts in plant functional composition following long-term drought in grasslands. *Journal of Ecology* 107, 2133-2148.

Taube F., Gierus M., Hermann A., Loges R. and Schönbach P. (2014) Grassland and globalization-challenges for north-west European grass and forage research. *Grass and Forage Science* 69, 2-16.

Prospect of field margins to reintroduce plant species richness in intensive grassland production

Schmitz A.[1], Lott S.[2], Wellinghoff J.[1,2], Leuschner C.[2] and Isselstein J.[1]
[1]*Georg-August-University Göttingen, Grassland Science, von-Siebold-Str. 8, 37075 Göttingen, Germany;* [2]*Georg-August-University Göttingen, Albrecht-von-Haller Institute for Plant Sciences, Untere Karspüle 2, 37073 Göttingen, Germany*

Abstract

In the north German Plains, species-rich wet lowland hay meadows have largely disappeared as a result of agricultural intensification such as increased fertilisation and frequent defoliation. Extensification measures often fail to restore species diversity due to depleted soil seed banks. Field margins and grassland strips adjacent to ditches seem promising for the establishment of species-rich swards without compromising the overall productivity of the farm's grassland. In order to assess the potential of restoring species-rich margins, we analysed 72 intensively managed grasslands on 12 dairy farms located in the North German Plains. We measured species richness, soil seed bank and soil chemical characteristics of the centre and the margins of each paddock. Our results confirm that species of a higher nature value were extremely rare both in the above ground vegetation and in the seed bank. Field margins had a higher species number and lower nutrient concentration than the referring centre. However, to reintroduce species of flower-rich lowland hay meadows, active seed addition of most target species is required.

Keywords: intensive grassland production, biodiversity, seed banks, restoration, agri-environment schemes

Introduction

In the north German Plains, extensively managed wet lowland hay meadows have become rare. Species of higher nature value (HNV-species) are almost lost as a result of increased fertilisation and cutting frequency (Krause *et al.*, 2011). Awareness of the urgent need to promote biodiversity is growing. However, large-scale extensification measures would pose a severe risk for profitability of the farming enterprise, and so they are not preferred by farmers. In addition, restoration measures often fail because of depleted soil seed banks and high nutrient concentration of the soils (Bakker and Berendse, 1999). Field margins adjacent to ditches have recently been suggested as niches for meadow species in marshlands (Rasran and Vogt, 2018) because they are usually less intensively managed. Therefore, field margins seem promising target areas to re-establish species-rich swards at a small spatial scale. In the present study we aim to analyse: (1) the status quo of species richness and target species of a higher nature value (HNV) in intensively managed grasslands, and (2) evaluate the potential of field margins as target areas for measures to re-establish species-rich grassland swards.

Materials and methods

We analysed 72 intensively managed grassland paddocks at 12 dairy farms in the north German Plains. Grasslands were located in two habitat types, marshlands (n=36) and moorlands (n=36), and have a long-term history of intensive grassland management with frequent cutting and heavy fertilisation. Herbage yields range from 8.5 to 11.5 Mg dry matter (DM) ha^{-1} $year^{1}$. We analysed the soil seed bank, the above ground vegetation and topsoil chemical characteristics (pH, plant available phosphorus mg^{-1} g^{-1} soil DM (P)) on each paddock. Measurements were taken along transect lines with one transect at the border (0-2 m from the field border), one transect within the field margin (2-8 m from the border) and one transect trough the central area of each paddock. The length depended on the size of each paddock. On smaller paddocks, the transect was 2×100 m and on larger paddocks 2×200 m. Seed banks were sampled in

December 2018 in the central and the border transect. Per transect, one litre soil was sampled randomly with a soil auger (1.5 cm diameter, sampling depth 0-10 cm). Soil was stored at 3 °C for six weeks and processed following Ter Heerdt (1996). Samples were then transferred to a greenhouse (~18 °C day, 15 °C night) and seedling emergence recorded over a period of 10 weeks. Sward species inventory and soil was sampled in May 2019 on all 3 transects per paddock (central, margin, border). HNV-species were used as an indicator for nature value and classified following a regional list (Bundesamt für Naturschutz BfN, 2019). Per transect, 500 ml soil were sampled to 10 cm depth and analysed for pH and plant available nutrients (CAL extraction). We modelled species richness (SR), HNV-species richness (HNV-SR) of swards and seed banks, as well as pH and P as a function of habitat type (marsh and moorlands) and transect location (central, margin, border, respective central and border for seed banks). Additionally, we modelled SR and HNV-SR of swards and seed banks as a function of pH and P. The farm was included as random factor (lme in nlme package). Tukey test was applied on significant effects (emmeans package) and conditional R^2 was calculated based on fixed and random effects within the piecewiseSEM package. Length of transects was tested for significant effects on dependent variables but excluded since none was obtained. All statistics were performed in R (version 3.6.1).

Results and discussion

Our results confirmed a generally low SR and especially HNV-SR in the above ground vegetation as well as in the seed bank of intensively managed grasslands (Krause *et al.*, 2011), regardless of the habitat type (marshlands or moorlands). We found an average of 17 vascular plant species in swards of central transects, with only one HNV-species on average. *Cardamine pratensis* (in 51% of centres) and *Ranunculus acris* (in 15% of centres) were the most frequent HNV-species in swards. Out of 72 grasslands, we found a maximum of three HNV-species in a central transect. In seed banks of central transects, on average fewer than 15 species were found. However, we observed highly significant differences of SR in seed banks and of SR and HNV-SR in swards within grassland sites; compared with central areas, we found more species in seed banks and swards along the field borders. In swards, more HNV-species were frequent along borders than in central and margin transects (Figure 1A-D). While highly productive species (i.e. *Lolium perenne, Festuca arundinacea*) are dominants in swards of intensively managed grasslands on

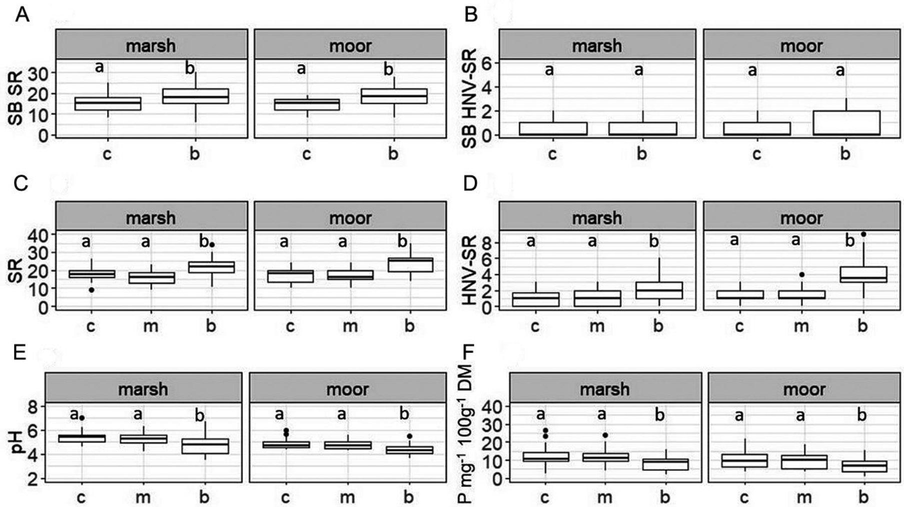

Figure 1. Species richness (SR) and High-Nature-Value indicator species richness (HNV-SR) of seed banks (A, B) and grasslands swards (C, D) as well as soil chemistry (E, F) of 72 grasslands. Lower case letters indicate significant differences ($P<0.05$) between locations within sites (c = central, m = margin, b = border) of the model-based estimates.

nutrient-rich soils, field borders provide niches (Rasran and Vogt, 2018) for more species, especially those indicating high nature value. This finding emphasises the potential of field margins, especially those areas close to the borders excluded from intensive management. The soil chemistry of these borders is significantly different from central swards and margins (Figure 1E-F). Field borders are more acidic and have significantly lower P concentrations than central areas. High P-concentration is a constraint of species diversity and restoration potential in grasslands (Bakker and Berendse, 1999). Therefore, the given soil chemistry might be more suitable for the establishment of species-rich swards. However, the observed higher SR of field margins is not limited to soil chemistry alone. Direct analysis of SR as a function of soil chemistry indicates an influence of pH (coefficient: 0.5, P=0.02) and P (coefficient: 0.33, P=0.001) on seed bank SR (conditional R^2=0.34) and of pH (coefficient. -1.3, P=0.01) on sward SR (conditional R^2=0.1). Regarding HNV-SR, we found an effect of pH coefficient: -0.78, P<0.001) on sward HNV-SR (conditional R^2=0.18) but not in seed banks. Very low R^2 values of those models stress the importance of other field-margin-related effects to explain SR and presence of HNV species.

Conclusions

The botanical diversity in the context of intensively managed grasslands in northern Germany is limited. Active seed addition of most target species is required to establish species-rich swards. To promote species richness in intensively managed grasslands, small-scale measures like improved field margins are promising. This is not only because they might be more attractive for farmers. The soil conditions are also better suited for the establishment of introduced species. Further research needs to address the establishment, practicability, and agronomic functionality of such small-scale measures.

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References

Bakker J.P. and Berendse F. (1999) Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends in Ecology & Evolution* 14, 63-68.

Bundesamt für Naturschutz BfN (2019) Kenntaxa für die regional differenzierte Bewertung von Grünlandflächen im Rahmen des HNV-Farmland-Indikators für Deutschland. URL: Available at: https://www.bfn.de/fileadmin/BfN/monitoring/Dokumente/14_02_26_Kennartenliste_HNV_barrfrei.pdf.

Krause B., Culmsee H., Wesche K., Bergmeier E. and Leuschner C. (2011) Habitat loss of flood-plain meadows in north Germany since the 1950s. *Biodiversity and Conservation* 20, 2347-2364.

Rasran L. and Vogt K. (2018) Ditches as species-rich secondary habitats and refuge for meadow species in agricultural marsh grasslands. *Applied Vegetation Science* 21, 21-32.

Ter Heerdt G.N.J., Verweij F., Bekker R.M. and Bakker J.P. (1996) An improved method for seed-bank analysis: seedling emergence after removing the soil by sieving. *Functional Ecology* 10, 144-151.

Leaf area index dynamics in a grass-willow alley cropping system

Sutterlütti R.[1,2], Tonn B.[1,2], Komainda M.[1], Kayser M.[1,3] and Isselstein J.[1,2]
[1]*Division of Grassland Science/Department of Crop Sciences, University of Göttingen, Germany;* [2]*Centre of Biodiversity and sustainable Land Use (CBL), University of Goettingen, Germany;* [3]*University of Vechta, Faculty II, Germany*

Abstract

In alley cropping systems, distance to the tree rows is a factor that potentially influences the Leaf Area Index (LAI) dynamics of the grassland between the tree rows. How LAI dynamics of grassland swards are affected in alley cropping is not well understood to date. In a field experiment, we determined temporal as well as spatial changes of LAI dynamics of grassland in an alley cropping system with willow (*Salix* sp.) under two cutting frequencies (two or four annual cuts) and five transect positions between two tree rows. We compared a direct approach (leaf area scans) with a light interception (ceptometer) method. The ceptometer readings were in good accordance with the directly measured leaf area values. Our results show that cutting frequency had the greatest effect on LAI over the course of the year. Transect position influenced LAI to a smaller degree. The influence of transect position was complex and interacted with management over time: it affected grassland LAI only at certain times and had a greater influence on differences in LAI at transect positions with an extensive cutting frequency.

Keywords: alley cropping, agroforestry, AccuPar

Introduction

Agroforestry is currently being rediscovered as a sustainable form of land use in the temperate climate zone. A frequently used practice is the combination of rapidly growing woody species such as poplar or willow planted in rows (alleys) and crops or grassland (alley cropping). Trees influence the adjacent grassland by altering microclimatic parameters such as relative humidity and temperature. By hydraulic lift trees might improve water availability for the grassland (Caldwell and Richards, 1989). On the other hand, competition for nutrients, light and water between trees and grassland might occur. This could influence LAI as a plant physiological parameter which affects the turnover of biomass and nutrients in the grassland. The LAI is an important measure for the productivity, heterogeneity and density of a grass sward and can also be used to estimate biomass (He *et al.*, 2007).

We assume that leaf area development during the growing season is influenced by the management as well as by distance and position in relation to the tree row and we test the accuracy of an indirect measurement compared with a direct measurement.

Materials and methods

Our experimental site is located in northwest Germany and consists of permanent grassland between two tree rows (*Salix* sp.) planted along a North-South axis with a spacing of 50 m. We used a two-factorial split-plot design with management and transect position (TP) as experimental factors (Figure 1). We compared an intensive (four annual cuts) with an extensive (2 annual cuts) management, each replicated in six main plots. Subplots were located at five TPs nested in a total number of 12 main plots with distances of 2, 6 and 25 m to the closest tree row (Figure 1). This enabled us to study growth processes dependent on the distance to the tree row as well as the exposition.

We monitored leaf area development from March until October 2019 at every one of the 60 TPs in bi-weekly intervals. Measurements were made with a ceptometer (AccuPar LP-80, METER Environment)

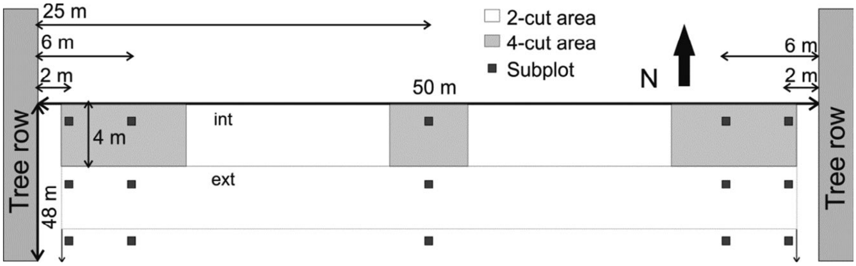

Figure 1. Experimental setup showing three of a total number of 12 main plots with five transect positions with defined distances to the tree row (small squares, area = 0.25 m^2).

which calculates LAI based on the interception of photosynthetically active radiation (PAR) by the canopy. To test the accuracy of the indirect method, we compared it to values obtained from a direct method. Therefore, we measured the herbage mass as an indicator of productivity by clipping the grass in subplots of 0.25 m^2 five times throughout the vegetation period. Direct leaf area measurements were taken on 20% of each biomass sample using the LAI3100 (LiCor). To compare methods, indirect LAI measurements by ceptometer were modelled as a linear function of the direct LAI measurements. The influence of the factors TP and T-M (a factor combining time and management) and their interaction on the LAI measured by ceptometer was evaluated with a linear mixed effects model (R Software, Version 3.3.0), with plot and main plot as nested random effects.

Results and discussion

Although the relationship between directly and indirectly measured LAI was reasonably close, we found that the indirect method underestimated LAI, particularly at higher values (Figure 2). This confirms findings of He *et al.* (2007). The development of LAI over time was influenced by T-M ($F_{27,611}$=129.9, P<0.0001), TP ($F_{4,44}$=18.7, P<0.0001) and also by an interaction between the two factors ($F_{108,611}$=2.8, P<0.0001). Our results show that management had the greatest effect on LAI over the course of the year. The effect of TP was complex: it affected grassland LAI at certain times, changing in the course of the season due to the interaction with T-M. The effect of the tree row was stronger during the primary growth in spring and more pronounced under the extensive management scheme (Figure 3).

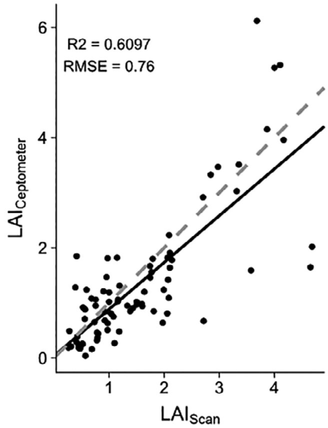

Figure 2. Leaf area index (LAI) values of calibration cuts from ceptometer readings (y-axis) in relation to LAI values derived from scans of harvested biomass (x-axis). The grey line represents the line of equality (y = x) between the two methods. The black line shows the relationship between the two methods derived from measurements: $LAI_{Ceptometer}$ = 0.85 LAI_{Scan} +0.04.

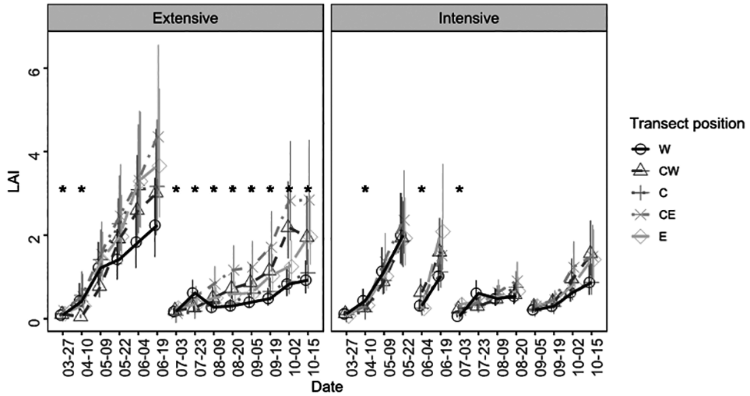

Figure 3. Leaf area index (LAI) values (model estimates) for the two-cut (extensive) and four-cut (intensive) treatment. Abbreviations for transect positions: W/E: tree row in the west/east of the plot, distance 2 m; CW/CE: tree row in the west/ east of the plot, distance 6 m; C: centre of the main plot, distance 25 m. Error bars represent calculated confidence intervals. Significant differences in LAI between transect positions are marked by asterisks.

Proximity to tree row influenced LAI, with lower LAI values at very close distances to the trees and maximum values at an intermediate distance that is still influenced by the trees. Due to the interaction between T-M and TP, the transects cannot be arranged in a consistent ascending/descending order with respect to their LAI values over the whole growing season.

Conclusions

We show that the biggest influence on LAI dynamics in the studied alley cropping system resulted from a combined factor of time and management. We assume that distance to the tree line alone is insufficient for explaining tree effects on grassland productivity, and that mediating factors, e.g. light quality and quantity and micrometeorological parameters influencing transpiration demand, must be studied directly.

Acknowledgements

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References

Caldwell M.M. and Richards J.H. (1989) Hydraulic lift: water efflux from upper roots improves effectiveness of water uptake by deep roots. *Oecologia* 79, 1-5.

He Y., Guo X. and Wilmshurst J.F. (2007) Comparison of different methods for measuring leaf area index in a mixed grassland. *Canadian Journal of Plant Science* 87, 803-813.

Pasture ecosystem services indicators: an expert based set of indicators of ecosystem services

Taugourdeau S.[1,2], Messad S.[1,2], Louault F.[3], Michelot-Antalik A.[4], Vigneron M.[1,2], Poisse L.[4], Yentur L.[4], Millet C.P.[3], Bastianelli D.[1,2], Carrère P.[3] and Plantureux S.[4]
[1]CIRAD, UMR SELMET, TA C-112 / A Campus de Baillarguet, 34398 Montpellier, France; [2]UMR SELMET, CIRAD Univ Montpellier SupAgro INRA, Campus de Baillarguet, Montpellier, France; [3]INRA, UMR UREP, Avenue du Brézet, Clermont Ferrand, France; [4]Univ Lorraine, UMR LAE, ENSAIA, Vandœuvre les Nancy, France

Abstract

Many recent studies show that grassland biodiversity is linked to ecosystem functioning (BEF Theory) and to its capacity to deliver a large diversity of ecosystem services (ES). However, till now no methods allow an easy evaluation of the ES provided (i.e. forage production, biodiversity conservation, pollination, carbon sequestration). Our goal was to develop indicators of ES using botanical surveys as a proxy for biodiversity, because botanical surveys can be achieved easily and are integrative of the spatiotemporal variability of grassland ecosystems. We used a multicriteria analysis tool to aggregate different criteria calculated from botanical survey to assess ES standardised scores (ranged from 0 to 1). This tool used a set of indicators, which combined academic knowledge and expertise. The first step was to select a set of criteria from botanical surveys which are standardised and combined together so as to assess five ecosystem services (1) forage production and quality, (2) flexibility of forage production, (3) nitrogen availability for vegetation, (4) biodiversity conservation and (5) resilience hazards and pollination. Finally, these sets of ES indicators were discussed and interactively improved with 20 experts during a workshop.

Keywords: botanical survey, ecosystem service, forage production, biodiversity, nitrogen

Introduction

Grassland, especially permanent grassland, provides many ecosystem services (ES), such as forage for animals, soil carbon sequestration, conservation of biodiversity, pollination. Grasslands are present in different climates and under different managements (grazing, cutting, fertilisation, etc.). This variability strongly affects the botanical composition of grassland and so the provision of ES. Many studies show for grassland a link between vegetation characteristics, especially biodiversity, and ecosystem functioning and therefore its services (De Bello *et al.*, 2010). Some studies have proposed to use this link between vegetation and ES in order to develop indicators of services (Lavorel *et al.*, 2011).

Our goal was to developed indicators of ES using the actual knowledge between grassland vegetation and ES. We used a multicriteria analysis tool to aggregate vegetation variables to produce scores for ES. The choice of the vegetation variables and the way to aggregate the different variables were defined by expert knowledge. One of the main constraints was that the variables can be calculated only from a botanical survey. Our main hypothesis was that botanical survey (species presence and absence) is enough to assess the variability of ecosystem services provision.

Materials and methods

We used the Tatale tools to develop the indicator (https://umr-selmet.cirad.fr/en/products-and-services/proposed-products/tatale) (Taugourdeau and Messad, 2017). This construction is based on two principles: (1) expertise on variation ranges and key relationships between variables, and (2) normalisation and aggregation of variables for scoring, which can be considered as latent variables. Variables such as percentage of legume or number of species from the botanical survey are first transformed into a score between 0 and 1 based on expert knowledge. The different scores are aggregated using mean values to produce a final ES score.

We chose to work on six different ES: forage provisioning in terms of quantity and quality, forage production stability, nitrogen availability, biodiversity conservation, resistance of the community to extreme events, and pollination. To be able to propose ES indicators, we had first to disaggregate these services into several component ecosystem functions and processes. Then, we selected vegetation variables related to the different functions and processes. For example, forage provisioning was disaggregated into forage annual production, forage quality and the percentage of the biomass available for animal feeding. Finally, we proposed transformation and aggregation coefficients for each vegetation variable. For example, we chose percentage of cover in the survey of species with pastoral value equivalent to 0 to evaluate the percentage of biomass available for animals feeding. Thus, a grassland cover of 100% undesirable species will have a score of 0. These indicators are the first prototypes of ES and are available on the website https://pesi-travail.cirad.fr/en. The creation of the indicators was made using literature surveys and expert meetings. We had a meeting with 20 experts in June 2018. The different indicators and the explanation for the different choices made are presented on a website (https://pesi-travail.cirad.fr/en).

We used the indicators developed in this work on the 1,754 botanical surveys from the eflorasys dataset (Plantureux and Amiaud, 2010). The surveys cover mostly France during 1973 to 2018. Functional traits were obtained from the TRY database (www.try-db.org).

Results and discussion

This example shows the possibility for using our indicators on large datasets to evaluate the trade-off between services and to see change over time. Figure 1 represented the principal component analysis (PCA) obtained for the 6 scores of ES together with latitude longitude and year of the survey as

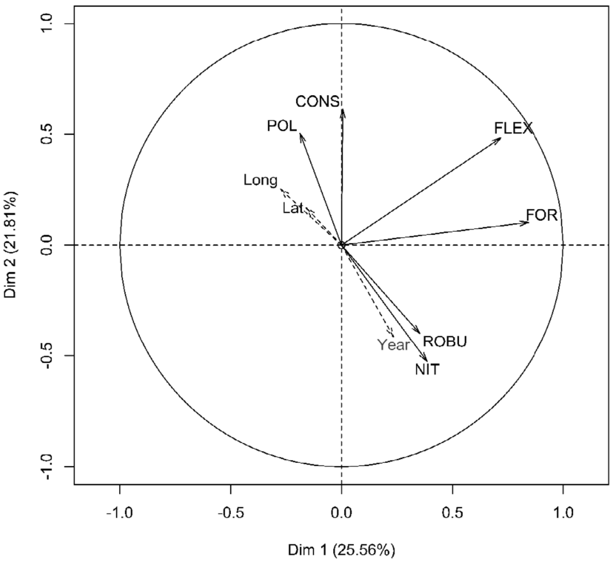

Figure 1. Principal component analysis on the score of ES (FOR forage provisioning, FLEX for flexibility in forage production, CONS for conservation of biodiversity, ROBU for Robustness to extreme events, NIT nitrogen availability, Pol pollination) and latitude (Lat), longitude (Long) and the year of the survey (Year) that were used as a supplementary variable.

supplementary variables. Nitrogen availability was positively correlated with the robustness to extreme events and negatively with pollination. Forage provisioning and forage flexibility were correlated together but were independent from other services. The average nitrogen availability seems to be higher in recent years, and opposite pollination and conservation of biodiversity. These results are quite logical in the context of intensification, and intensification of grassland management decreases plant diversity.

Conclusions

These indicators, produced using expert knowledge and based on botanical surveys, can be used on the data from historical botanical surveys to see the long-term changes in provision of ecosystem services of grasslands.

Acknowledgements

We thank all the experts that participated in the workshop for their precious input, and the Ecoserv INRA metaprogram for the funding.

References

De Bello F., Lavorel S., Diaz S., Harrington R., Cornelissen J.H.C., Bardgett R.D., Berg M.P., Cipriotti P., Feld C.K., Hering D., Da Silva P.M., Potts S.G., Sandin L., Sousa J.P., Storkey J., Wardle D.A. and Harrison P.A. (2010) Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodiversity Conservation* 19, 2873-2893.

Lavorel S., Grigulis K., Lamarque P., Colace M.-P., Garden D., Girel J., Pellet G. and Douzet R. (2011) Using plant functional traits to understand the landscape distribution of multiple ecosystem services. *Journal of Ecology* 99, 135-147.

Plantureux S. and Amiaud B. (2010) e-FLORA-sys, a website tool to evaluate agronomical and environmental value of grasslands. *Grassland Science in Europe* 15, 732-734.

Taugourdeau S. and Messad S. (2017) *TATALE: tools for assessment with transformation and aggregation using simple logic and expertise*. Manual. CIRAD-ES-UMR SELMET, Montpellier, France, 11. pp.

When root traits emerge: soaking solutions for washing perennial grass roots

Thivierge M.-N.[1], Royer I.[1], Halde C.[2], Chantigny M.H.[1], Bélanger G.[1], Lachance C.[1] and Lavergne S.[2]
[1]Quebec Research and Development Centre, Agriculture and Agri-Food Canada, Canada; [2]Département de Phytologie, Université Laval, Canada

Abstract

Root traits provide valuable information when studying grassland ecophysiology. With their fibrous architecture, roots of perennial grasses are particularly challenging to separate from the surrounding soil. We assessed the effect of five soaking solutions (sodium hexametaphosphate, sodium bicarbonate, sodium chloride, ethylenediaminetetraacetic acid, and distilled water) and three soaking durations (15 min, 2 h, and 16 h) on root recovery and root tissue elemental composition after washing. Root samples were collected by soil coring in a 3-yr-old timothy (*Phleum pratense* L.) sward on a silty loam with 24 replications of each combination of soaking solution and duration for a total of 360 samples. After soaking, roots were washed, dyed, and digitized to measure morphological traits and, then, analysed to determine their elemental composition. Soaking duration did not affect root recovery. The greatest recovery of ash-free root biomass and total root length was obtained with sodium bicarbonate, whereas sodium hexametaphosphate led to the lowest root contamination by soil particles, as measured by root tissue concentrations for ash and for major and trace elements. This study revealed a trade-off in selecting soaking solutions between root recovery and root contamination by soil particles.

Keywords: grass root, soaking solution, root biomass, root traits, root tissue elemental composition

Introduction

Root traits are of utmost importance when studying grassland ecophysiology. The accuracy and precision of root trait measurements are strongly dependent on the thoroughness of root washing. The washing procedure to separate roots from soil can lead to a loss of roots (Smucker *et al.*, 1982). Moreover, root contamination by soil can lead to an overestimation of root biomass and can compromise analyses of root tissue elemental composition (Azcue, 1996). With their fibrous architecture, roots of perennial grasses are particularly challenging to separate from the surrounding soil, which is why the greatest care must be taken while washing them. We assessed the effect of soaking solutions and durations on the recovery of roots from field-extracted soil cores in a timothy (*Phleum pratense* L.) sward, and the elemental composition of recovered root tissues.

Materials and methods

Soaking solutions, concentrations, and durations were selected following an exhaustive literature review. All combinations of five soaking solutions (sodium hexametaphosphate [$(NaPO_3)_6$] 0.16 M, pH 6.3; sodium bicarbonate [$NaHCO_3$] 1.20 M, pH 8.3; sodium chloride [NaCl] 1.00 M, pH 5.8; ethylenediaminetetraacetic acid [EDTA $C_{10}H_{14}N_2O_8Na_2$, $2H_2O$] 0.01 M, pH 4.6; and distilled water [H_2O], pH 6.4) and three soaking durations (15 min, 2 h, and 16 h) were tested in a factorial experiment, for a total of 15 treatment combinations. A total of 360 field-extracted soil cores from the 8 to 13 cm top soil layer (24 replications for each of the 15 treatment combinations) were collected in 2017 in a 3-year-old timothy pure stand on a silty loam in Quebec, Canada (46°44'N, 71°30'W). The volume of each soil core was 221 cm^3. Each soil core was individually placed in a 2 l container with 300 ml of soaking solution. After soaking, separation of roots from soil was performed using a hydropneumatic elutriation washing machine (Smucker *et al.*, 1982) with a 760 µm primary sieve and a 290 µm secondary sieve. After separation from the soil, root samples were soaked for 2 h in a 0.5 g l^{-1} neutral red solution ($C_{15}H_{17}ClN_4$)

to improve the contrast between roots and the white background used for image acquisition. Dyed roots were carefully placed on a 30×40 cm transparent tray and were digitized (Epson Expression 10000XL scanner, Epson Canada, Markham, ON) at a resolution of 600 dots per inch (dpi). Images were analysed using the WinRhizo Pro Arabidopsis software (Regent Instruments Inc., Quebec City, QC) to measure root length and diameter. After digitization, root samples (n=360) were dried at 50 °C for 72 h, weighed, ground, and analysed for C, N, and S concentrations by dry combustion and for ash concentration with a thermogravimetric analyser (LECO CNS-1000 and TGA701, respectively, Leco Corp., St. Joseph, MI, USA). Root biomass is expressed on an ash-free dry mass basis. The remaining material was then pooled by soaking solution (irrespectively of soaking duration) and analysed for elemental composition. Major and trace elements in roots (Al, Co, Fe, Mg, Mn, Ni, P, and Zn) were extracted by acid digestion with HNO_3:HCl (5:2) (US EPA, 1996) and their total concentration was determined in the extract by ICP-OES. All data were subjected to analysis of variance using the MIXED procedure in SAS Statistical Analysis System, Release 9.3, 2010 (SAS Institute Inc., Cary, NC, USA). When a significant effect was found, multiple comparisons were performed. Differences were considered significant at $P\leq0.05$.

Results and discussion

Soaking duration did not affect the mass, length or diameter of recovered roots, but the 16 h soaking duration had a significant but small reducing effect on root ash concentration (136.7 vs 146.6 g kg^{-1} dry matter (DM) with the shorter durations). Regardless of soaking duration, sodium bicarbonate resulted in the greatest recovery of ash-free root biomass and total root length (Figure 1). Root diameter was not affected by the soaking solution (P=0.09; data not shown).

The sodium bicarbonate solution in contact with soil samples had a rapid effervescent reaction (with CO_2 production) resulting in an homogeneous mixture, unlike all other soaking solutions that resulted in a soil deposit at the bottom of the container. This soil deposit possibly imprisoned roots and prevented them from being fully recovered during the washing procedure, which could explain the lower ash-free root biomass and root length recovered in solutions other than sodium bicarbonate. With sodium bicarbonate, roots were easy to separate from the soil, but then clean roots tended to stick together and took longer to prepare for digitization, which is a factor that should be taken into consideration when a high number of samples is to be processed. Distilled water did not lead to a smaller root recovery than sodium hexametaphosphate, sodium chloride, or EDTA (Figure 1), and therefore should not be discarded as a soaking solution when it comes to washing grass roots.

Roots washed with sodium bicarbonate, however, had the greatest ash concentration (Figure 1), suggesting that a greater proportion of fine soil particles stayed attached to the roots after washing.

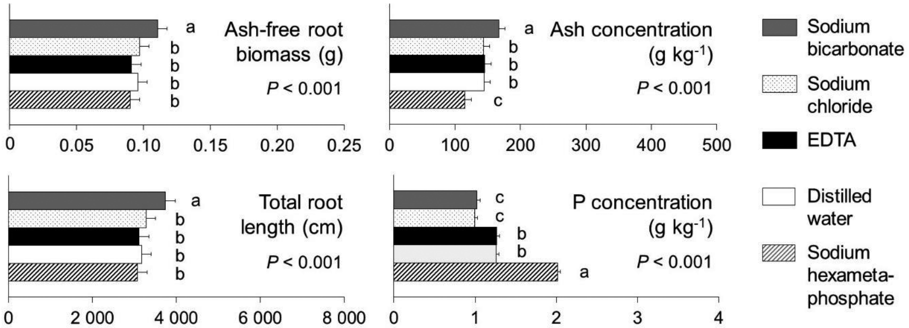

Figure 1. Effect of the five soaking solutions on four of the measured root traits. Concentration units (g kg^{-1}) are expressed on a dry matter basis. Bars represent standard error.

Consequently, the greatest concentrations of Al, Co, Fe, Mg, Mn, Ni, and Zn were found in roots washed with sodium bicarbonate (data not shown). The high pH of the sodium bicarbonate solution (8.3) may have promoted the sorption of the elements present on the soil particles still attached to the roots, compared to the other solutions that had a more acidic pH (4.6 to 6.4). An acidic pH solution would most likely favour the leaching of elements adsorbed on root surfaces. This contamination could lead to a significant overestimation of root biomass unless ash mass is measured and root biomass is expressed on an ash-free basis.

Root separated with sodium hexametaphosphate had some of the lowest concentrations in ash (Figure 1) and several elements (Al, Co, Fe, Mg, Mn, and Ni; data not shown). Nevertheless, phosphates contained in this solution [$(NaPO_3)_6$] clearly contaminated root tissue (2.0 vs 1.1 g P kg^{-1} DM with other solutions; Figure 1). Sodium chloride and EDTA showed no clear advantage over distilled water that would justify their preferential use.

Conclusions

These results show that the choice of the soaking solution will differ depending on the objectives being pursued. For studies focusing on root morphological traits, sodium bicarbonate may be a suitable soaking solution as long as root biomass is corrected for ash concentration. Consideration should also be given to the longer time required to process samples for digitization with this soaking solution. In contrast, if root tissue elemental composition is the prime interest, sodium hexametaphosphate would be a better option, with the exception of P-related studies. Distilled water should not be disregarded as a soaking solution, at least in silty loams. Further studies are necessary to see if results could be extended to other soil textures.

Acknowledgements

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References

Azcue J.M. (1996) Comparison of different cleaning procedures of root material for analysis of trace elements. *International Journal of Environmental Analytical Chemistry* 62, 137-145.

Smucker A.J.M., McBurney S.L. and Srivastava A.K. (1982) Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. *Agronomy Journal* 74, 500-503.

US EPA (1996) *Method 3050B (Revision 2): Acid digestion of sediments, sludges, and soils*. Washington, DC, USA.

Organic and mineral fertilisation of grassland compared: a 50-year field trial in Germany

Thumm U.[1], Breinlinger C.[1], Boob M.[1,2] and Elsaesser M.[2]

[1]*Biobased Products and Energy Crops, Institute of Crop Science, University of Hohenheim, Stuttgart, Germany;* [2]*Agricultural centre for cattle production, grassland management, dairy management, wildlife and fisheries Baden-Wuerttemberg (LAZBW), Aulendorf, Germany*

Abstract

Fifty years ago, fundamental questions about grassland fertilisation were of particular scientific interest. In 1966, a field trial was established at the University of Hohenheim on a mesotrophic *Arrhenatheretum* grassland, a traditional hay meadow cut twice a year. The aim was to assess the effects of organic and mineral fertilisation on sward composition and dry matter (DM) yield, and their development over time. The following variants were tested: annual PK fertilisation; annual NPK fertilisation; alternating annual PK and NPK fertilisation; annual fertilisation with farmyard manure; alternating succession of annual PK, NPK, manure fertilisation. The annual fertilisation quantities were: 100 kg N ha^{-1}, 35 kg P ha^{-1}, 250 kg K ha^{-1} and 30 Mg manure ha^{-1}. There were large year-to-year variations in DM yield. Over the entire length of the trial, the mean DM yield was highest for NPK (7.56 Mg ha^{-1}) and manure (7.55 Mg ha^{-1}), and lowest for PK (6.03 Mg ha^{-1}). Both herb proportion and plant species number decreased with N fertilisation. No differences were observed between mineral and organic fertilisation. Irrespective of N supply, there was also a tendency for a decline in species number and increase in proportion of grasses over the 50-year trial period.

Keywords: long-term trial, meadow, manure, mineral fertiliser, alternating application

Introduction

In the 1960s, grassland fertilisation was a topic of particular scientific interest. Fertilisation trials were carried out at various sites. Some are still ongoing, such as the German long-term fertilisation trials in Rengen and Steinach (Hejcman *et al.*, 2007, 2014). In many cases, the focus was the comparison of mineral fertiliser application with the traditional use of manure (Schröder *et al.*, 2007). In 1966, the University of Hohenheim also started an experiment to compare the effect of organic and mineral fertilisation. The aim was to investigate fertilising effects on the botanical composition and dry matter (DM) yield of a traditional hay meadow. The long duration of the experiment enables long-term effects to be studied.

Materials and methods

The experiment was set up in 1966 on a mesotrophic *Arrhenatheretum* grassland cut 2-3 times per year at the experimental station 'Ihinger Hof' (southern Germany, 25 km west of Stuttgart, 480 m above sea level). The soil is a Luvisol, consisting of 21% clay, 75% silt and 4% sand (Poeplau *et al.*, 2018). The long-term average annual precipitation is 690 mm and average annual temperature is 7.9 °C (with rising temperatures in the recent years). Four mineral fertilisation treatments and one organic treatment were tested: annual PK fertilisation, annual NPK fertilisation, alternating annual PK and NPK fertilisation, annual fertilisation with farmyard manure (ORG); alternating succession of annual PK, NPK, ORG fertilisation. The amounts of fertiliser applied were N: 100 kg ha^{-1} (CAN, in two applications), P: 35 kg ha^{-1} (SSP), K: 250 kg ha^{-1} (KCl), ORG: 30 Mg ha^{-1} farmyard manure. The five different treatments were arranged in a Latin rectangle design with four replications and a plot size of 15.8 m^2. The meadow was cut two or three times per year depending on the weather conditions, and the DM yield measured at harvest. The botanical composition of the sward was determined at irregular intervals before the first cut. Yield proportions of the various plant species were estimated visually as a percentage of total dry matter.

The data were analysed in a general mixed model using the SAS procedure Mixed. For analysis of the yield trend over the years, a linear regression was run for each plot over all years. In a second step, the slope and intercept values were analysed using Proc Mixed.

Results and discussion

At the end of the observation period, the swards were dominated by grasses (Figure 1), with *Arrhenatherum elatius* having the highest proportions in all plots. *Alopecurus pratensis* was high in plots receiving N fertilisation. The proportion of legumes was only 1 to 3% in all treatments. The proportion of forbs (especially *Plantago lanceolata*) was highest in the PK variant. Nitrogen fertilisation had reduced the legume and herb proportions over the years.

During the experimental period, a sharp increase in grasses and decrease in forb proportions were observed. This change is associated with concurrent declines in the number of species in all treatments (Figure 2). The N fertilisation promoted the competitiveness of the grasses and led to a displacement of less competitive species. There was no difference in influence on species number between organic and mineral fertilisation.

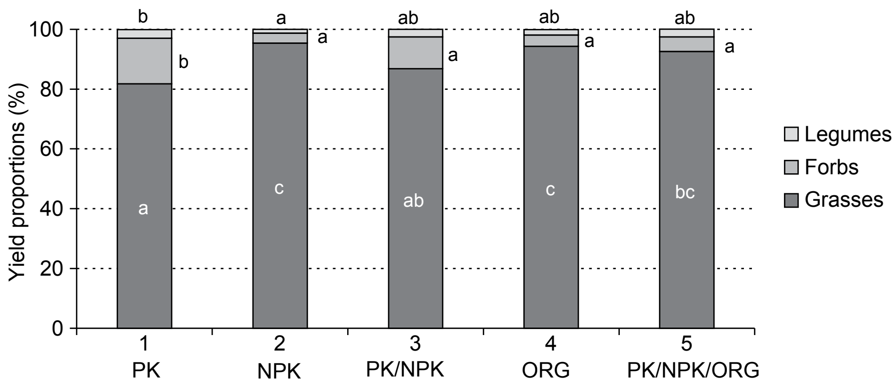

Figure 1. Proportion of grasses, forbs and legumes in spring 2018. Letters indicate significant differences between fertilisation treatments within a species group (P<0.05).

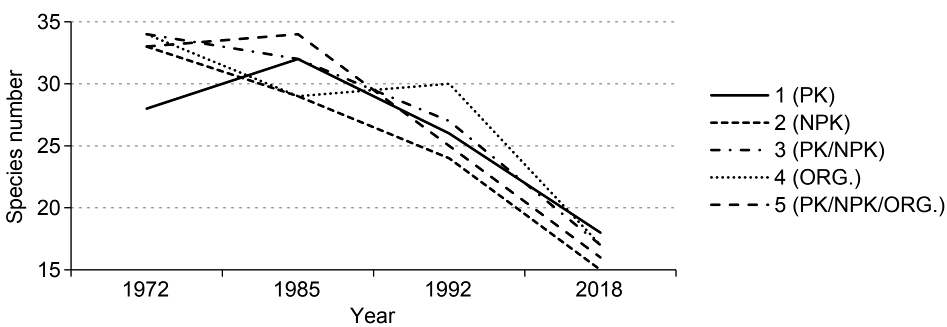

Figure 2. Development of plant species numbers over the years.

The average dry matter yields over the entire test period were highest for NPK (7.56 Mg ha^{-1}) and ORG (7.55 Mg ha^{-1}), and lowest for the PK treatment (6.03 Mg ha^{-1}). The PK/NPK and PK/NPK/ORG were intermediate at 6.74 and 7.15 Mg ha^{-1}, respectively.

There were marked differences in yields between the years (Figure 3), possibly due to annual variability in precipitation. Further evaluations of the data set are planned to investigate this interdependence. Over the years, there was a significant trend towards increasing yields with NPK, ORG and PK/NPK/ORG fertilisation, while the PK and PK/NPK treatments showed either a negative or constant trend.

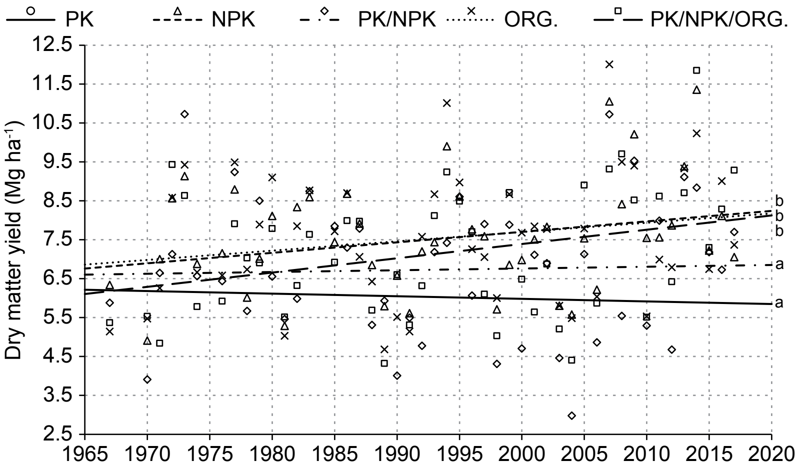

Figure 3. Dry matter yields and associated regression lines for the period 1967-2017. Letters indicate significant differences in the slope of yield regression lines.

Conclusions

No long-term differences were observed between the mineral and organic fertilisation of a *Arrhenatheretum* grassland. During the 50-year trial period, a tendency was found towards a decline in species number and an increase in grass proportions, regardless of the nutrient supply. This could be an indication of other factors such as changed temperature and precipitation patterns due to climate change.

References

Hejcman M., Klaudisová M., Schellberg J. and Honsová D. (2007) The Rengen grassland experiment: plant species composition after 64 years of fertilizer application. *Agriculture, Ecosystems and Environment* 122, 259-266.

Hejcman M., Sochorová L., Pavlů V., Štrobach J., Diepolder M. and Schellberg J. (2014) The Steinach grassland experiment: soil chemical properties, sward height and plant species composition in three cut alluvial meadow after decades-long fertilizer application. *Agriculture, Ecosystems and Environment* 184, 76-87.

Poeplau C., Zopf D., Greiner B., Geerts R., Korvaar H., Thumm U., Don A., Heidkamp A. and Flessa H. (2018) Why does mineral fertilization increase soil carbon stocks in temperate grasslands? *Agriculture, Ecosystems and Environment* 265, 144-155.

Schröder J.J., Uenk D. and Hilhorst G.J. (2007) Long-term nitrogen fertilizer replacement value of cattle manures applied to cut grassland. *Plant and Soil* 299, 83-99.

Was vegetation equilibrium achieved after 74 years of fertiliser applications in the Rengen Grassland Experiment?

Titěra J.[1,2], Pavlů V.[1,2], Pavlů L.[2], Hejcman M.[1], Gaisler J.[2] and Schellberg J.[3]
[1]Department of Ecology, Czech University of Life Sciences Prague, 165 21, Czech Republic; [2]Department of Weeds and Vegetation of Agroecosystems, Crop Research Institute, 460 01, Czech Republic; [3]University of Bonn, Institute of Crop Science and Resource Conservation, Bonn, Germany

Abstract

Vegetation equilibrium reflects the long-term stable ecological conditions. However, the equilibrium in guild composition is a dynamic one, continually perturbed by climate. The changes in plant species composition in the Rengen Grassland Experiment, Germany (established in 1941) were studied over a 10-year period (2005-2014). It is a complete randomised block design with five treatments and five replications. The treatments are: Calcium (Ca); Ca and nitrogen (N); Ca, N, phosphorus (P); Ca, N, P, potassium chloride (KCl); Ca, N, P, potassium sulphate (K_2SO_4); and one control, with zero fertiliser. All treatments were cut twice a year in late June/early July and in mid-October. Percentage cover of individual plant species was estimated by visual observation over the study period. Despite year-to-year variability in the cover of the individual vascular plant species, the redundancy analyses revealed relatively stable response of plant community. However, the control treatment had lower temporal residual variability and a higher temporal explained variability compared with the other treatments. In conclusion, the vegetation equilibrium of grassland community was achieved in all treatments with any fertiliser application, but not in unfertilised control treatment.

Keywords: botanical composition, fertiliser applications, sward, temporal trend

Introduction

Long-term fertiliser application together with constant defoliation management can lead to vegetation equilibrium. The number and relative abundances of plant species in a community at equilibrium are determined by competition for resources such as for soil nutrients and irradiance penetrating the canopy (Tilman, 1982). However, the equilibrium in guild composition is a dynamic one, continually perturbed by climate (Silvertown, 2006). In order to find if the vegetation equilibrium was achieved under different treatments in the long-term fertilised (74 years) Rengen Grassland Experiment (RGE), ten years of (2005-2014) vegetation mapping was conducted.

Materials and methods

The RGE was established in 1941 in extensively grazed heathland in the Eifel mountains (Germany, 50°13' N, 6°51' E; 475 m above sea level). The mean annual temperature is 6.9 °C (Rengen meteorological station). The mean annual precipitation is 811 mm. The soil is classified as a Stagnic Camisole. The experimental area was mown twice per year (in late June/early July and October) since 1962. The experiment is arranged into five randomised blocks with five fertilised treatments (applied nutrients in kg ha^{-1}) B: Ca (Ca=715, Mg=67); C: Ca/N (Ca=752, N=100, Mg=67); D: Ca/N/P (Ca=936, N=100, P=35, Mg=75); E: Ca/N/P/KCl (Ca=936, N=100, P=35, K=133, Mg=90); F: Ca/N/P/ K_2SO_4 (Ca=936, N=100, P=35, K=133, Mg=75) and one control (not fertilised) treatment (A). The percentage cover of all vascular plant species was estimated visually from the centre (1.8×3.2 m) of each plot. Nomenclature of vascular plant species follows the regional flora (Rothmahler *et al.,* 2000). For multivariate data analysis CANOCO 5 program (Ter Braak and Šmilauer, 2012) was used. Principal component analysis (PCA) was used to illustrate differences between treatment responses within individual years (2005-2014). As part of redundancy analysis (RDA), variability partitioning was counted from sum of all eigenvalues to reveal if there is some type of temporal trend within individual treatments and to see residual variability in vegetation data (%).

Results and discussion

PCA analysis showed temporal pattern of all treatments throughout the ten years study period (Figure 1). Based on RDA, the temporal variability eigenvalues decomposition showed that the control treatment (A) had lower temporal residual variability and higher temporal explained variability in comparison with the other treatments (B, C, D, E, F) (Figure 2). Despite the inter-annual variability in the cover of the individual vascular plant species, the redundancy analyses revealed relatively stable response of plant community to the different fertiliser applications throughout the ten years. In the unfertilised control, the temporal trend and low residual variability pointed out vegetation development. This was probably caused by an ongoing oligotrophication due to nutrient removal, even after 74 years, as there was more plant-available P and K in the soil than in B and C treatments (Hejcman *et. al.,* 2010). It seems that vegetation equilibrium in all fertilised treatments was caused by stable nutrient levels in the soil.

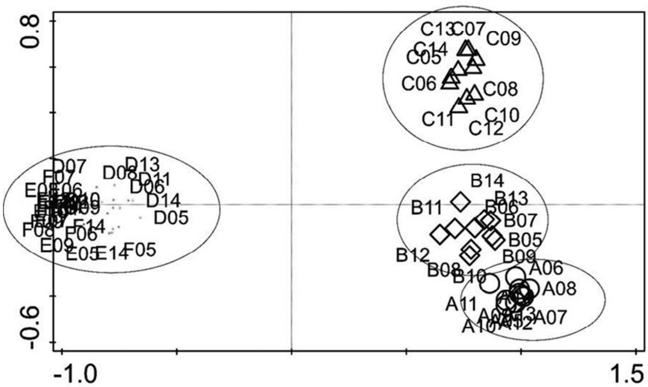

Figure 1. Results of principal component analysis for the treatments over the study period 2005-2014. Treatments abbreviations are explained in the Materials and methods section.

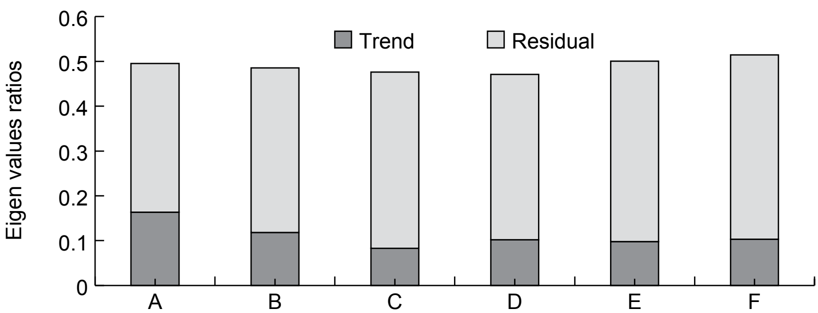

Figure 2. Temporal variability eigenvalues decomposition of individual treatments (A-F) over the study period 2005-2014. Treatments abbreviations are explained in the Materials and methods section.

Conclusions

The main finding of this study was that multivariate data analyses (PCA) revealed a relatively stable response of plant community to all treatments throughout the ten years study period, despite year-to-year variability in the cover of individual vascular plant species. However, in the control treatment without any fertiliser application, a trend in plant species composition was revealed through variability decomposition as part of RDA analysis. Different residual variability of control treatment was probably response to ongoing oligotrophication.

Acknowledgements

The authors are grateful for technical support of the staff in Rengen Grassland Research Station and to many students from Czech University of Life Sciences for assistance with data collection. Then they are grateful to Jan Lepš for his help with data analysis. The experiment was conducted by the financial support of the University of Bonn. Preparation and data analyses were funded by IGA (FES; 20184239) of Czech University of Life Sciences and by MACR (RO0417).

References

Hejcman M., Češková M., Schellberg J. and Pätzold S. (2010) The Rengen Grassland Experiment: effect of soil chemical properties on biomass production, plant species composition and species richness. *Folia Geobotanica* 45, 125-142.

Rothmahler W., Jager E.J. and Werner K. (2000). *Exkursionsflora von Deutschland, Bd. 3, Gefäßpflanzen*: Atlasband. [Flora from excursions in Germany, Bd. 3, Vascular plants]. Spectrum Akademischer Verlag Heidelberg, Berlin, D.

Silvertown J., Poulton P., Johnston E., Grant E., Heard M. and Biss P.M. (2006) The Park Grass Experiment 1856-2006: its contribution to ecology. *Journal of Ecology* 94, 801-814.

Ter Braak C.J.F. and Šmilauer P. (2012) Canoco 5, Windows release (5.00). [Software for canonical community ordination]. Microcomputer Power, Ithaca, NY, US.

Tilman D. (1982) *Resource Competition and Community Structure*. Princeton University Press, Princeton.

Contribution of High Nature Value farming areas to sustainable livestock production: A pilot case in Finland

Torres-Miralles M.[1], Särkelä K.[1], Koppelmäki K.[1,2], Tuomisto H.L.[1,3,4] and Herzon I.[1, 3]
[1]*Department of Agricultural Sciences, PO Box 27, 00014 University of Helsinki, Finland;* [2]*Farming Systems Ecology Group, Wageningen University & Research, the Netherlands;* [3]*Helsinki Institute of Sustainability Science (HELSUS), University of Helsinki, Finland;* [4]*Natural Resources Institute Finland (Luke), Finland*

Abstract

Animal production, and particularly ruminant livestock, has risen to be among the key issues in the agricultural sustainability discourse. This study addresses the topic through a specific and seldom-explored focus – High Nature Value (HNV) farming systems. These are mainly pastoral systems using semi-natural grasslands in production of high-quality animal-based food. The ongoing study aims to assess the environmental sustainability of production on HNV farms, focusing on greenhouse gas emissions, nitrogen balance and land use. We chose Finland as the pilot study to develop a methodological approach, and aim to extend the analysis to HNV farms within five regions in Europe. We measured the environmental impacts of HNV farms and their alternative states, that is, the same production but without semi-natural grasslands. The HNV farm impacts were comparable to that of the alternative field-based production in terms of their environmental impacts related to production amounts. Using semi-natural grasslands tended to minimize the arable land area, but maximised nitrogen cycling and maintained unique biodiversity.

Keywords: biodiversity, farming systems, livestock, sustainability

Introduction

Considerable environmental impacts of livestock production have been well demonstrated. However, livestock are an important part of nutrient cycling in agriculture, as they are able to utilise resources otherwise unsuitable for human consumption and may also support unique biodiversity (Röös *et al.*, 2006). The highest sustainability benefits could be provided by the production systems that have the least overall adverse impact but highest benefits – the lowest possible trade-off situation. This study explores the above through focusing on High Nature Value (HNV) farmlands. These are areas where 'agriculture is a major land user, and where agriculture supports, or is associated with, either a high species and habitat diversity or the presence of species of European conservation concern or both' (Andersen *et al.*, 2003). This has led to the inclusion of HNV farmland indicator as part of the EU sustainability indicator framework. HNV farming systems have so far received only minor research attention in the food system assessments. Most of the Life Cycle Assessment (LCA) studies have been done for intensive animal production systems of limited or no biodiversity, or for other non-material benefits (e.g. pig fattening). In this ongoing project, we collect data from 50 HNV farms across Europe to assess the environmental sustainability of production on HNV farms. We use Finland as the pilot study to test a methodological approach.

Materials and methods

We collected data from nine HNV farms in Finland: five beef cattle, two sheep and two sheep and cattle farms. The farms correspond to HNV farming system type 1, that is, farms that utilise semi-natural vegetation for grazing and/or haying. We applied the Carbon Calculator (CC) from the Joint Research Centre of the European Commission (Tuomisto *et al.,* 2015). We measured four environmental indicators: greenhouse gas (GHG) emissions and nitrogen balance (from the CC) as well as arable land use and biodiversity value. For each sample farm, we built an alternative state that maintains the production without use of the semi-natural grassland, taking into account the feed demand for the same animal numbers. This resulted mainly in additional area of cultivated grassland and associated inputs. We used the best available national estimations of arable land required by the respective livestock type, and its productivity.

There are several approaches for estimating the biodiversity value of a farm for LCA (Winter *et al.*, 2017). In Finland, the semi-natural grasslands represent the single most biodiverse land use on farmland with unique and highly threatened communities (Raatikainen *et al.,* 2017). Therefore, we assumed that the proportion of the managed semi-natural grasslands of a farm indicates presence of such unique biodiversity, while other fields and elements have relatively common farmland species. A farm with no semi-natural grassland thus got 0 value and a farm with half of the land area as semi-natural got 50. We used ANOVA to test the environmental impacts between the HNV farms and their alternative state.

Results and discussion

The HNV farms have higher GHG emissions (GHGs) at the product level compared with their alternatives states (Figure 1). When such farms sell only small amounts of animal-derived products, presumably keeping livestock mainly for the agricultural subsidies, their GHGs at the product level are high (160.8 and 193.7 Mg CO_2-eq for two farms with the highest values). HNV farms have lower GHGs at the farm level compared to their alternative states. However, there was no statistically significant difference in GHGs at the farm level (P=0.1). The average nitrogen balance value in HNV farms was close to zero. There were no statistically significant differences between the HNV and alternative state farms (P=0.2, 0.2 and 0.5 for arable land use, nitrogen balance and GHGs at the product level, respectively). The HNV management options are low or zero applications of external inputs and use of on-farm resources (manure), thus maximizing the nutrient cycling at farm level. HNV farms also use zero-input pastures as potential carbon sinks.

We assumed that farming practices of the alternative state farms would remain equivalent to those from HNV farms. Only the allocation of arable land use would change without the access to semi-natural grassland. With an increase of arable land use, the nitrogen inputs also grow. The negative average values for the alternative state farms demonstrate that it is not sufficient to maintain the nitrogen balance through legumes in cultivated grasslands (Karlsson and Röös, 2019). Five out of nine farms would need to increase their arable land or imports of feed in order to maintain their level of production. Since most semi-natural grasslands cannot be used for the arable cropping (due to the environmental constrains

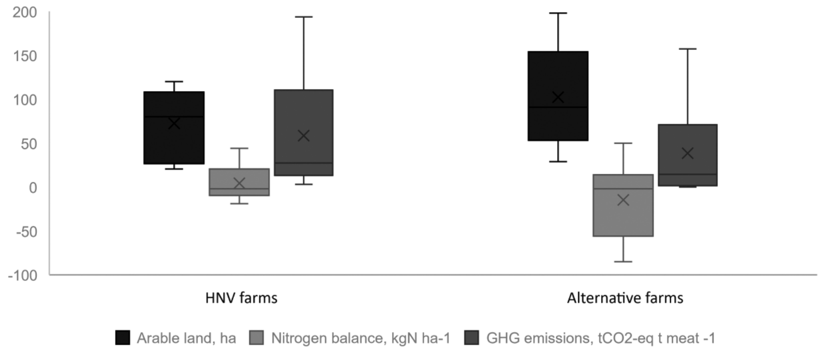

Figure 1. Median, average and quartile values from nine High Nature Value (HNV) farms and their alternative state without semi-natural grasslands (A) for three environmental indicators: arable land, nitrogen balance and GHG emissions at product level.

such as being on coastal areas or forested land, or legislative ones such as protection under Natura 2000), utilising them in production releases arable land for growing crops for direct human consumption or other uses such as bioenergy production. Biodiversity values of the HNV farms averaged at 52% (range 28-83%). They were assumed zero here for non-HNV farms though such farms can have considerable biodiversity for other reasons (i.e. landscape complexity). Our approach focused specifically at semi-natural grassland while assuming other farm parameters being equal.

Our results provide evidence for HNV farming systems being compatible with the mainstream production in terms of their environmental impacts: while producing animal-derived food, they also maintain unique biodiversity, tend to minimise use of arable land area and maximise nutrient cycling. They also illustrate a high trade-off in cases where there is exceptionally low farm productivity: the biodiversity values come at a high environmental cost and small contribution to production. However, reliance on HNV production systems may require changes in the current dietary pattern towards lower levels of animal products. Other plausible benefits of HNV systems – carbon sequestration on semi-natural grasslands, quality of product in terms of healthy composition and taste – have never been studied in Finland.

Conclusions

The preliminary results suggest that HNV farming systems in Finland play their role in combining production with maintenance of biodiversity but only when maintaining a certain level of production output. Two issues need to be resolved: methodologically, development of methods dealing with impacts of different nature (e.g. biodiversity vs GHG emissions); and, from the production perspective, how to maintain or increase the capacity of these systems in supplying animal products.

Acknowledgements

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References

Andersen E., Baldock D., Bennett H., Beaufoy G., Bignal E., Brouwer F. *et al.* (2003) Developing a high nature value indicator. *Report for the European Environment Agency.* Copenhagen.

Karlsson J.O. and Röös E. (2019). Resource-efficient use of land and animals – environmental impacts of food systems based on organic cropping and avoided food-feed competition. *Land Use Policy* 85, 63-72.

Raatikainen K.J., Mussaari M., Raatikainen K.M. and Halme P. (2017) Systematic targeting of management actions as a tool to enhance conservation of traditional rural biotopes. *Biological conservation* 207, 90-99.

Röös E., Patel M., Spångberg J., Carlsson G. and Rydhmer L. (2016) Limiting livestock production to pasture and by-products in a search for sustainable diets. *Food Policy*, 58, 1-13.

Tuomisto H.L., De Camillis C., Leip A., Nisini L, Pelletier N. and Haastrup P. (2015) Development and testing of a European Union-wide farm-level carbon calculator. Integr. *Integrated Environmental Assessment and Management* 11, 404-416.

Winter L., Lehmann A., Finogenova N. and Finkbeiner M. (2017) Including biodiversity in life cycle assessment – State of the art, gaps and research needs. *Environmental Impact Assessment Review* 67, 88-100.

Trends in soil organic matter in long-term grassland experiments under grazing in the Netherlands

Van Middelkoop J.C., Regelink I.C., Holshof G. and Ehlert P.A.I.
Wageningen University and Research, Wageningen, the Netherlands

Abstract

Grasslands can contribute to carbon sequestration as a mitigation method for climate change. Here the evolution of soil organic matter (SOM) was evaluated in a long-term trial (>16 yrs) testing combined nitrogen and phosphorus fertilisation levels (240 and 370 kg N ha^{-1} yr^{-1}, with 24, 33 and 44 kg P ha^{-1} yr^{-1}) for different soil types (i.e. sandy soil and young marine clay). The study was carried out in a random plot design under a combined grazing-cutting regime. During the trial, SOM was annually measured for 4 layers in 0-30 cm below surface. Analyses showed that at sand1 site, soil layers slightly changed in SOM, while the SOM of the 0-30 cm was unchanged. At the sand2 site, SOM did not change during the measuring period. On the young marine clay, an increase was observed in SOM in the top layers. Overall, results suggested that the sandy grassland soil were saturated with SOM, while the young marine clay was building up carbon stock (i.e. 0.06% units SOM annually, ~3.5 Mg CO_2 ha^{-1} yr^{-1}). Different SOM saturation levels and history of agricultural use possibly contributed to these differences.

Keywords: soil organic matter, grazing, grassland, long-term experiment

Introduction

Soil organic matter (SOM) is an important characteristic for soils. There is widespread awareness that soils with a sufficient content of SOM have a better water holding capacity, a better structure and a higher cation buffering capacity than soils that have SOM contents below a certain threshold (Janssen, 1984). There is also the additional function of SOM as carbon(C)-sink as a mitigation measure for climate change. Grasslands are considered to sequester large amounts of C. However, knowledge about how management of grazing systems affects the C sequestration rate is under development (Van den Pol-van Dasselaar, 2018). To develop knowledge about C sequestration potential, SOM data of a long-term experiment were analysed.

Materials and methods

The experiment was set up in 1997 on three permanent grassland sites in the Netherlands (Van Middelkoop *et al.*, 2016) and comprised different soil types: sandy soil (sand1 and sand2), and young marine clay. The site sand1 ended after 16 years, sand2 after 17 years and young marine clay is still running after 23 years. At each location, six plots were randomly assigned to a combination of nitrogen (N) and phosphorus (P) fertilisation, without replicates. Plots were supplied with 240 and 370 kg N ha^{-1} yr^{-1}, and with 24, 33 and 44 kg P ha^{-1} yr^{-1}. Fertilisation comprised cattle slurry, applied in early spring and before the 4th cut, and mineral N fertiliser, applied throughout the whole season. Per site, all plots received the same dosage of cattle slurry. The deposition of excreta during grazing was not accounted for as this was considered to be part of an internal cycle. On all locations the first and fourth cuts were taken for silage, but on the dates of the other 'cuts' the swards were grazed by heifers or dry cows. Dry matter yield and grass contents were determined. In 2002, the management on sand1 was converted from conventional to organic farming. On the high N fertilisation plots, white clover (*Trifolium repens*) was introduced. After the growing season, soil was sampled by stratified sampling: plots were divided in 20 squares from which a sub-sample was taken. The sub-samples were mixed and analysed for, amongst others, SOM. Samples were taken from the soil layers of 0-5, 5-10, 10-20 and 20-30 cm below surface. SOM of 0-30 cm was calculated by a weighted average of the four soil layers. Differences between treatments in SOM were statistically analysed in a linear model with a fixed and a random part with the Restricted Maximum Likelihood (Reml) method (Harville, 1977), using Genstat (19th edition). The fixed part comprised site, N and P fertilisation, number of years (for trend in time), and interactions. The random part comprised year×plot×soil layer (year as factor).

Results

The change of SOM over time was positive and significant on sand1 in 0-5 cm and on young marine clay in 0-5 and 5-10 cm (Table 1, Figure 1). N and P fertilisation levels did not affect SOM and therefore SOM data were averaged over all treatments. The SOM contents of the sandy soils were comparable and amounted to about 6% in 0-5 cm and about 4% in 5-30 cm below surface. The SOM content of the young marine clay in 0-5 cm was 10 to 15% and in 5-30 cm below surface 4 to 6%. However, on sand1 in the layers below 5 cm the SOM decreased slightly, though not significantly. Averaged over 0-30 cm, SOM did not change on sand1 and increased by 0.06%-units SOM per year on young marine clay.

Discussion

Over a period of 17 years, no changes were observed in the amount of SOM stored in the upper 30 cm of sandy soils despite the addition of animal manure and excreta of grazing animals. N and P fertilisation levels did not affect SOM in 0-30 cm. On the sandy sites mean SOM was 4.1% on both sites and N levels. On the clay site SOM was 6.5% on low N and 6.4% on high N, in spite of differences in yields. Dry matter yields for the high N level were about 1 Mg ha^{-1} higher on sand, and 3 Mg ha^{-1} higher on clay, than for the low N level. The sandy soils had possibly reached an equilibrium in SOM, since both sandy soils had been grassland for at least three decades and probably for centuries in agricultural use before the start of the trial. On the other hand, the increase on the clay site might be partly a consequence of the relatively young age of the clay. The land was reclaimed from the sea in the 1950s and in agricultural use since the 1970s. At the end of the trial the SOM in clay was higher than in sand, though management was equal and dry matter yield levels comparable, 10 to 12.5 Mg ha^{-1} yr^{-1}. An equilibrium level for SOM on the clay site might be higher than on the sandy sites because clay contains smaller particle-size fractions (less than 2 μm) that most efficiently protect SOM (Dignac *et al.*, 2017). During the experiment no grassland renewal took place. In the Netherlands it is normal practice that grassland is reseeded on a regular basis. It

Table 1. Results of statistical analyses; Reml estimates of trend in time affecting soil organic matter (%-units year^{-1}); means by treatments.[1]

Site (number of years)	0-5 cm	5-10 cm	10-20 cm	20-30 cm	0-30 cm[2]	LSD[3]
Sand1 (16)	**0.082**	0.013	-0.049	-0.037	-0.013	0.079
Sand2 (17)	0.083	0.044	-0.012	-0.016	0.012	0.071
Young marine clay (23)	**0.245**	**0.143**	0.001	-0.020	**0.058**	0.052

[1] $P \leq 0.05$; bold means significant.

[2] 0-30 cm calculated as (soil organic matter from (0-5 cm + 5-10 cm + 2 × 10-20 cm + 2 × 20-30 cm) / 6.

[3] LSD = least significant difference.

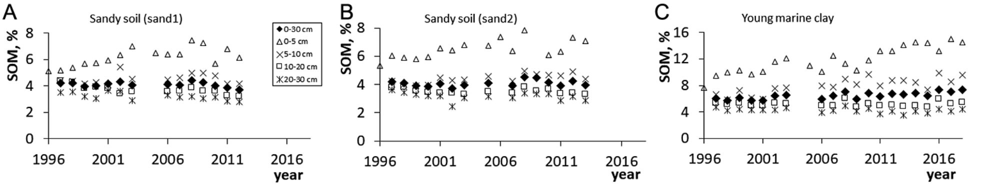

Figure 1. Mean annual soil organic matter (SOM) content at three sites (sand1, sand2, young marine clay), as function of time and soil layer below surface. NB: Y axis scales differ between sites.

can be expected that tillage is used during grassland renewal, and SOM will be lower at least temporarily due to increased mineralisation.

Conclusions

Grassland soils at three sites under similar grazing management and slurry application showed different responses towards the C sequestration rate. On sandy soil, there was no increase in SOM stored in the upper 30 cm of the soil over a period of 16 or 17 years, whereas SOM increased by 0.06%-units annually (~3.5 Mg CO_2 ha^{-1} yr^{-1}) in the upper 30 cm of young marine clay. Possibly soil type, and more specifically the ability of the soil to protect and sequester C, and historical use of soil is of great importance for the potential C sequestration rate.

References

Dignac M.-F., Derrien D., Barré P., Barot S., Cécillon L., Chenu C., Chevallier T., Freschet G.T., Garnier P., Guenet B., Hedde M., Klumpp K., Lashermes G., Maron P.-A., Nunan N., Roumet C. and Basile-Doelsch I. (2017) Increasing soil carbon storage: mechanisms, effects of agricultural practices and proxies – a review. *Agronomy for Sustainable Development* 37, 14.

Harville D.A. (1977) Maximum Likelihood approaches to variance component estimation and to related problems. *Journal of American Statistical Association* 72(358), 320-338.

Janssen B.H. (1984) A simple method for calculating decomposition of 'young'soil organic carbon. *Plant and Soil* 76, 297-304.

Van den Pol A., Chabbi A., Cordovil C.M.D.S., De Vliegher A., Die Dean M. and Hennessy D. (2018) Grazing for carbon. *Grassland Science in Europe* 23, 682-684.

Van Middelkoop J.C., Van der Salm C., Ehlert P.A.I., De Boer I.J.M. and Oenema O. (2016) Does balanced phosphorus fertilisation sustain high herbage yields and phosphorus contents in alternately grazed and mown pastures? *Nutrient Cycling in Agroecosystems* 106, 93-111.

Theme 4.
Novel technologies in farm management

Precision agriculture in practice – utilisation of novel remote sensing technologies in grass silage production

Honkavaara E.[1], Näsi R.[1], Oliveira R.A.[1], Niemeläinen O.[2], Viljanen N.[1], Hakala T.[1] and Kaivosoja J.[2]
[1]Finnish Geospatial Research Institute in National Land Survey of Finland; [2]Natural Resources Institute Finland

Abstract

Modern remote sensing by satellites and unmanned aerial vehicles (UAVs) offer new tools for serving crop information needs required in precision agriculture (PA). The objective of this paper is to review novel remote sensing technologies and evaluate their suitability for PA tasks in grass silage production. We also present the UAV-based remote sensing system developed at the Finnish Geospatial Research Institute and give results of quality and quantity estimation of grass silage in trial sites and on a real production farm. Our assessment showed that UAVs especially offer an efficient approach for capturing timely information for all the essential PA tasks required in silage management. UAVs provide high-resolution and multi-temporal data, and are a valuable alternative to overcome laborious field and laboratory measurements or the scale and availability limitations when employing satellite data. The focus in our experiments was the estimation of the fresh and dry biomass as well as digestibility of organic matter in dry matter (D-value). The reference measurements showed high correlations with the calibrated remote sensing datasets, and trained estimators provided accurate estimates for these parameters.

Keywords: remote sensing, unmanned aerial vehicle, satellite, hyperspectral imaging, photogrammetry, machine learning, grass, biomass, digestibility

Introduction

Precision agriculture (PA) is a farming management concept based on observing, measuring and responding to inter and intra-field variability in crops. A core asset in PA is a decision support system (DSS) for the farm management with the goal of maximising outputs while minimizing the inputs. In the future, it can be seen that farm management will be based on a holistic, artificial intelligence-based management approach utilising a cyber-physical systems concept (Wolfert *et al.*, 2017), which is expected to enable better environmental, economic and cultural sustainability of agriculture and food production via overall optimisation, automation, and robotization.

Important PA tasks in grass silage management include harvesting of the crop, fertilising, weed control, and maintenance (Nikander *et al.*, 2018). In northern countries, grass silage swards are harvested two to four times a season, and fertiliser is applied once for each harvest when the aim is to achieve maximum yields of a desired quality. The feed quality (digestibility) in grass swards declines rapidly, especially in the primary growth period, while the yield is increasing. Therefore, timing of the primary harvest is important to facilitate harvest of high yield at adequately high quality. Knowledge of nitrogen uptake is necessary to determine the fertiliser needed for the regrowth. The most important maintenance operation is supplementary seeding; this is often necessary if the stand is not dense enough for optimal production, e.g. due to winter damage. Weed control is usually carried out by chemical treatments during the early establishment phase of the grass sward, and it may be required also in old stands (Nikander *et al.*, 2018).

The objective of this paper is firstly to make a brief review of novel remote sensing technologies and consider their suitability for the PA tasks in the management of grass silage production. In the second part we present a UAV-based remote sensing approach for grass quality and quantity assessment developed at the Finnish Geospatial Research Institute (FGI) and results of case studies in trial sites and on a real production farm.

Remote sensing techniques for grass silage production management

Timely information about the crop status is required in order to carry out PA tasks in an optimal way. Recent revolutions in remote sensing technologies offer efficient tools for this (Ali *et al.*, 2016; Hassler and Baysal-Gurel, 2019; Shafi *et al.*, 2019). The principle idea is to capture information about the crop status using remote sensing and combine this information with agronomic knowledge and other information, such as farming history, weather and soil data, in order to optimise the management operations (Kaivosoja *et al.*, 2013).

Remote sensing data can be captured from various platforms, including satellites, manned and unmanned aerial vehicles (UAV), as well as using proximal remote sensing with terrestrial vehicles, robots, and tractors (Shafi *et al.*, 2019). Measurements of plant geometric traits provide information about the crop height, density, biomass, etc., and they can be carried out reliably using light detection and radar techniques (LiDAR) or photogrammetrically using images and structure-from-motion techniques (Bareth and Schellberg, 2018; Viljanen *et al.*, 2018). Analysis of spectral characteristics can provide information about plant health, nitrogen uptake, biomass, and diseases, among others; colour, multispectral, and hyperspectral cameras are used for the spectral measurements (Aasen *et al.*, 2018). Satellites and UAVs can be considered as highly interesting tools, because they have no direct contact and thus can visit the fields numerous times without damaging the crops or causing soil compaction.

The satellite programmes, especially the European Sentinels (https://sentinel.esa.int) are highly relevant for PA as they offer free radar and optical multispectral datasets at weekly or even higher temporal resolution (Punalekar *et al.*, 2018). Utilising satellite datasets, services such as Cropsat have been developed (https://cropsat.com/). The Cropsat is updated with new satellite images when they become available, and it provides vegetation index maps that can be used to create prescription files to control, for example, the application rate of the fertiliser sprayer. In the future, the availability of space data will increase via multiple satellites and cubesat programmes that are under preparation. The advantages of satellite monitoring include the affordability (especially in cases of free satellite datasets) and large coverage (Ali *et al.*, 2016); the major challenges include the limited usability of images due to clouds, and the relatively low resolution when considering small-scale phenomena in fields, such as pest or weed detection (Näsi *et al.*, 2017). The more frequent visit times of novel small-satellite constellations with increasing spectral and spatial resolutions could reduce these limitations.

UAV remote-sensing technologies have developed rapidly in recent years (Manfreda *et al.*, 2018; Hassler and Baysal-Gurel, 2019; Tsouros *et al.*, 2019). UAVs are still mostly operated in a visual line of sight (VLOS), but also more efficient beyond visual line of sight (BVLOS) systems are evolving, and will take over as soon as the progress of legislation allows. Miniaturized sensor technologies, such as still and video cameras, multi- and hyperspectral sensors and LiDAR for UAV use are developing rapidly (Aasen *et al.*, 2018; Hassler and Baysal-Gurel, 2019). The UAV-based technologies can capture 3D and spectral datasets with ultrahigh spatial resolution, mm to cm levels, producing a precise characterization of the object.

Remote sensing analysis is based on estimators and classifiers that are trained using machine learning techniques (Liakos *et al.*, 2018). The machine learning tasks in crop management can be grouped in tasks such as yield prediction, disease detection, weed detection, crop quality analysis, and species recognition. The estimation methods can be used in relative or absolute mode. In the relative mode, for example, the inter-field variability is analysed utilising various vegetation indices calculated from the data. In the absolute mode, the crop parameters are estimated using analysers that are trained using machine learning techniques, such as regression techniques, random forest, or artificial neural networks.

An essential task in the remote-sensing processing chain is the geometric and radiometric calibration. The precise calibration is crucial, especially if the objective is to make absolute measurements, e.g. to provide exact information about plant geometry to derive dry matter yield (DMY), or to relate the spectral data to existing library values to provide absolute quality information, or when the objective is to compare multitemporal datasets or to perform collaborative work (e.g. UAV and tractor). For the geometric calibration, the Global Navigation Satellite Systems (GNSS) and inertial measurements offer efficient tools (Aasen *et al.*, 2018; Viljanen *et al.*, 2018). Radiometric calibration techniques are also under development, but the varying weather conditions and tilting UAV platform often make the task challenging (Aasen *et al.*, 2018; Honkavaara and Khoramshahi, 2018). Fast cloud-based processing services are already available (for example, DroneDeploy https://www.dronedeploy.com/) and real-time techniques are developing (Oliveira *et al.*, 2018).

Different remote sensing techniques are optimal for different silage management tasks. The optimisation of the harvesting time and fertilisation are time-critical tasks, requiring a few days' time resolution. In Scandinavia, the cloud cover often disturbs the utilisation of satellite data; thus the UAV-based techniques can be considered as the most feasible (Näsi *et al.*, 2017). In weed detection, the spatial resolution of the satellite data might not be good enough; thus UAV-based approaches are the most promising. Maintenance tasks, such as supplementary seeding, are usually not so time critical and therefore satellite-based techniques have potential for providing information for these tasks. Furthermore, satellite monitoring provides an interesting approach for developing models for grass growth (Ali *et al.*, 2016; Persson *et al.*, 2019).

In this study, we demonstrate the use of UAV remote sensing for optimising the harvesting time. For this task, it is essential to estimate the grass biomass and its digestibility. Recent studies have indicated that UAV-based techniques are efficient for the grass height, fresh yield (FY) and DMY estimation (Bareth and Schellberg, 2018; Viljanen *et al.*, 2018). The studies concerning the quality parameters have focused on proximal measurements (Pullanagari *et al.*, 2012) or remote sensing by manned aircrafts (Pullanagari *et al.*, 2018). Our recent studies have developed an approach for estimating grass quantity and quality based on UAV remote sensing. Viljanen *et al.* (2018) developed a machine learning technique for the estimation of canopy height, DMY and FY utilising features extracted from the low-cost remote sensing data by combining a photogrammetric canopy height model (CHM), red, green, blue, and near-infrared intensity values, as well as different vegetation indices (VI) derived from the intensity values. The analysis methodology was extended in Näsi *et al.* (2018) to combine hyperspectral, RGB, and 3D features. Finally, the methodology was used to estimate various grass quantity and quality parameters (Oliveira *et al.*, unpublished data). The estimation method is presented in the next section, and results of case studies are given in the Results and discussion section.

Materials and methods

Test areas

Experiments were carried out in two areas. The first dataset comprises a trial area that was established in Jokioinen, located in southwest Finland, in the summer of 2017 by the Natural Resources Research Institute, Finland (Luke). The experimental site of the primary growth (J-1st_cut) was established on a second-year silage production field using six different nitrogen fertilisation rates (0-150 kg ha^{-1}) and sampled at four different dates to generate a large variation in the reference data (Figure 1A). The experiment had a total of 96 samples (Viljanen *et al.* 2018). The site of the regrowth (J-2nd_cut) (Figure

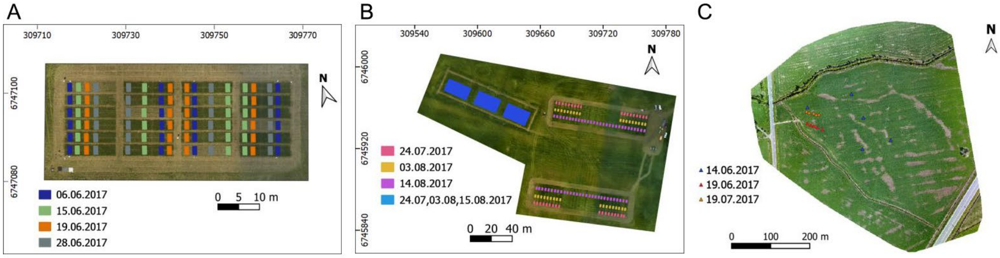

Figure 1. Jokioinen test areas in summer 2017. (A) The primary growth site and delineation of four harvests, (B) the regrowth study area and plots used as testing data. (C) The real farm test site in Liminka and distribution of field samples.

1B) was established on another silage production field about one km from the primary growth trial. The site was sampled three times resulting in a total number of samples of 108 (Oliveira *et al.*, unpublished data). Samples were taken from each harvested plot for quantity and quality analyses. The FY and DMY represented the quantity parameters. Digestibility of organic matter in the dry matter (D-value) was selected as the quality parameter, and was measured by the Valio Ltd feed laboratory, using a Near Infrared Spectroscopy (NIRS) technique. D-value and DMY were strongly negatively correlated (correlation coefficient -0.85 in the primary growth) (Oliveira *et al.*, unpublished data).

The second test area was a second-year silage production field on a real farm in Liminka, 450 km north from the Jokioinen trial site (Figure 1C). Luke carried out field sampling simultaneously with UAV data collection, which was captured twice during the primary growth (L-1st cut1, L-1st_cut2) and once during the regrowth (L-2nd_cut). Five samples from an area of 50×50 cm were collected each time and the FY and DMY were measured. Details of the experiments are given in Table 1.

Remote sensing datasets

Remote sensing datasets were captured using different UAV systems by the FGI (Figure 2). In Jokioinen, UAV datasets were captured four times in the primary growth and three times in the regrowth trial site. A quadcopter drone was equipped with a multisensory remote sensing system consisting of an RGB camera, a hyperspectral 2D camera, a GNSS receiver, and a Raspberry Pi single-board computer. The flight height was 50 m giving a GSD of 5 cm for the hyperspectral data and 0.64 cm for the RGB data (Viljanen *et al.* 2018, Oliveira *et al.*, unpublished data). Agisoft Photoscan Professional software was used for geometric processing. Canopy height models (CHMs) were created from the photogrammetric digital surface models (Viljanen *et al.*, 2018). The radiometric processing of the hyperspectral data was performed using the in-house software RadBA (Honkavaara *et al.*, 2018). The reflectance transformation was computed using panels with nominal reflectance of 0.03, 0.10 and 0.50.

Table 1. Overview of the reference datasets.[1]

Acronym	Location	Sampling dates	Growth	n	DMY (kg ha^{-1})			FY(kg ha^{-1})		
					min	max	ave	min	max	ave
J-1st_cut	Jokioinen	6 - 28 June	Primary	96	336	6,135	2,653	1,022	25,459	11,046
J-2nd_cut	Jokioinen	24 July-14 Aug	Regrowth	108	368	7,229	5,427	1,378	32,391	24,926
L-1st_cut1	Liminka	14 June	Primary	5	676	2,290	1,597	5,200	12,180	9,193
L-1st_cut2	Liminka	20 June	Primary	5	305	3,330	2,200	1,344	21,552	12,917
L-2nd_cut	Liminka	19 July	Regrowth	5	128	1,176	542	672	6,652	3,090
L-1st+2nd_cut	Liminka	14 June-19 July	Both	15	128	3,330	1,446	672	21,552	8,400

[1] n = number of reference samples; minimum (min), maximum (max) and average (ave) of dry matter yield (DMY) and fresh yield (FY).

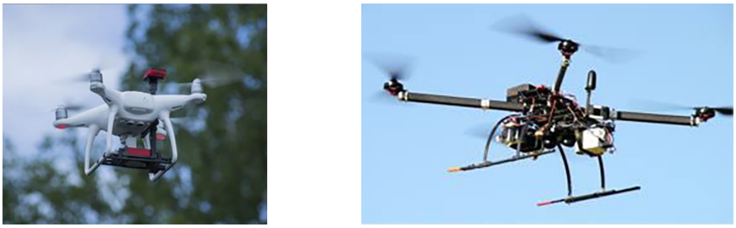

Figure 2. Two UAV systems used in this study: the DJI Phantom 4 with a RGB and multispectral camera and the FGI's quadcopter UAV with hyperspectral and photogrammetric camera payload.

The Liminka dataset was captured using low-cost multispectral and RGB cameras. Two datasets were captured during the primary growth and one during the regrowth. During the first flight of the first cut, the DJI Phantom 4 equipped with a Parrot Sequoia multispectral camera was used and in other flights, the standard DJI Phantom 4 RGB camera (12 megapixels, f/2.8) was used. The flight height of the Liminka campaigns was 140 m, which led to GSDs of 5.5 cm for the RGB data and 14 cm for the Sequoia multispectral data. Geometric processing was carried out using the Pix4D software and the reflectance transformation was similar to Jokioinen datasets.

Analysis methods

The estimation process used in this study follows the methods proposed by Näsi *et al.* (2018) and Viljanen *et al.* (2018). From the datasets, various features were extracted, including the spectral bands and VIs from the RGB, multi- and hyperspectral cameras, and 3D features from the CHMs. We obtained 47, 5, and 16 features, from hyperspectral, RGB, and CHM datasets, respectively. These features were used in various combinations to assess the performance of a simple RGB-camera, multispectral camera, hyperspectral camera and their combination; the most extensive feature set included 68 features (Figure 3). In Jokioinen, all feature sets were used; in Liminka, we only used spectral and VI features from the multispectral and RGB cameras. The features were calculated for the sample plot areas. The Random Forest (RF) and single linear regression (SLR) were used.

We used the leave-one-out and independent test data methods to assess the performance. In the leave-one-out method, the model was trained using all reference samples, excluding one, which was used as an independent test sample to evaluate the estimator. This process was repeated for all samples and sample statistics were calculated utilising the individual error measures. In the independent test data method,

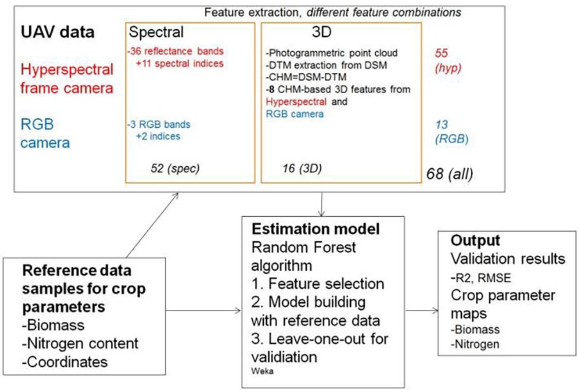

Figure 3. Workflow to estimate crop parameters using UAV-based spectral and 3D features (from Näsi *et al.*, 2018).

the estimator was trained using reference data from one site and tested using reference data from another site. Information about the sample used in each estimation is given in the Results and discussion section.

Results and discussion

Estimators using Jokioinen trial datasets

SLR models were estimated for DMY and D-value using the Jokioinen data. The 3D features had the best Pearson correlation coefficients with the DMY; they were at best 0.9 for the primary, and 0.8 for the regrowth (Figure 4B). The correlations of DMY and spectral and VI features were at best on level of 0.8 (Figure 4). Overall, the correlations were higher for the primary growth than for the regrowth. The D-value reached the highest correlations of over 0.7 in the green spectral bands (500-550 nm) and at red edge (700-740 nm), and with VIs with the green band (RGBVI, ExG). The correlations were higher for the FY and DMY than for the D-value.

Machine learning estimators for FY, DMY and D-value were calculated for the primary and regrowth. The primary growth estimators combining the hyperspectral and 3D data gave NRMSEs of 13.4 and 14.7% for the FY and DMY, respectively; for the regrowth, the corresponding values were 17.2 and 16.1% (Table 2). The estimators utilising hyperspectral data provided lower NRMSEs than the estimators based on the RGB data. The D-value estimators trained with the hyperspectral and 3D-data provided NRMSEs of 2.11 and 2.53% for the primary and secondary growth, respectively. With the RGB and 3D features the corresponding values were 2.48 and 3.25%, respectively. A visualisation of the estimated D-value for the entire site in different dates is shown in Figure 5. Plots of the measured and estimated FY, DMY and D-value using RF estimator indicated excellent fit (Figure 6).

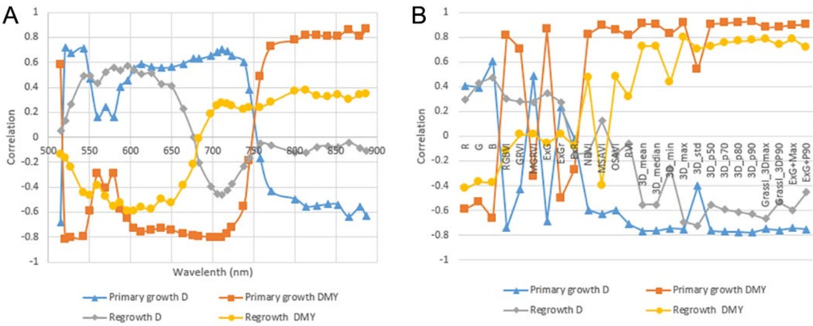

Figure 4. Correlation coefficients of D-value and DMY with UAV based (A) spectral features and (B) 3D features and MS indices.

Table 2. The normalised root mean square error (NRMSE) in % with the leave-one-out estimation for the primary and regrowth.[1]

Data	Growing stage	FY (%); corr	DMY (%); corr	D-value (%); corr
RGB, 3D	Primary	15.63; 0.97	16.05; 0.96	2.48; 0.90
HSI, 3D	Primary	13.39; 0.98	14.66; 0.97	2.11; 0.93
RGB, 3D	Regrowth	26.79; 0.83	21.19; 0.88	3.25; 0.76
HSI, 3D	Regrowth	17.24; 0.94	16.08; 0.94	2.53; 0.86

[1] FY = fresh yield; DMY = dry matter yield; D-value = digestibility of organic matter in dry matter. The remote sensing datasets were the RGB-images with the 3D features (RGB, 3D) and the hyperspectral images with the 3D features (HSI, 3D).

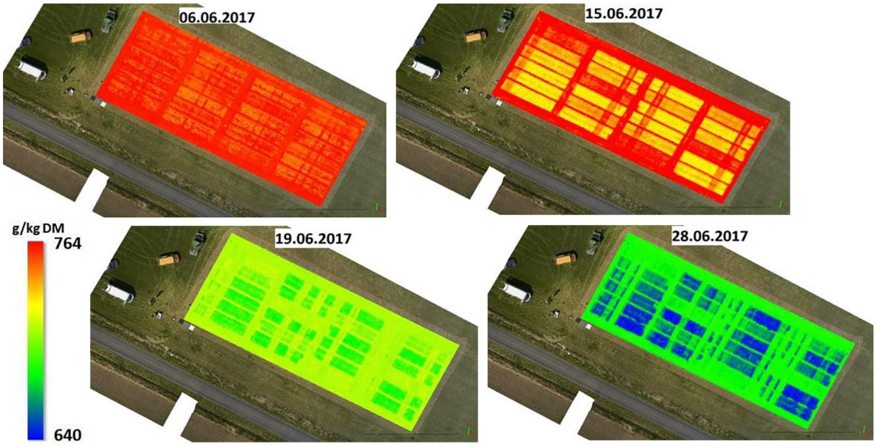

Figure 5. Visualisation of the development of the digestibility of organic matter in dry matter (D-value) in the Jokioinen trial site during the primary growth.

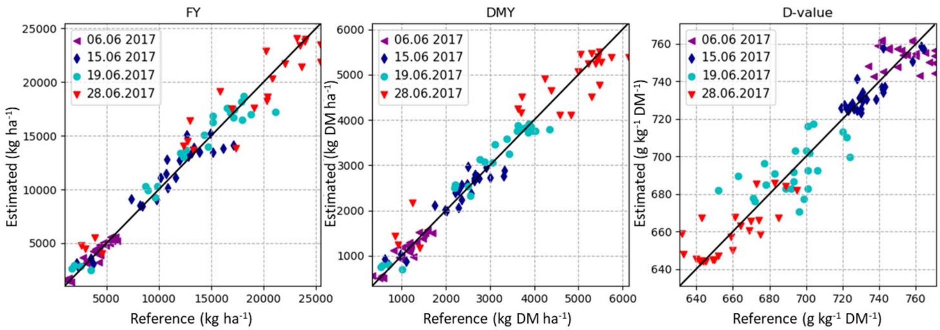

Figure 6. Plots of the estimated and measured values for the fresh yield (FY), dry matter yield (DMY), and digestibility of organic matter in dry matter (D-Value) for the primary growth. Random forest with hyperspectral and 3D data. DM = dry matter.

Grass quantity estimations in a farm

In the real farm dataset, correlation analysis utilising band and VI features showed that the best correlations on the level of 0.7 were obtained with the RGBVI, GRVI, MGRVI, ExGr (regrowth) indices (Figure 7). The primary and regrowth provided quite similar results. The correlations were slightly lower than with the trial site data (Figure 4). The plots of the estimated and reference biomass values showed relatively good correspondence (Figure 8). The NRMSE and correlation were 54.4% and 0.66, respectively, with RF, and 55.0% and 0.67, respectively, with SLR. The yield estimators for the real farm datasets thus had considerably larger NRMSEs and lower correlations than the trial sites (Table 2). The visual assessment indicated that the biomass maps were of good quality (Figure 8C). These maps provide spatially distributed information about the yield and growth over the entire field, which supports the farmers while implementing PA tasks.

We assessed the performance of the estimators trained using the Jokioinen datasets with all available datasets (Table 3). We used three different feature combinations: the RGB features, the RGB and multispectral features, and a combination of hyperspectral and 3D features. Unfortunately, all the features were not available in all cases. The best results were obtained when using the estimators trained in Jokioinen with Jokioinen datasets. The most comprehensive remote sensing dataset including hyperspectral and 3D

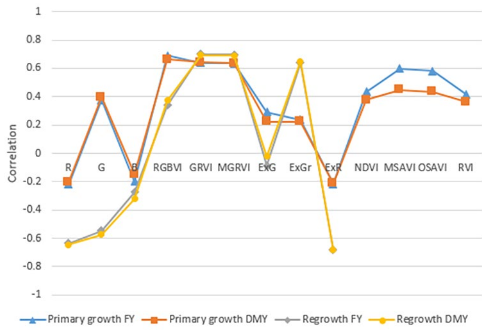

Figure 7. Correlation coefficients between RGB and MS features (primary) and fresh yield (FY) and dry matter yield (DMY) in the primary and regrowth stages in the Liminka dataset.

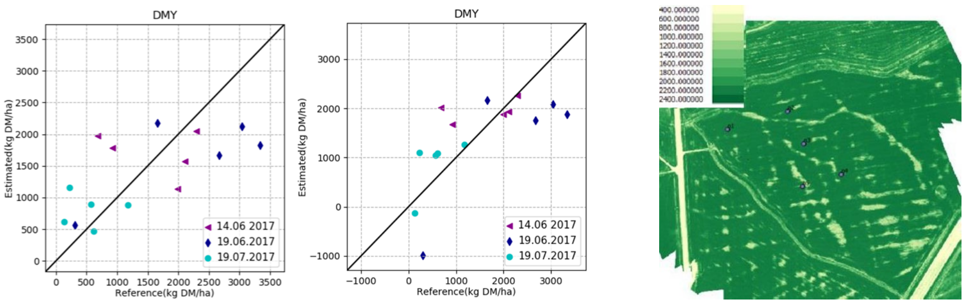

Figure 8. The relationship between the estimated and measured values of the dry matter yield (DMY) using (A) random forest estimator and (B) and single linear estimator with RGB data. (C) Estimated yield in kg ha^{-1}. DM = dry matter.

Table 3. Normalised root mean square error (NRMSE) in % for the DMY with the primary (J-1st_cut) and regrowth (J-2nd cut) models estimated in the Jokioinen trial site RGB, multispectral (MS) and hyperspectral images and 3D features (HSI+3D).

	Testing data	Training data					
		RGB		MS		HSI+3D	
		J-1st_cut	J-2nd_cut	J-1st_cut	J-2nd_cut	J-1st_cut	J-2nd_cut
RF	J-1st_cut	23.8	129.1	15.9	65.5	14.7	27.2
	J-2nd_cut	59.5	29.9	56.5	22.3	29.3	16.1
	L-1st+2nd_cut	140.2	325.8	-	-	-	-
SLR	J-1st_cut	30.5	150.4	27.3	65.2	23.0	36.7
	J-2nd_cut	53.5	43.7	421.6	41.6	24.1	18.1
	L-1st+2nd_cut	384.5	361.0	-	-	-	-

features provided the best results; the Jokioinen primary growth estimator had a NRMSE of 29.3% with the Jokioinen regrowth dataset and the Jokioinen regrowth estimator had a NRMSE of 27.2% with the Jokioinen primary growth dataset. When using only RGB spectral data, the corresponding values were 59.5% and 129.1%, respectively; the use of the multispectral features improved the results slightly. The Jokioinen estimators did not work in Liminka; the NRMSEs were over 100%. These results indicated that higher quality remote sensing data provided better results in challenging estimation tasks.

Conclusions

This paper presents a review on satellite and unmanned aerial vehicle- (UAV) based remote sensing techniques for precision agriculture (PA) and considers their suitability for grass silage management. In the second part we have presented a UAV-based remote sensing method for the grass sward quantity and quality estimation and demonstrated its use in a trial site and on a real farm. The trial site provided high accuracy for the estimations. One reason for the better performance at the trial site was the use of a high performance remote sensing system with a hyperspectral camera and 3D-features providing comprehensive information of the object, whereas on the production site, a customer grade system with multispectral and RGB-colour cameras were used. Secondly, the reference measurements for the farm were not as comprehensive as those in the trial site. The best results were obtained when the estimator was trained using reference data from the site; this is not a practical approach for use in real conditions. Thus, more general estimators have to be developed to make the method operational in practical farming conditions. This can be achieved by collecting and generating more training data from different conditions.

The results have shown that UAV remote sensing is a promising approach to help farmers in optimising the management of silage production. UAVs provide information of sward quality and quantity faster and more comprehensively than existing state-of-the-art techniques, such as rising plate meters and laboratory analysis. This will give a better basis for scheduling of harvesting dates of different fields. During harvesting, real-time information about the crop quality could be used for classifying harvest to different quality classes in the storage for feeding. Also, other yield parameters such as the nitrogen uptake could be estimated to support fertilisation planning. The remote sensing imagery also produces information about the growth all over the field, and by this way it provides information to support maintenance operations. It also provides farmers with parcel-specific productivity information about their fields that would help them to manage cultivation; on large farms this can be as much as 30 to 50 scattered silage field parcels.

In the future, it will be relevant to develop more complete systems that integrate the continuous satellite monitoring with drone-based precision monitoring to enable holistic implementation of the smart farming approach.

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References

Aasen H., Honkavaara E., Lucieer A. and Zarco-Tejada P. (2018) Quantitative remote sensing at ultra-high resolution with uav spectroscopy: a review of sensor technology, measurement procedures, and data correction workflows. *Remote Sensing* 10, 1091.

Ali I., Cawkwell F., Dwyer E., Barrett B. and Green S. (2016) Satellite remote sensing of grasslands: from observation to management. *Journal of Plant Ecology* 9, 649-671.

Bareth G. and Schellberg J. (2018) Replacing manual rising plate meter measurements with low-cost UAV-derived sward height data in grasslands for spatial monitoring. *PFG – Journal of Photogrammetry, Remote Sensing and Geoinformation Science* 86(3-4), 157-168.

Hassler S.C. and Baysal-Gurel F. (2019) Unmanned aircraft system (UAS) technology and applications in agriculture. *Agronomy* 9, 618.

Honkavaara E. and Khoramshahi E. (2018) Radiometric correction of close-range spectral image blocks captured using an unmanned aerial vehicle with a radiometric block adjustment. *Remote Sensing* 10, 256.

Kaivosoja J., Pesonen L., Kleemola J., Pölönen I., Salo H., Honkavaara E., Saari H., Mäkynen J. and Rajala A. (2013) *A case study of a precision fertilizer application task generation for wheat based on classified hyperspectral data from UAV combined with farm history data*. SPIE Remote Sensing, 23-26 September 2013, Dresden, Germany.

Liakos K.G., Busato P., Moshou D., Pearson S. and Bochtis D. (2018) Machine learning in agriculture: a review. *Sensors* 18, 2674.

Manfreda S., McCabe M.F., Miller P.E., Lucas R., Pajuelo Madrigal V., Mallinis G., Ben Dor E., Helman D., Estes L., Ciraolo G., Müllerová J., Tauro F., De Lima M.I., De Lima J.L.M.P., Maltese A., Frances F., Caylor K., Kohv M., Perks M., Ruiz-Pérez G., Su Z., Vico G. and Toth B. (2018) On the use of unmanned aerial systems for environmental monitoring. *Remote Sensing* 10, 641.

Nikander J., Huitu H., Koistinen M., Laajalahti M., Niemeläinen O. and Kaivosoja J. (2018) Application of drone-based maps in planning farm operations for sward management in silage production. 7 p. European Society of Agricultural Engineers (EurAgEng).

Näsi R., Viljanen N., Kaivosoja J., Hakala T, Pandzic M., Markelin L. and Honkavaara E. (2017) Assessment of various remote sensing technologies in biomass and nitrogen content estimation using an agricultural test field. *International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences XLII-3/W3*, pp. 137-141.

Näsi R., Viljanen N., Kaivosoja J., Alhonoja K., Hakala T., Markelin L. and Honkavaara E. (2018) Estimating biomass and nitrogen amount of barley and grass using UAV and aircraft based spectral and photogrammetric 3D features. *Remote Sensing* 10, 1082.

Oliveira R.A., Khoramshahi E., Suomalainen J., Hakala T., Viljanen N. and Honkavaara E. (2018) Real-time and post-processed georeferencing for hyperpspectral drone remote sensing. *International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences XLII-2*, pp. 789-795.

Persson T., Höglind M., Van Oijen M., Korhonen P., Palosuo T., Jégo G., Virkajärvi P., Bélanger G. and Gustavsson A.M. (2019) Simulation of timothy nutritive value: a comparison of three process-based models. *Field Crops Research* 231, 81-92.

Pullanagari R.R., Yule I.J., Tuohy M.P., Hedley M.J., Dynes R.A. and King W.M. (2012) In-field hyperspectral proximal sensing for estimating quality parameters of mixed pasture. *Precision Agriculture* 13, 351-369.

Pullanagari R., Kereszturi G. and Yule I. (2018) Integrating airborne hyperspectral, topographic, and soil data for estimating pasture quality using recursive feature elimination with random forest regression. *Remote Sensing* 10(7), 1117.

Punalekar S.M., Verhoef A., Quaife T.L., Humphries D., Bermingham L. and Reynolds C.K. (2018) Application of Sentinel-2A data for pasture biomass monitoring using a physically based radiative transfer model. *Remote Sensing of Environment* 218, 207-220.

Shafi U., Mumtaz R., García-Nieto J., Hassan S.A., Zaidi S.A.R. and Iqbal N. (2019) Precision agriculture techniques and practices: from considerations to applications. *Sensors* 19, 3796.

Tsouros D.C., Bibi S. and Sarigiannidis P.G. (2019) A review on UAV-based applications for precision agriculture. *Information* 10, 349.

Viljanen N., Honkavaara E., Näsi R., Hakala T., Niemeläinen O. and Kaivosoja J. (2018) A novel machine learning method for estimating biomass of grass swards using a photogrammetric canopy height model, images and vegetation indices captured by a drone. *Agriculture* 8(5), 70.

Wolfert S., Ge L., Verdouw C. and Bogaardt M.-J. (2017) Big data in smart farming – a review. *Agricultural Systems* 153, 69-80.

Machine learning forecasting model for grass yield estimation in Ireland

Marwaha R.[1,2], Cawkwell F.[1], Hennessy D.[3] and Green S.[2]
[1]*Department of Geography, University College Cork, Cork, Ireland;* [2]*Agrifood Business and Spatial Analysis, Teagasc, Dublin, Ireland;* [3]*Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland*

Abstract

Measuring grassland biomass (or dry matter, DM) for grazing management on livestock farms is an important but time-consuming task. Using satellite data and a machine learning approach potentially offers a cost- and time-effective approach. A grass yield (kg DM ha^{-1}) estimation model in Ireland was developed using medium resolution satellite data and machine learning. The normalised difference vegetation index (NDVI) was derived from Landsat-8 data for eight sites in Ireland. NDVI, along with meteorological data and growing degree days (GDD), were used as predictors within the grass yield model. Two years of conventional paddock-scale grass biomass data (2017 and 2018), supplied from the PastureBase Ireland service were used to build two predictive machine learning models, i.e. random forest (RF) and adaptive neuro fuzzy-inference system (ANFIS). Grass growth rate prediction was made using 70% of the predictors and 30% was used for testing the model. R^2, RMSE and SMAPE values obtained from the RF model were about 0.69, 17.48 and 10.60, respectively, in testing stage. RF performed better than ANFIS for grass growth rate estimation. The accuracy of the machine learning model developed shows that it is a promising method for grass yield estimation at a farm scale.

Keywords: grassland, machine learning, Landsat-8, NDVI, grass growth rate, growing degree days, random forest, artificial neuro-fuzzy inference system

Introduction

Ireland is located in a temperate maritime zone. Out of 6.9 million hectares (ha) of land in Ireland, 4.4 million ha are dedicated to agriculture. Grasslands form 80% of that agricultural area (CSO, Farm Structures Survey 2013). Livestock form an important part of agriculture providing milk and meat and, hence, contributing directly to total food production. Livestock in Ireland are predominantly grass fed, and grassland management, including weekly paddock-scale grass growth measurements, has been proven to be the key to economic and environmental sustainability of dairy and beef enterprises (Conant *et al.*, 2010).

There are various methods to estimate grass biomass, such as the cut and dry method (which is a destructive method), visual estimation, and rising platemeter, etc. These methods cannot be used at a national level as they are time-consuming and expensive. Traditionally, remote sensing is used to estimate crop yield, especially for arable systems, and is increasingly used to estimate grass biomass in pastoral landscapes (Kumar *et al.*, 2015). Various empirical and mechanistic models use weather data as input to predict crop yield. For accurate predictions, not only the weather data but also the actual conditions on the ground are important. Grass growth is dependent on the accumulation of the energy in the plant. Growing degree days (GDD) is an estimate of the accumulated heat energy in the plant. Satellite data capture the grass growth conditions using various indices which can further be used for grass yield prediction. Thus, satellite data provide complementary information for grass yield estimation. The crop models for yield prediction employing statistical models, such as multiple linear regression, are unable to capture non-linearity in the input datasets (Johnson *et al.*, 2016). Hence, in this study the machine-learning algorithms better able to handle non-linear relationship between variables are explored for weekly grass growth rate predictions.

Materials and methods

The study area is composed of 8 Teagasc farms. The farms are classified into Dairy, Beef and Sheep farms, and distributed across the south and west of Ireland. Therefore, the study area is representative of different soil types and weather conditions. The average farm size in the Western region is approximately 27.1 ha, and in the Southern and Eastern regions 38.6 ha. The data used were the weekly grass growth rates (kg DM ha^{-1} per day) for all the farms.

Landsat-8 (level-2, surface reflectance) remote sensing images with 30 m spatial resolution were used. The data covered the 2017 to 2018 years and were provided by USGS Earth Explorer data portal (https://earthexplorer.usgs.gov/). The images were inspected for cloud and shadows, and removed where present and the images were converted into normalised difference vegetation index (NDVI) scores using red and near-infrared bands. NDVI is used as a proxy for biomass and is the ratio of near infrared band minus the red band to infrared band plus the red band. Higher values of NDVI represent healthy and dense vegetation. The images at this level of detail are capable of capturing spatial and temporal variations of grass growth at farm scale. The average NDVI values for each farm were calculated. Ground based data included weekly grass growth data (measured using a plate meter), average farm cover and cutting and grazing dates for each farm. Meteorological data used in this study included temperature, precipitation data downloaded from Irish Metrological Agency, Met Éireann (https://www.met.ie/). Hence, the input variables used in the model were mean temperature, GDD, rainfall data (mm), NDVI and grass growth rate. In designing the model 70% of the data were used to train the model and 30% to validate the model. The dependent variable chosen was grass growth and rest of the data was independent variable. The GDD was calculated based on the formula given in Fealy and Fealy (2008).

Machine learning uses black-box algorithms, which are increasingly used for agricultural applications such as crop classification, yield estimation, etc. In this paper we applied two machine learning algorithms for predicting grass growth at a farm scale, i.e. adaptive neuro-fuzzy inference system (ANFIS) and random forest (RF). All the datasets were normalised to have a mean of zero and standard deviation of 1. Five-fold cross validation was applied on the data. Along with the agro-meteorological and satellite data, the location information and day of the year is also used as an input to the model. This is done to include temporal and spatial variability in the model.

Results and discussion

We used two machine-learning algorithms: random forest (RF) and adaptive neuro-fuzzy inference system (ANFIS). The performance of the model was evaluated by the cross-validated mean-squared error (*MSE*), correlation coefficient (R^2), root mean squared error (*RMSE*) and Symmetric Mean Absolute Percent Error (*SMAPE*) as shown in Table 1. For RF, R^2 is the highest for training data, i.e. 0.92, and for testing data it is 0.64. For ANFIS, R^2 was 0.52 for training data and 0.48 for testing data. RMSE and SMAPE for ANFIS was 27 (kg DM ha^{-1} per day) and 16.62, and for RF was 17.48 (kg DM ha^{-1} per day) and 10.60 respectively. The scatter plot between the observed and the predicted grass growth rate is shown in Figure 1 for testing data. RF performed better than ANFIS model for 2017 and 2018. The model underperforms during the latter half of 2018 because 2018 was a drought affected year.

Table 1. Results for random forest (RF) and adaptive neuro-fuzzy inference system (ANFIS) for testing stage.

	ANFIS	RF
R^2	0.48	0.69
RMSE	27	17.48
SMAPE	16.62	10.60

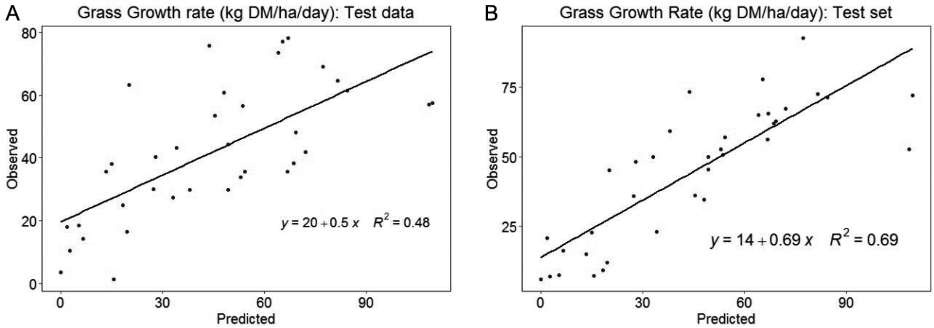

Figure 1. Scatter plot for testing data for (A) adaptive neuro-fuzzy inference system (ANFIS) and (B) random forest (RF).

Conclusions

Biomass is an important indicator of grass growth. The method used in this study allows the prediction of grass growth rate using Random Forest and ANFIS model which can be converted into biomass. To include the temporal and spatial variability in the model, date of the year and latitude and longitude were included. We developed a model combining both agro-metrological variables and satellite data at a farm scale. Modelling with weather and satellite data integrated is better than considering only meteorological data, or satellite data alone (Cai *et al.*, 2019). Also, the model was trained using all types of farms: sheep, dairy and beef farms. The results showed that RF model outperformed ANFIS model. The *RMSE* and *SMAPE* values obtained from the RF model were about 17.48 (kg DM ha^{-1} per day) and 10.60, respectively, in testing stage. To improve the model further, the use of Sentinel-2 with better spatial and temporal resolution is recommended. Nevertheless, the results shows that the model developed can be used as a significant tool for grassland monitoring.

References

Cai Y., Guan K., Lobell D., Potgieter A.B., Wang S., Peng J., Xu T., Asseng S., Zhang Y., You L. and Peng B (2019) Integrating satellite and climate data to predict wheat yield in Australia using machine learning approaches. *Agricultural and Forest Meteorology* 274, 144-159.

Conant R.T. (2010) Challenges and opportunities for carbon sequestration in grassland systems: A technical report on grassland management and climate change mitigation. *Integrated Crop Management* 9.

CSO (2013) Farm structure survey. *Central Statistics Office*, Dublin, Ireland.

Fealy R. and Fealy R.M. (2008) The spatial variation in degree days derived from locational attributes for the 1961 to 1990 period. *Irish Journal of Agricultural and Food Research* 47, 1-11.

Johnson M.D., Hsieh W.W., Cannon A.J., Davidson A. and Bedard F. (2016) Crop yield forecasting on the Canadian Prairies by remotely sensed vegetation indices and machine learning methods. *Agricultural and Forest Meteorology* 218-219, 74-84.

Kumar L., Sinha P., Taylor S. and Alqurashi A.F. (2015) Review of the use of remote sensing for biomass estimation to support renewable energy generation. *Journal of Applied Remote Sensing* 9, 097696.

Towards a dual-polarisation radar vegetation index for Sentinel-1 for grassland monitoring

Holtgrave A.[1,2], Ackermann A.[1], Röder N.[1] and Kleinschmit B.[2]

[1]*Institute of Rural Studies, Thünen Institute, Bundesallee 64, 38116 Brunswick, Germany;*

[2]*Geoinformation in Environmental Planning Lab, Technische Universität Berlin, Straße des 17. Juni 145, 10623 Berlin, Germany*

Abstract

Optical vegetation indices (VI) have long been used for grassland monitoring in remote sensing. Studies with radar VIs have value for vegetation monitoring as well. Nevertheless, there are no studies available for the specific configurations of the Sentinel-1 radar satellite. Active radar remote sensing is independent of light and cloud cover, and thus has a higher temporal resolution than optical sensors. This is an advantage for intra-annual monitoring of grassland vegetation. Our current study investigates the potential of converting Sentinel-1 radar data to indices to represent vegetation development on grassland sites. For this purpose, we deploy different optical VIs from Sentinel-2 as a proxy for on-site biomass development. Overall, 8,420 grassland parcels in Lower Saxony are used in this study. Dates with simultaneous Sentinel-1 and Sentinel-2 pass-overs in 2018 with no cloud cover were considered. We calculate correlations between all radar and optical VIs to get knowledge of the possibility to use solely radar VIs for vegetation monitoring. Further, we include additional geo-data like soil information and a digital elevation model in a multiple regression and a random forest regression model. First results show R^2 between 0.17 and 0.52 for variations of radar to optical VIs depending on the method.

Keywords: satellite remote sensing, SAR, radar vegetation index, Sentinel-1, biomass

Introduction

For grassland monitoring over large areas, satellite remote sensing is the only suitable method. For a lot of questions regarding grassland monitoring, the biomass on a field is an important factor (Kumar *et al.,* 2013). Optical Vegetation Indices (VIs) have long been used to model vegetation biomass for agricultural areas (Bannari *et al,.* 1995).

The biggest disadvantage of optical VIs though, is their dependence on illumination and absence of clouds. Thus, the temporal resolution of optical satellite images is often not good enough for intra-annual monitoring. An alternative is active radar remote sensing as it is independent of light and can pass through clouds (Barrett *et al.,* 2014). Studies with quad-pol synthetic aperture radar (SAR) VIs confirm its value for vegetation monitoring (Kim and Van Zyl, 2004). Although there are some studies with dual-pol SAR VIs (Kumar *et al.,* 2013), there are none available for the specific configurations of the Sentinel-1 (S1) SAR satellite yet. This study aims at finding a radar VI from S1 to model grassland biomass.

Materials and methods

For the year of 2018, land parcel identification system (LPIS) data with crop type and parcel delineation of field grass, meadow and hay meadow parcels in Lower Saxony, Germany were used in this study. There were 22 randomly distributed 10×10 km study sites considered, with a total of 8,420 grassland parcels covering 14,125 ha of land.

The Sentinel satellites are earth observation satellites from the Copernicus programme of the European Space Agency. S1 consists of two SAR satellites and Sentinel-2 (S2) of two optical satellites. For every test site, S2 images with 0-5% cloud cover for the year 2018 were selected. The resulting scenes were then filtered for scenes with a corresponding S1 scene at the same date. For the remaining dates, three to fourteen S1 and S2 scenes were downloaded for every test site – 35 scenes each in total. S1 and 2 images were downloaded via the Sentinel Hub. S1 was downloaded as Ground Range Detected (GRD) orthorectified Interferomic Wide Swath (IW) Gamma Backscatter in high resolution VV + VH polarisation with a resolution of 10 m. Pre-Processing steps of the Sentinel Hub include calibration and thermal noise reduction. S2 scenes were downloaded as L1C images with full atmospheric correction with a resolution of 10 m for the satellite bands used in this study.

The original bands from both satellites were processed into different indices. For S2, we used different and well-known vegetation indices and adapted them to S2 bands: enhanced vegetation index (EVI) (Huete *et al.*, 2002), normalised difference vegetation index (NDVI) (Rouse *et al.*, 1974), normalised difference red edge index (NDRE) (Barnes *et al.,* 2000), normalised difference water index (NDWI) (Gao, 1996), soil adjusted vegetation index (SAVI) (Huete, 1988). We adapted existing quad-pol VIs to be used with the VV+VH dual-pol characteristics of S1: radar forest degradation index (RFDI) (Mitchard *et al.,* 2011) and radar vegetation index (RVI) (Kumar *et al.,* 2013). We assumed HH≈VV and HV≈VH. Additionally, we used a simple band ratio (VH/VV) and single polarisations. For every date, the median value of all pixels was calculated for every parcel respectively, which resulted in a total of 55,000 observations.

Linear regression, multiple linear regression and random forest regression were calculated for every optical and radar VI combination with the R packages *base* and *randomForest*. The latter two regression analyses included additional data like height, slope, aspect from 10×10 m Digital Elevation Model, soil type and soil landscape type, as well as the test site ID and acquisition date. The data set was divided into training and test data sets. Hereby, 5,000 observations were used for training.

Results and discussion

The highest Pearson Correlations can be found between RFDI and NDVI or RVI and NDVI (both r=0.41, R^2=0.17) which shows that there is no direct linear regression between the optical and radar VIs. RFDI and RVI are very highly correlated themselves with R^2=1. Including more dependent variables into a Multiple Regression analysis improves the result whereas the highest correlation can be found between RDFI or RVI and SAVI with R^2=0.36.

Applying the Random Forest Regression on a test data set, results in R^2 of up to 0.52 for SAVI. RFDI, RVI and VH/VV Ratio are equally good in predicting the optical VI (Figure 1). This shows that the combination of VV and VH improves the model. The most important variable when modelling SAVI from RVI is the Radar Index followed by the date variable and height and test area ID information (Figure 2).

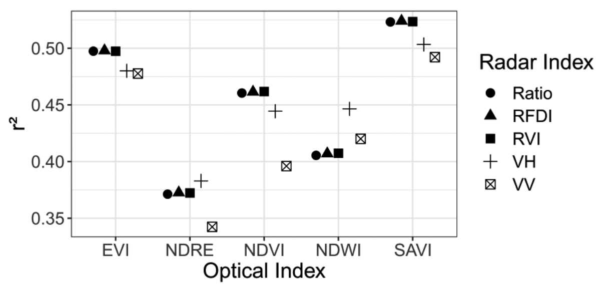

Figure 1. R^2 of predicted vs actual values for all optical vegetation indices (VIs) modelled from different radar VIs with random forest regression.

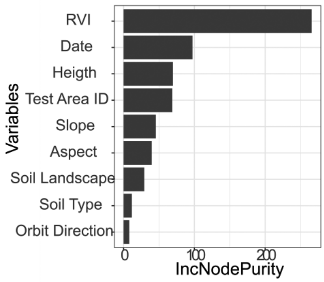

Figure 2. Variable importance for the random forest regression model.

Conclusions

Different radar VIs are capable of approaching different optical VIs moderately well. So far, they cannot be used for absolute biomass modelling. The optical VIs were predicted differently well. They were used as a proxy for the actual biomass in this study. As we do not know the exact absolute biomass, it is not clear whether the radar VIs would perform better or worse with real biomass data. For many applications, it is not the real biomass but the biomass development that is the most important factor. Further research will include the possibility of radar VIs to describe relative biomass development over time.

References

Bannari A., Morin D., Bonn F. and Huete A.R. (1995) A review of vegetation indices. *Remote Sensing Reviews* 13 (1-2), 95-120. https://doi.org/10.1080/02757259509532298

Barnes E.M., Clarke T.R., Richards S.E., Colaizzi P.D., Haberland J., Kostrzewski M. *et al.* (2000) Coincident detection of crop water stress, nitrogen status and canopy density using ground-based multispectral data. In: *Proc. 5th Int. Conf. Precis Agric*.

Barrett B., Nitze I., Green S. and Cawkwell F. (2014) Assessment of multi-temporal, multi-sensor radar and ancillary spatial data for grasslands monitoring in Ireland using machine learning approaches. *Remote Sensing of Environment* 152, 109-124. https://doi.org/10.1016/j.rse.2014.05.018

Gao B. (1996) NDWI – a normalized difference water index for remote sensing of vegetation liquid water from space. *Remote Sensing of Environment* 58(3), 257-266. https://doi.org/10.1016/S0034-4257(96)00067-3

Huete A., Didan K., Miura T., Rodriguez E.P., Gao X. and Ferreira L.G. (2002) Overview of the radiometric and biophysical performance of the MODIS vegetation indices. *Remote Sensing of Environment* 83(1-2), 195-213. https://doi.org/10.1016/S0034-4257(02)00096-2

Huete A.R (1988) A soil-adjusted vegetation index (SAVI). *Remote Sensing of Environment* 25(3), 295-309. https://doi.org/10.1016/0034-4257(88)90106-X

Kim Y. and Van Zyl J. (2004) Vegetation effects on soil moisture estimation. In: Proceedings. IEEE International Geoscience and Remote Sensing Symposium. Anchorage, AK, USA, 20-24 Sept. 2004, pp. 800-802.

Kumar D., Srinivasa Rao S. and Sharma J.R. (2013) Radar vegetation index as an alternative to NDVI for monitoring of soyabean and cotton. *Indian Cartographer* XXXIII, 91-96.

Mitchard E.T.A., Saatchi S.S., Lewis S.L., Feldpausch T.R., Woodhouse I.H., Sonké B. *et al.* (2011) Measuring biomass changes due to woody encroachment and deforestation/degradation in a forest-savanna boundary region of central Africa using multi-temporal L-band radar backscatter. *Remote Sensing of Environment* 115(11), 2861-2873. https://doi.org/10.1016/j.rse.2010.02.022

Rouse J.W., Haas R.H., Scheel J.A. and Deering D.W. (1974) Monitoring vegetation systems in the Great Plains with ERTS. In: *Proceedings. 3rd Earth Resource Technology Satellite (ERTS) Symposium*, pp. 48-62.

Grassland forage quality and quantity estimation with UAV-borne imaging spectroscopy

Wijesingha J.S.J., Schulze-Brüninghoff D., Wengert M., Astor T. and Wachendorf M.
Grassland Science and Renewable Resources, Universität Kassel, Steinstraße 19, 37213 Witzenhausen, Germany

Abstract

Grassland forage quality and quantity information is essential for management of feedstocks for ruminants. Spectral data from unmanned aerial vehicle (UAV) borne remote sensing is a promising technology for estimating field-scale grassland biomass and forage quality compared with traditional ground-borne observations. In this study, we examined data from eight grasslands, differing in vegetation type and management, located in northern Hesse, Germany. The goal of this study was to utilise UAV-borne imaging spectroscopy data to build generalized models for estimating (1) grassland dry matter yield (DM) and (2) two important grassland forage quality parameters: crude protein (CP) and acid detergent fibre (ADF). The imaging spectroscopy data were acquired from a hyperspectral snapshot camera attached to the UAV. Machine learning regression models were employed to address the high variability and multicollinearity of the spectral data. Grassland DM was estimated with 11.2% median relative root mean square error (rRMSE) and quality parameters CP and ADF were predicted with 10.6 and 13.4% median rRMSE, respectively. Overall, the UAV-borne imaging spectroscopy provides a promising tool for a precise grassland forage quality and quantity prediction and mapping for multiple grasslands.

Keywords: grassland, imaging spectroscopy, unmanned aerial vehicle, dry matter yield, forage quality, machine learning regression

Introduction

Approximately 30 to 35% of the utilised agricultural area in Europe is grassland (Huyghe *et al.*, 2014). Provision of forage for ruminants is one of the primary ecosystem services provided by grasslands. Accurate and timely knowledge of the amount and quality of the forage is critical for meeting demands in animal feeding. Traditional ground-based methods to determine the quantity and quality of grassland forage are labour intensive and time consuming. Application of unmanned aerial vehicle (UAV) borne spectral data can overcome the limitations of ground-based methods and easily cover larger fields. However, the applicability of spectral data models depends on the variability of the calibration data, which can be surmounted by the inclusion of a variety of grassland types and management practice in the model development. The principal aim of the presented work was to build generalised models using UAV-borne spectral data to estimate (1) grassland dry matter yield (DM), and (2) grassland forage quality values namely crude protein (CP), and acid detergent fibre (ADF) irrespective to grassland type or management practices.

Materials and methods

The study examined data from four extensive grasslands in the Rhön biosphere reserve (Hesse, Germany) and four grasslands with various intensities near Witzenhausen (Hesse, Germany). All grasslands differed in terms of species composition and cutting regime. A 30×50 m plot was selected as a study plot in each grassland. In the summer of 2018, UAV-borne imaging spectroscopy data were collected in each grassland study plot using a Cubert Firefleye S185 SE (Cubert GmbH, Germany) hyperspectral snapshot camera (450-950 nm spectral range) which was attached to an RTK-X8 octocopter (Copter Squad AUS UG, Germany). Spatially randomly distributed 1 m^2 subplots were utilised to collect reference samples after each UAV data collection for determination of DM, CP, and ADF. In total, DM was determined from 320 subplots and CP, ADF from 194 subplots.

Machine learning (ML) based regression models were built to overcome multicollinearity in the spectral data and to get the advantage of the reflectance data from the whole spectrum. Different ML regression algorithms were tested to identify the best algorithm for each forage parameter, namely partial least squares regression (PLSR), random forest regression (RFR), support vector regression (SVR) and the rule-based cubist regression (CBR). R statistical software with classification and regression training (caret) package was employed for regression model building (Kuhn *et al.*, 2018; R Core Team, 2019).

For each forage parameter (DM, CP, and ADF), the same procedure was followed to build regression models using spectral data. The dataset was divided into two parts, with 80% for model training and 20% for model testing. To evaluate model robustness, 100 different models from each ML algorithm were trained and tested for each forage parameter. The best ML model was identified according to the 100 models' prediction root mean square error (RMSE) (Equation 1), and observation range based relative RMSE (rRMSE) for the test datasets. The model with lowest median RMSE and lowest standard deviation of RMSE was considered as the best model with highest accuracy and highest stability respectively. These models were then utilised to generate field-level forage quantity and quality maps for each grassland.

$$\mathrm{RMSE}=\sqrt{\frac{\sum_{j=1}^{n}\left(y_j-\hat{y}_j\right)^2}{n}}$$

where y is the observed value, ŷ is the predicted value, and n is the number of observations.

Results and discussion

DM values fluctuated between 0.008 and 0.62 kg m^{-2}. CP concentrations varied between 5.1 and 23.3% DM, while ADF varied between 22.5 and 38.5% DM. From four ML algorithms, CBR was the best for estimating DM, ADF while SVR was the best for CP estimation (Table 1). All three forage parameters were estimated with median relative prediction errors of less than 14%. The relative estimation errors were comparable to results from studies with terrestrial field spectroscopy in the temperate region (Biewer *et al.*, 2009; Psomas *et al.*, 2011; Pullanagari *et al.*, 2012; Safari *et al.*, 2016).

The plot of fit based on the best ML algorithm shows how the prediction value changed for each observed value when 100 different models were used (Figure 1). Moreover, higher DM and CP values showed a clustering pattern in respective plots. The high variation of DM and CP between the cuts in grasslands due to intensive management practice and drought condition might be the reason for the mentioned pattern. However, the observed against predicted plot of fit for ADF did not highlight a similar pattern.

Table 1. Summary of the identified best machine learning (ML) regression models for dry matter yield (DM), crude protein (CP) and acid detergent fibre (ADF).[1]

Forage parameter	ML model	Median RMSE	Median rRMSE (%)
DM	CBR	0.07 kg m^{-2}	11.2
CP	SVR	1.90% DM	10.6
ADF	CBR	2.20% DM	13.4

[1] SVR = support vector regression, CBR = cubist regression.

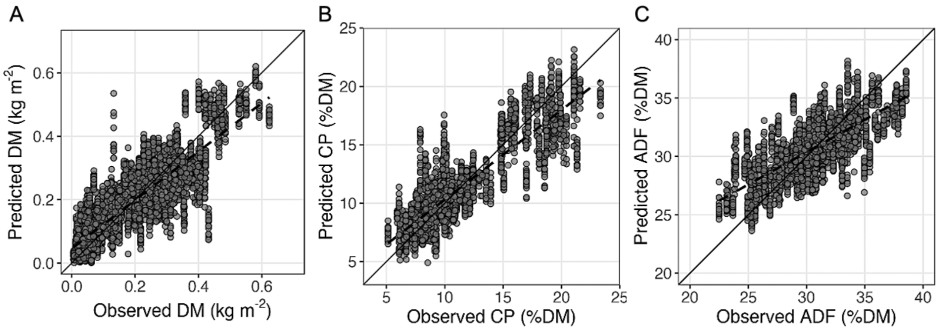

Figure 1. Observed versus predicted value scatter plots for (A) dry matter yield (DM), (B) crude protein (CP), and (C) acid detergent fibre (ADF). The black line is the 1:1 line, and the broken line represents the linear regression line between observed.

Conclusions

The study intended to estimate forage quantity and quality of a multitude of grasslands with different botanical composition and cutting regimes using UAV-borne imaging spectroscopy data. It was found that the resulting models could accurately estimate DM, CP and ADF irrespective of the grassland type, and accuracy of the models were in the same range as those obtained with the use of field spectroscopy. With such models, forage quantity and quality maps can be generated, which may provide benefits for sustainable grassland management. Further, adjusting the models with additional different grasslands will be a way forward to obtain even more generalised model regardless of the grassland type or management practice.

References

Biewer S., Fricke T. and Wachendorf M. (2009) Determination of dry matter yield from legume-grass swards by field spectroscopy. *Crop Science* 49, 1927-1936.

Huyghe C., De Vliegher A., Gils B.V. and Peeters A. (2014) *Grasslands and herbivore production in Europe and effects of common policies* 287 pp.

Kuhn M., Wing J., Weston S., Williams A., Keefer C., Engelhardt A., Cooper T., Mayer Z., Kenkel B., the R Core Team, Benesty M., Lescarbeau R., Ziem A., Scrucca L., Tang Y., Candan C. and Hunt T. (2018) Caret: classification and regression training. Available at: https://cran.r-project.org/

Psomas A., Kneubühler M., Huber S. and Itten K. (2011) Hyperspectral remote sensing for estimating aboveground biomass. *International Journal of Remote Sensing* 32, 1-25.

Pullanagari R.R., Yule I.J., Tuohy M.P., Hedley M.J., Dynes R.A. and King W.M. (2012) In-field hyperspectral proximal sensing for estimating quality parameters of mixed pasture. *Precision Agriculture* 13, 351-369.

R Core Team, 2019. R: a language and environment for statistical computing.

Safari H., Fricke T. and Wachendorf M. (2016) Determination of fibre and protein content in heterogeneous pastures using field spectroscopy and ultrasonic sward height measurements. *Computers and Electronics in Agriculture* 123, 256-263.

Suitability of non-destructive yield and quality measurements on permanent grassland

Klingler A.[1], Schaumberger A.[1], Vuolo F.[2] and Poetsch E.M.[1]

[1]*Institute of Plant Production and Cultural Landscape, Agricultural Research and Education centre Raumberg-Gumpenstein, Austria;* [2]*Institute of Geomatics, University of Natural Resources and Life Sciences, Vienna, Austria*

Abstract

There is an increasing interest in non-destructive measurements for comprehensive grassland monitoring. The present study aims to assess the ability of crop height (CH), leaf area index (LAI), chlorophyll content (CC) and mean developmental stage measurements to estimate dry matter yield (DMY), crude protein content (CP) and neutral detergent fibre content (NDF) of permanent grassland. Therefore, LAI from hyperspectral reflectance acquired by a field spectrometer and from the AccuPAR LP-80 was recorded weekly during an entire growing season. CH was measured with a rising plate meter (RPM) and a yardstick (YS) before destructive sampling for DMY, CP and NDF determination. The most abundant grass species were cut for measuring mean developmental stage and CC. Starting with a null model, the best predictors for DMY, CP and NDF using an exhaustive search were determined. Including design effects and the cuts in a mixed model approach, CH and LAI showed an R^2 of 0.93 for DMY estimation. CC from *Alopecurus pratensis* and CH were the best predictors for CP (R^2=0.86) and CC along with mean developmental stage from *A. pratensis* for NDF (R^2=0.81). The tested parameters and in particular parameter combinations led to promising results for DMY, CP and NDF estimation.

Keywords: grassland monitoring, leaf area index, crop height, yield, forage quality

Introduction

Grassland swards are well known for their high spatial and temporal variability due to abiotic and biotic influences during each growth (Schut *et al.*, 2002). To guarantee optimal and comprehensive grassland management during the entire growing period, information about yield and quality development are of utmost importance (Wachendorf *et al.*, 2018). Non-destructive methods for the estimation of yield and forage quality have become more common in recent years. These methods offer the possibility to monitor growth characteristics within particular cuts and thus optimise grassland management. Non-destructive observations mostly refer to the relationship of sward height, phenological stage, species composition or the interaction between leaves and radiation with yield and forage quality. In situ measurements provide valuable information at the local level, but are not very suitable for large scale observations due to their mostly labour-intensive execution (Ali *et al.*, 2016). In recent years, satellite data have proved to be very helpful for observing grasslands regionally. Especially the freely available Sentinel-2 data provide a sufficiently high temporal and spatial resolution (Drusch *et al.*, 2012). Few methods, and especially method combinations for the estimation of DMY, CP and NDF have been tested for species-rich permanent-grassland vegetation. This research aims to test common non-destructive measurements for their ability to predict the yield and quality of permanent grassland during an entire vegetation period.

Materials and methods

A field experiment was conducted in 2018 on permanent grassland in the Enns Valley, Austria (47°30'35.4' N; 14°05'03.5'E; 643 m above sea level). The experimental setup was established as a split-plot design with three replicates (2.25 m^2 per plot) before the start of the growing period in 2018. The sampling was carried out at weekly intervals during the entire vegetation period, which resulted in 32 campaigns, each following a precise workflow.

Initially, CH was measured using an RPM and a YS. Four hyperspectral reflectance measurements were acquired by a field spectrometer (HandySpec/tec5), and three AccuPAR readings were obtained at each plot. The hyperspectral reflectance data were resampled into Sentinel-2 bands according to the Spectral Response Functions (ESA, 2018). After resampling, LAI was calculated using a neural net algorithm from Baret *et al.* (2010). This algorithm is specifically tailored for Sentinel-2 data and was trained with radiative transfer simulations from the PROSPECT and SAIL models (Jacquemoud *et al.*, 2009; Verhoef, 1984). Following the non-destructive measurements, an area of one square meter was harvested on each plot. After drying the samples for DMY determination, CP and NDF were analysed chemically. Representing the abundance of the three most dominating species in the sward, fifteen individual plants of *Alopecurus pratensis,* ten of *Dactylis glomerata* and five of *Festuca pratensis* were manually cut on the remaining edges of the harvested plots for the following analyses. CC measurements were carried out using a chlorophyll meter (SPAD 502) and were then separated into observations on the flag leaf and observations on randomly selected leaves. Subsequently, the mean developmental stage of each grass species according to the BBCH-scale (Meier *et al.*, 2009) was calculated by the following two equations:

$$MSC = \sum_{i=1} \frac{S_i * N_i}{C}$$

where MSC = mean stage count, S_i = growth stage index, N_i = number of plants in stage S_i and C = total number of plants in the sample population (Moore *et al.*, 1991).

$$MSW = \sum_{i=1} \frac{S_i * D_i}{W}$$

where MSW = mean stage weight, S_i = growth stage index, D_i = weight of plants in stage S_i and W = total weight of plants in the forage sample (Fick and Mueller, 1989). The best and the two best predictor variables were determined using exhaustive search from the function 'regsubsets' of the 'leaps' R-package. The response variables were DMY, CP and NDF. Explanatory variables included in the models were: crop height (RPM and YS), LAI from AccuPAR and field spectrometer, CC from *A. pratensis*, *D. glomerata* and *F. pratensis* (flag leaves and random leaves of each species), MSC and MSW of *A. pratensis*, *D. glomerata* and *F. pratensis* and average MSC and MSW of all three species. After defining the best explanatory variables, a mixed model regression was set up using the function 'lmer' of the 'lme4' R-package. The dataset was split into 67% training data and 33% test data for the calculation of *RMSE* and R^2.

Results and discussion

CH (RPM) was identified by the exhaustive search method to be the best predictor for DMY. It showed an R^2 of 0.90 and an *RMSE* of 434 when testing the mixed model regression on the test data set. The two best response variables for DMY estimation were CH (RPM) and LAI from AccuPAR. The predictor combination led to an improved *RMSE* (Figure 1). The combination of CH (RPM) and LAI from field spectrometer showed a high prediction accuracy as well (R^2=0.92, *RMSE*=363), indicating the high potential of remote sensing data for grassland yield estimation. CH (YS) was the best predictor for CP (R^2=0.75, *RMSE*=22). The combination of the two best predictors, CH (YS) and CC from *A. pratensis* (random leaves) improved the prediction accuracy again (Figure 1). MSC from *F. pratensis* served as the best predictor for NDF (R^2=0.55, *RMSE*=50) whereas MSW from *A. pratensis* and CC from *A. pratensis* (random leaves) were the best predictors for NDF. The parameter combination again improved the prediction accuracy (R^2=0.81, *RMSE*=42). The prediction accuracy increased in all three cases (DMY, CP and NDF) when two explanatory variables were included in the model. This shows that the combination of morphological, phenological and optically sensed parameters significantly improves the model performance. LAI, CC, CH, mean developmental stage and in particular, their combination prove their ability for the prediction of yield and forage quality.

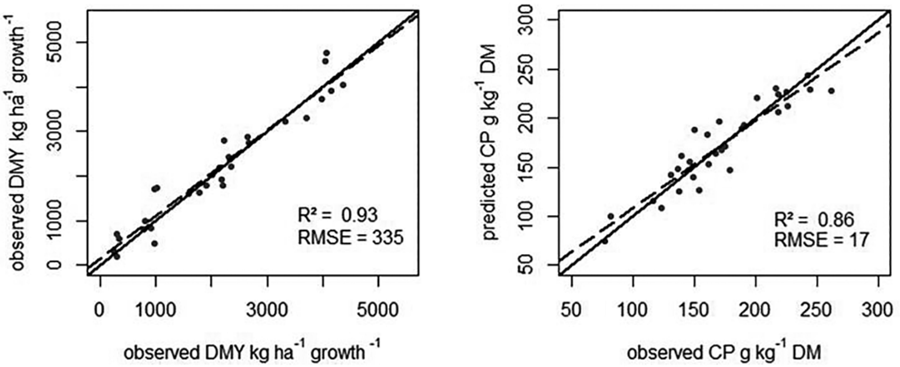

Figure 1. Observed vs predicted dry matter yield (DMY) kg ha^{-1} using rising plate meter (RPM) and leaf area index (LAI) from AccuPAR (left) and observed vs predicted crude protein (CP) g kg^{-1} dry matter (DM) using crop height and chlorophyll content (CC) from *Alopecurus pratensis* (random leaves) on the right side.

Conclusions

Non-destructive measurements on high abundant plants, as well as on the total sward, and a combination of both, show a high ability to estimate cost-efficiently and expeditiously some important yield and quality parameters of species-rich permanent grassland. The results demonstrate the possibility of continuous and comprehensive growth monitoring from small plots to regional scale. The planned extension of the experiment to several sites will provide reliable and robust results for a larger range of grasslands.

References

Ali I., Cawkwell F., Dwyer E., Barrett B. and Green S. (2016) Satellite remote sensing of grasslands: from observation to management. *Journal of Plant Ecology* 9, 649-671.

Baret F., Weiss M., Bicheron P. and Berthelot B. (2010) Sentinel-2 MSI products WP1152 algorithm theoretical basis document for product group B. INRA-EMMAH, Avignon, France.

Drusch M. *et al.* (2012) Sentinel-2: ESA's optical high-resolution mission for GMES operational services. *Remote Sensing of Environment* 120, 25-36.

ESA (2018) Sentinel-2 spectral response functions. Available at: https://earth.esa.int/web/sentinel/user-guides/sentinel-2-msi/document-library/-/asset_publisher/Wk0TKajiISaR/content/sentinel-2a-spectral-responses.

Fick G.W. and Mueller S.C. (1989) Alfalfa: quality, maturity, and mean stage of development. *Information Bulletin Cornell University* 217, 16.

Jacquemoud S. *et al.* (2009) PROSPECT + SAIL models: a review of use for vegetation characterization. *Remote Sensing of Environment* 113, 56-66.

Meier U. *et al.* (2009) The BBCH system to coding the phenological growth stages of plants – history and publications. *Journal für Kulturpflanzen* 61, 41-52.

Moore K.J., Moser L.E., Vogel K.P., Waller S.S., Johnson B.E. and Pedersen J.F. (1991) Describing and quantifying growth stages of perennial forage grasses. *Agronomy Journal* 83, 1073-1077.

Schut A.G.T., Ketelaars J.J.M.H., Meuleman J., Kornet J.G. and Lokhorst C. (2002) AE – automation and emerging technologies: novel imaging spectroscopy for grass sward characterization. *Biosystems Engineering* 82, 131-141.

Verhoef W. (1984) Light scattering by leaf layers with application to canopy reflectance modeling: the SAIL model. *Remote Sensing of Environment* 16, 125-141.

Wachendorf M., Fricke T. and Möckel T. (2018) Remote sensing as a tool to assess botanical composition, structure, quantity and quality of temperate grasslands. *Grass and Forage Science* 73, 1-14.

Evaluation of remote-sensing and labour-saving technologies to measure pasture biomass

Huson K.M., Scoley G., Barr S. and McConnell D.A.
Agri-Food and Biosciences Institute, Hillsborough, Co. Down, NI, United Kingdom

Abstract

With expanding herd sizes, labour use efficiency is an increasing challenge for many Northern Ireland (NI) farms. New remote-sensing and labour-saving technologies were assessed for their accuracy and reliability in estimating pasture biomass on both plots and grazing paddocks. In a 2018 plot experiment, pasture biomass estimations calculated using a standard rising plate meter (RPM), the GrassHopper® RPM (GH RPM), and the C-Dax trailed pasture meter (TPM) were compared with total plot dry matter (DM) yields. Each approach was then repeated at the paddock scale in 2019, with the addition of satellite-based estimations. In 2019, pasture biomass was estimated using a quadrat cut-and-weigh approach to determine DM yield. Both RPMs produced consistent results, strongly correlated with total plot DM yields (R^2=0.79). Normalised difference vegetation index values were obtained by unmanned aerial vehicle, but no correlation with pasture biomass values was identified from plot measurements in 2018, but an R^2 of 0.46 was recorded from paddocks in 2019. Results from measurements collected over a greater area (satellite and TPM), gave more consistent biomass estimates. Less-established technologies required bespoke calibration equations to accurately predict pasture biomass for NI grazing swards.

Keywords: remote sensing, herbage biomass estimation, grazing

Introduction

Accurate estimation of available pasture biomass is crucial for ensuring that herbage offered to ruminant livestock is sufficient to meet animal demands while also maximising herbage utilisation and grazing efficiency. Currently only 13.5% of dairy farmers in Northern Ireland (NI) regularly measure pasture biomass in the grazing season (McConnell *et al.*, 2020), and a key barrier to increasing farmer adoption of regular pasture measurement has been identified as the perceived labour requirement. The time required to walk all grazing paddocks on a large holding may extend to several hours. New technologies offer the potential to reduce the time and labour required to assess pasture biomass. In turn, this may support greater uptake of measurement, enabling improvements in grass utilisation grazing efficiency (Murphy *et al.*, 2018). The current study aimed to assess the accuracy and reliability of three emerging technologies for measuring pasture biomass in NI; the GrassHopper® rising plate meter (GH RPM), which uses a micro-sonic sensor to measure compressed sward height (CSH) and GPS for geo-referencing, the C-Dax trailed pasture meter (TPM), which measures sward height (SH) through a light emitting and sensing photodiode array at 20 mm spacing, measuring the height of grass passing between these sensors, and is also GPS enabled, and a normalised difference vegetation index (NDVI) camera mounted to unmanned aerial vehicle (UAV), as NDVI has previously shown potential for inferring biomass in grassland systems (Flynn, 2006). These methods were compared to the standard ratchet RPM and quadrat cut-and-weigh (C&W) techniques at both the experimental plot and grazing paddock scale. For paddocks, satellite-based estimations of pasture biomass ('MiGrass', via a proprietary calculation within the Precision Decisions (UK) platform) were also compared.

Materials and methods

Two trials were undertaken at the Agri-Food and Bioscience Institute (AFBI) research farm, Hillsborough, Northern Ireland (54°27' N; 06°04' W). In the autumn of 2018, plots measuring 15×2 m were established to capture 4 different growth stages (7, 14, 21, and 28 days regrowth after cutting) in six replicate blocks, each containing 16 plots (n=192). Plots were established on a predominantly (>80%) perennial ryegrass

(*Lolium perenne*) pasture. Initial cutting dates were staggered to ensure all 4 stages of regrowth were cut simultaneously to mitigate against any effect of varying grass dry matter (DM), weather conditions or light levels on the date of measurement. Arial images were collected by a DJI Phantom 4 UAV equipped with a Sentera High-Precision NDVI Single Sensor camera (Sentera, MN, USA), at a flying height of 25 m. Sentera Field Agent software generated NDVI values for individual plots/paddocks. Each plot was measured with a single lengthways pass at >5 km/h with the TPM. For each RPM 30 drops were recorded, as per industry recommendations, and for the standard RPM a previously optimised conversion equation; Biomass = CSH (cm) × 124 + 608, was used to generate kg DM ha^{-1} readings (Dale, 2010). With the GH RPM software, grass DM for the calculation of herbage biomass was set as 17%. Total plot yields were calculated from the fresh herbage mass >4 cm, and oven DM% of plot sub-samples. Herbage biomass <4 cm in height was assumed to be 1,500 kg DM ha^{-1}. Data collection at the paddock scale was conducted weekly in late summer 2019 from 6×0.4 ha paddocks (33.5×120 m). Measurements were taken as above, but with 6×lengthways passes made of each paddock with the TPM, and the C&W approach was adapted to use three 1 m^2 quadrat clips per paddock, with DM calculated for each clip to obtain an estimate of average paddock kg DM ha^{-1}. Satellite-based estimations of herbage biomass from the 'MiGrass' function within the Precision Decisions online portal (Precision Decisions, UK) were included with paddock measurements. Paddocks were grazed to a target post-grazing residual of 1,700 kg DM/ha by lactating dairy cows 10 days prior to week 1 of data collection, and 0-2 days after week 2 and week 5 measurements. For week 5, no MiGrass data were available for comparison due to cloud cover. All UAV imagery was taken at approximately midday, and prior to all other measurements to ensure images were of an untouched sward. Data were analysed by linear regression in Excel, and RMSE calculated to give the residual spread where appropriate.

Results and discussion

In 2018, harvested plot yields ranged from 1,598-2,740 kg DM ha^{-1}, and in 2019 paddock estimates ranged from 1,282-3,081 kg DM ha^{-1}, as determined by C&W. All regression analyses, except between plot C&W DM mass and UAV NDVI values, indicated a significant positive correlation between the control (C&W, $P \leq 0.001$) and other methods of herbage mass estimation (Table 1). Results from the GH RPM proprietary equation were comparable to the recommended NI RPM equation for determining herbage mass from CSH. Results from the plot experiment showed a correlation of 0.50 between total plot (2018) DM yield and the SH measurement from the TPM. The regression equation for this relationship was; Biomass (kg DM ha^{-1}) = (SH (cm) × 83.9) + 1,217.7, and this equation was used to calculate herbage biomass estimates with the TPM in 2019. Readings of NDVI obtained from plots in 2018 did not correlate with herbage mass (R^2=0.001). Despite this, others using UAV-mounted NDVI cameras have previously reported favourable results (Flynn, 2006), and R^2=0.46 was found between paddock C&W measures and NDVI values obtained from paddocks in 2019. Further investigation and adaptation of this technique is therefore warranted, and may include combining estimates of herbage height alongside NDVI in regression equations to calculate herbage biomass (Gebremedhin *et al.*, 2019). In 2019, the quadrat C&W measures taken from paddocks gave highly variable results (Table 2), likely influenced by high

Table 1. Correlation and RMSE between experimental herbage mass measurements and control measurements of plot (2018) or pasture (2019) cover (C&W).[1,2]

Equipment	Plots – 2018		Paddocks – 2019	
	R^2	RMSE (kg DM ha^{-1})	R^2	RMSE (kg DM ha^{-1})
GH	0.79	335.2	0.46	300.8
TPM	0.50	163.8	0.63	297.8
NDVI	0.01	*	0.46	*
RPM	0.79	363.8	0.53	527.6
MiGrass	N/A	N/A	0.48	377.2

[1] * = with NDVI values, no calculation to kg DM ha^{-1} was possible, and RMSE was not estimated.

[2] DM = dry matter; GH = grass height; TPM = trailed pasture meter; RPM = rising plate meter; NDVI = normalised difference vegetation index; MiGrass = satellite-based estimation.

paddock heterogeneity and the comparatively small area sampled. The cutting of only three quadrats at each sampling was selected to represent common on-farm practices, but the poor consistency of results obtained here highlights a known weaknesses of the C&W quadrat approach. This variability likely contributed to the lower R^2 seen between C&W and each RPM in 2019 compared with 2018. However, the correlation between both RPMs in 2019 was strong (R^2=0.74). Time-savings were observed with both the GH RPM and TPM through automatic generation of pasture biomass readings and a grass wedge, compared to the ratchet RPM which required manual data input and calculations. The TPM and UAVs (if optimised for pasture biomass quantification) could offer additional time savings on large grazing platforms as the speed at which data can be captured (5-20 km h^{-1} and ~24 km h^{-1} respectively) is much faster than walking speed (~5 km h^{-1}). Furthermore, satellite technology removes the labour requirement on-farm completely. However, whilst the TPM and GH RPM and some satellite biomass measures are currently commercially available, they can be much more costly than purchasing a ratchet RPM, and further work is required to estimate the cost efficiency of these tools, with accuracy and reliability also considered.

Table 2. Mean (Av., kg DM ha^{-1}) grass covers estimated weekly (Wk) across all 6 grazing paddocks in 2019, and the range (kg DM ha^{-1}) of estimations obtained for each method of measurement.[1]

Wk	C&W		RPM		GH		TPM		MiGrass	
	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range
1	1,904	501	2,149	595	2,206	239	1,925	132	1,956	196
2	2,651	971	2,617	897	2,453	749	2,236	236	2,773	303
3	1,972	633	1,867	413	1,879	467	1,772	162	1,981	229
4	1,951	288	2,095	541	2,078	463	1,977	159	2,300	308
5	2,322	369	2,187	587	2,281	756	2,066	217	-	-
6	1,573	707	1,876	541	1,915	400	1,786	154	2,211	254

[1] DM = dry matter; C&W = quadrat cut and weigh; GH = GrassHopper rising plate meter; TPM = trailed pasture meter; MiGrass = satellite-based estimation.

Conclusions

Both RPMs produced results which correlated well to total plot DM yields (R^2=0.79), and between each RPM at the paddock scale (R^2=0.74). Future trials of these technologies on large grazing platforms will enable clear quantification of time-saving potential. The equation identified for converting TPM average SH measures to kg DM ha^{-1} for NI recorded a marginally poorer correlation to total plot yields than the standard RPM, but showed improved consistency between paddocks, and further work may optimise this equation for NI pastures. With UAV NDVI imagery further work is needed calibrate NDVI to herbage mass.

Acknowledgements

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References

Dale A. (2010) A review of herbage mass estimation techniques appropriate for Northern Ireland, and suggested developments to improve adoption and accuracy of grassland management assessments. Belfast.

Flynn, E.S. (2006) *Using NDVI as a pasture management tool*, 412 pp.

Gebremedhin A., Badenhorst P., Wang J., Giri K., Spangenberg G. and Smith K. (2019) Development and validation of a model to combine NDVI and plant height for high-throughput phenotyping of herbage yield in a perennial ryegrass breeding program. *Remote Sensing* 11.

McConnell D.A., Huson K.M., Gordon A. and Lively F.O. (2020) Identifying barriers to improving grass utilisation on dairy farms. *Grassland Science in Europe* 25, ###-###.

Murphy D.J., O'Brien B. and Murphy M.D. (2018) Development of a labour utilisation decision support tool to efficiently measure grass herbage mass using a rising plate meter. *ASABE Annual International Meeting*

How cows compete with human nutrition – assessing feed-food and land-use competition of Swiss dairy production

Ineichen S.[1], Zumwald J.[2], Nemecek T.[2] and Reidy B.[1]

[1]*Bern University of Applied Sciences, School of Agricultural, Forest and Food Sciences HAFL, 3052 Zollikofen, Switzerland;* [2]*Agroscope, Agroecology and Environment, Life Cycle Assessment Research Group, 8046 Zurich, Switzerland*

Abstract

Ruminants are able to convert feed sources not directly usable by humans into valuable human-edible food. If, however, ruminants are fed with feedstuff that could have been consumed directly as food by humans (i.e. cereals), or which were produced on land that could be used to grow arable crops for direct human consumption, competition arises between feed production for ruminants and food production for humans. We developed and applied two different methods to assess feed-food and land-use competition on Swiss dairy farms. Depending on the amount and type of concentrate used, limited feed-food competition was found. All farms showed a larger edible output (in terms of milk and meat) than input (in terms of feedstuffs). Contrastingly, strong competition for land-use was found, as most farms (depending on the proportion of arable land) could have produced more edible energy and protein, if the land had been used instead for direct human food production.

Keywords: feed-food competition, land-use competition, by-products, dairy production

Introduction

Ruminants are able to convert fibre-rich feed sources into valuable human-edible food. This unique ability of ruminants may be relevant for ensuring future global food demands, as decreasing arable land area meets an ever-growing world population. Having scarce resources such as land and therefore feedstuffs in mind, ruminants need to convert feed into food (as milk and meat) efficiently. A higher feed conversion efficiency can be achieved by feeding more concentrated feedstuffs (meaning higher density of energy or protein content per kg of dry matter). However, many of these concentrate feeds would also be suitable for direct human consumption (e.g. wheat or soybeans). If the potential human edibility of feeds is being considered, ruminants may directly compete with human nutrition (CAST, 1999) or indirectly, when feed is being produced on land that would be suitable for crop production as well (Van Zanten *et al.*, 2016). In this paper, we combined two existing methods to quantify feed-food and land-use competition on Swiss dairy farms.

Materials and methods

Twenty-five dairy farms, located on the Swiss Central Plateau as well as in mountainous regions, were chosen. They depict a variety of traits (climate zone, milk yield, organic/conventional, feeding strategy, arability of the land used) that may be suitable to explain differences in the degree of competition between feed/food and land use respectively. The farms managed 33 (±16) ha land and held 46 (±26) cows. All farm-specific data (feed consumed by cows, yields per hectare, purchased feeds, milk production, replacement rates, etc.) were collected on farm by individual interviews during 2018. The system boundaries were set at the farm gate, meaning all animals, feeds and land area necessary to produce the milk and sustain the herd were included. Land area or animals kept on farm that were neither producing milk nor rearing were excluded. Surplus calves were counted as meat output (75 kg live weight) as well as culled cows (individual farms mean cows live weight). Boneless meat yield of calves was set at 410 g kg^{-1} of live weight, for culled cows at 310 g kg^{-1} of live weight. Protein content was assumed to be at 32, 170 and 190 g kg^{-1} for milk, veal and beef respectively. All calculations were conducted for protein (with quality correction by the digestible indispensable amino acid score (DIAAS), FAO 2013) as well as energy. In this paper we focus on protein, as animal products usually contribute more to protein than energy demands of humans. Most findings were equivalent for both nutritional aspects but differed only numerically.

Feed-food competition is being expressed as the ratio of all human edible feeds (numerator) divided by all edible products (milk and meat, denominator) as proposed by Wilkinson (2011). Values below one indicate that there was more human edible food produced than consumed by the dairy system and vice versa. Edibility of feedstuffs was assumed to be 'low', meaning a poor recovery rate of human-edible energy and protein from feeds according to Ertl *et al.* (2015).

Land-use competition was assessed according to Van Zanten *et al.* (2016) by quantifying the land area occupied by dairy production and assessing its suitability for the production of arable crops for direct human consumption. In opposition to Van Zanten *et al.* (2016) four different crop rotations were defined to maximize protein (or energy) output for 'favourable' or 'less favourable' climatic conditions. Poor soil quality leads to a reduction in the potential yield per ha.

Results and discussion

The annual milk yield was 7616 (±1,636) kg energy corrected milk (ECM) per cow. The mean annual diet of cows consisted of 78% (±16%) grass (pasture, fresh cut, silage or hay), 10% (±10%) corn (silage or dried), 10% (±7%) concentrates and 2% (±2%) of other feeds (such as sugar beet pulp or straw). Concentrate feed intensity was at 123 (±71) g kg^{-1} ECM, where concentrate was defined as all feeds with a crude fibre content below 120 g kg^{-1} dry matter.

Feed-food competition was below one for all farms, meaning that more edible protein and energy was produced than was fed to the cows (Figure 1). Competition was low for farms feeding exclusively grass (fresh cut, pasture, hay or silage). Feed-food competition may be reduced on all farms by replacing food crops by co-products as well as increasing overall productivity. Except for farms in the mountain area, land-use competition was generally greater than one, an indication that more food could have been produced with arable crops for direct human consumption. The degree of land-use competition depends on the share of arable land as well as the type and origin of bought feeds. The land-use competition may be decreased by using the less-favourable land area for dairy production as well as by improving the feed conversion ratio.

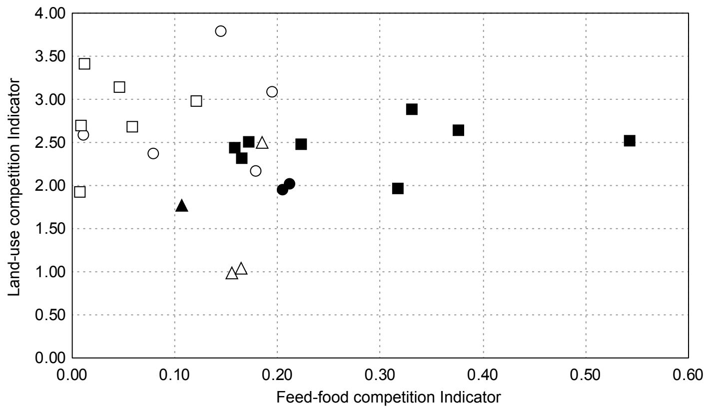

Figure 1. Feed-food and land-use competition for 25 Swiss dairy farms located in the Valley- (■,□), Hill- (●,○) and Mountain-Zone (▲,△), open symbols refer to milk yield of <8,000 kg energy corrected milk (ECM) cow^{-1}, filled symbols to a milk yield of >8,000 kg ECM cow^{-1}.

Conclusions

Feed-food and land-use competition indicators are not correlated and deliver a complementary and holistic view on the feed-food competition. Improving overall productivity decreases feed-food as well as land-use competition if animal's diets remain constant.

References

CAST (1999) *Animal agriculture and global food supply*. Task Force Report No. 135, Council for Agricultural Science and Technology, USA, 92 pp.

Ertl P., Klocker H., Hörtenhuber S., Knaus W. and Zollitsch W. (2015) The net contribution of dairy production to human food supply: the case of Austrian dairy farms. *Agricultural Systems* 137, 119-125.

FAO (2013) Dietary protein quality evaluation in human nutrition. In: *FAO Food and Nutrition Paper*, Rome; Italy,79 pp.

Van Zanten H.H.E., Mollenhorst H., Klootwijk C.W. Van Middelaar C.E. and De Boer I.J.M. (2016) Global food supply: Land use efficiency of livestock systems. *International Journal of Life Cycle Assessment* 21(5), 747-758.

Wilkinson J. (2011) Re-defining efficiency of feed use by livestock. *Animal* 5(7), 1014-1022.

Biogas from grass – sustainable or not?

Rasi S., Timonen K., Joensuu K., Regina K., Virkajärvi P., Pulkkinen H., Tampio E., Pyykkönen P. and Luostarinen S.
Natural Resources Institute Finland (Luke), Finland

Abstract

The aim of the new renewable energy directive (RED II) is help the EU to meet its emissions reduction commitments under the Paris Agreement. The new directive is now considering also solid and gaseous biofuels, in addition to liquid fuels. The aim of this work was to calculate greenhouse gas (GHG) emission values for grass silage in Finland and use the values to calculate GHG emissions of biogas plant. In this example, biogas is upgraded to biomethane and used as vehicle fuel. Calculations were made to biogas plant operating with grass only and, for comparison, with a grass-manure mix. When using only grass silage as substrate for biogas production and the grass is cultivated for energy purpose, the emission reduction targets are not easy to achieve because of high emissions from the cultivation phase. This is the case especially if the cultivation is done on organic land. The reduction targets can be achieved if grass is cultivated because of crop rotation (e.g. for fertilising or soils structure maintaining purposes) when the emissions from cultivation are not included in the calculations. Another option to get to the targets is to use grass in co-digestion with manure.

Keywords: biogas, grass silage, RED II

Introduction

The new renewable energy directive (RED II; Directive (EU) 2018/2001 of the European Parliament and of the Council of 11 December 2018 on the promotion of the use of energy from renewable sources) for years 2021-2030 was released December 2018. The directive sets the overall EU target for Renewable Energy Sources consumption by 2030 to 32%. The final agreement includes a transport sub-target that Member States must require fuel suppliers to supply a minimum of 14% of the energy consumed in road and rail transport by 2030 as renewable energy. New targets are expanded also to consider solid and gaseous biofuels, in addition to liquid fuels. The RED II defines a series of sustainability and GHG emission criteria that fuels used in transport must comply with to be counted towards the overall 14% target and to be eligible for financial support by public authorities. This work concentrates to greenhouse gas (GHG) emissions from biogas used as vehicle fuel. According to directive, biogas used as vehicle fuel is renewable (and can be counted in overall target) if the emission reduction compared to fossil fuels is over 65% (from biogas plants in operation from 2021 or later), the fossil fuel comparator being 94 g CO_{2eq} MJ^{-1}. In the directive, there are typical and default GHG emissions for several different raw materials, e.g. biogas from manure or maize silage. The plants that need to verify the emissions can use these values from the directive, or calculate the emissions by themselves using the methods provided in the directive. The aim of this work was to calculate GHG emission values for grass and clover silage from mineral and organic soils in Finland and use the values to calculate GHG emissions of biogas plant when gas is used as vehicle fuel. The example cases were calculated for biogas plant operating with grass only and with grass-manure mix. Also using grass only from third harvest or from green manuring was taken into account.

Materials and methods

The life cycle assessment (LCA) was used to calculate the climate impact of grass cultivation as well as emissions from the production of biomethane. The calculation was done according to REDII and IPCC

(2006) rules. In the calculation, carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) were taken into account.

Cultivation emissions were calculated from inputs (fertilisers, liming, preservatives, and seeds for sowing, fuels and plant protection products) and fuels from machines. In addition, the direct N_2O emission from the soil due to fertilising and decomposition of crop residues and organic matter in organic soils, and indirect N_2O emissions from nitrogen leaching and other nitrogen air emissions were calculated, and also CO_2 emissions from lime application. In the case of grass from buffer zones, only the emissions related to harvesting (production and use of fuel in field machinery, preservative) were taken into account.

The emissions from biogas plant and the production of biomethane included the transport of grass from fields to plant as well as the energy input needed in biogas plant and upgrading facility. All the produced biogas was assumed to be used as vehicle fuel so the energy needed for biogas production and upgrading is bought outside (average Finnish electricity and heat from wood chips). It was assumed that biomethane is sold next to the plant so there were no emissions from biomethane transportation. Four cases of different feed compositions were compared: (1) grass silage alone (from mineral and organic soil), 62,000 Mg a^{-1}; (2) clover silage, 74,000 Mg a^{-1}; (3) mix of cow liquid manure, 27,000 Mg a^{-1}, cow dry manure 27,000 Mg a^{-1}, pig manure 54,000 Mg a^{-1}, and grass silage (from mineral soil) 27,000 Mg a^{-1}; (4) grass silage from green manuring 48,000 Mg a^{-1}. Sensitivity analysis was done for examples 1 and 2, where 20% of grass silages were replaced with (cow liquid) manure. Also using grass only from third harvest or from green manuring was taken into account (when only the emissions from harvesting are taken into account).

The emission reduction (stated in directive to compare the emissions from fossil fuels) was calculated by Equation 1:

$$\text{Emission reduction} = \frac{\text{fossil fuel comparator} - \text{biomethane total emissions}}{\text{fossil fuel comparator}} \qquad (1)$$

Results and discussion

The emissions from grass cultivation are presented in Table 1.

The total emissions from biomethane production and emission reductions compared to fossil fuels (stated in directive) are in Table 2. When grass or clover-grass silage is used in mono-digestions, the emissions from cultivation phase are too high when total emissions are compared with the fossil fuel comparator. In the case where grass is cultivated in organic soils for energy purpose alone, the emissions can be even higher than with fossil fuels. If grass is only additional raw material with manure or grass is cultivated for fertilisation purposes (as green manuring), the emissions reductions are high enough. Also, manure as additional material can be used in co-digestion with grass but the amount of manure has to be over 20%

Table 1. The emissions (g CO_{2ekv} MJ^{-1}) from grass cultivation for different yields.

	Yield level (kg total solids ha^{-1})		
	7,530	**5,550**	**3,040**
Grass silage (mineral soil)	28.2	32.0	37.3
Grass silage (organic soil)	75.3	96.0	154.0
Clover silage (mineral soil)	20.1	22.3	27.0
Third harvest	-	-	6.1
Grass from green manuring	-	-	6.3

Table 2. The total biogas emissions and emission reductions compared to fossil fuel.

Plant example	Total emission $g\ CO_{2ekv}\ MJ^{-1}$	Emissions after manure bonus $g\ CO_{2ekv}\ MJ^{-1}$	Emission reduction %
Grass silage (mineral soil)	47.2		50
Grass silage (organic soil)	105.9		-13
Clover silage (mineral soil)	36.3		61
Manure + grass silage	29.4	3.8	96
Grass silage from green manuring	19.6		79
Grass silage + 20% manure	46.0	33.7	64
Clover silage + 20% manure	37.4	23.5	75

with grass silage cultivated in mineral soils to achieve the targets. For clover, smaller amount of manure can be used to achieve the targets set in REDII.

Conclusions

When using only grass silage as the substrate for biogas production, and the grass is cultivated for energy purposes, the emission reduction targets set in REDII are not easy to achieve. The reduction targets can be achieved if grass is cultivated because it is part of a crop rotation (e.g. for fertilisation or for maintaining soil structure) when the emissions from cultivation are not included in calculations, or when used from the third harvest of grass-only. Another option to achieve the targets is to use grass in co-digestion with manure.

Acknowledgements

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References

IPCC (2006) *Guidelines for national greenhouse gas inventories*. Volume 4: Agriculture, Forestry and Other Land Use. Intergovernmental panel on climate change.

Grass silage for biorefinery – silage juice as a dietary component for growing pigs

Keto L.[1], Perttilä S.[1], Särkijärvi S.[1], Kamppari K.[2], Immonen I.[3], Kytölä K.[3], Ertbjerg P.[2] and Rinne M.[1]
[1]Natural Resources Institute Finland, 31600 Jokioinen, Finland; [2]Department of Food and Nutrition, University of Helsinki, 00014 Helsinki, Finland; [3]A-Rehu Ltd., P.O. Box 908, 60061 Atria, Finland

Abstract

Using grass-based feeds in intensive pork production is a revolutionary idea. By biorefining, innovative feed products can be produced. These are also suitable for pigs, as water-soluble nutrients including protein, carbohydrates, fermentation products and minerals are released from the fibre matrix. If the grass is ensiled, it can be used in the biorefinery all year around. In the current experiment, silage juice was produced using a screw press. The experiment used 208 pigs, both barrows and gilts, which were kept in pens (sexes separately). The pen served as the experimental unit. The juice was fed at a maximum of 3 l per pig per day during the last 7 weeks of the growing period, and the growth performance and meat quality traits were compared with a conventional liquid diet without silage juice addition. Growth rate, carcass quality and meat quality traits were not affected by dietary treatments, showing that juice extracted from grass silage can be included successfully in the diets of growing pigs. However, fat oxidation during cold storage in high oxygen modified atmosphere was slightly higher in meat originating from silage juice fed pigs.

Keywords: liquid-solid separation, pork, press juice, grass silage, liquid feed

Introduction

In a green biorefinery approach, liquid feed can be produced from grass silage by simple liquid-solid separation. The silage juice contains soluble components from the grass silage, the main components being minerals (ash), soluble crude protein, fermentation end-products (lactic acid, volatile fatty acids, ethanol) and possibly water-soluble carbohydrates depending on the concentration in the original silage. The pH is typically in the range 4.0-4.5. Silage juice can be used for pig feeding, where it provides nutrients, stabilises pH of the liquid feed, and may help with maintaining intestinal health of the pigs. Inclusion of grass-derived feeds in pig diets provides opportunities to include grass into crop rotations on pig farms (Tampio *et al.*, 2019) and brings a new source of feed, which is not edible for humans, into the diet of pigs.

Mechanically separated silage liquid has not previously been tested as a fattening-pig feed. However, spontaneously separated silage effluent and mechanically separated grass juice have been seen as promising ingredients for fattening-pigs feeds (Patterson and Walker, 1979a,b; Patterson, 1990, Adler *et al.*, 2018). The objective of the present study was to investigate the suitability of mechanically separated grass silage juice as a feed for fattening pigs.

Materials and methods

The grass silage used was made from a timothy-meadow fescue sward, which was precision chopped and ensiled into a clamp using a formic acid-based additive at Jokioinen, Finland. The silage was fractionated into liquid and solid fractions at farm scale using a twin-screw press (Haarslev Industries A/S, Søndersø, Denmark). For details about juice production and the use of the solid fraction as a feed for dairy cows, see Savonen *et al.* (2020).

Altogether 208 pigs, both barrows and gilts, were reared on a commercial farm, in pens of 10.8 m^2, 8 pigs per pen, sexes separately. Feed consumption of the pigs was recorded on a weekly basis per feeding vent (two pens per vent) in order to calculate the average feed consumption and feed conversion ratio (FCR) in the two treatments. In both treatments the pigs were first fed standard starter liquid feed (11.1 MJ net energy (NE), 196 g kg^{-1} dry matter (DM) crude protein (CP)) for 5 weeks from 27 to 63 kg live weight. After that, the pigs were fed with control finisher liquid feed (11.4 MJ NE, 187 g CP kg^{-1} DM) and max. 3 litres per pig per day silage juice containing liquid feed (11.1 MJ NE, 197 g kg^{-1} DM CP). Pigs were slaughtered at an average live weight of 124 kg and carcass quality was measured with Autofom. Meat pH was analysed from six and colour (L*, a*, b*) and oxidation indicator TBARS on 12 LD (*M. longissimus dorsi*) muscles in each treatment. For TBARS analyses the meat was packaged in either vacuum or modified atmosphere (MA) (80% O_2, 20% CO_2) and stored for 2, 4, 7 and 9 days. Due to errors in pig live weight recordings, the gilts of control group produced only one observation and these results will serve as reference results for average daily gain (ADG) and FCR. The ADG and FCR results from control and juice feeding barrows and juice feeding gilts were compared using one-way ANOVA. TBARS were analysed with generalised linear model and feed, sex, package type and storage time were set as fixed factors.

Results and discussion

The ADG was over 1.150 kg d^{-1} for all groups, including the reference gilts and no difference was observed between the groups (P>0.05). The NE consumption of the pigs was lowest in silage juice fed barrows and highest in control fed barrows (P<0.05). The FCR was the best in silage juice fed gilts (P<0.05). Carcass weight was similar in all groups (P>0.05) but as expected, gilts were leaner than barrows (P<0.05). Gilts in the silage juice group had the leanest carcasses. Silage juice DM content varied during the 7-week trial period. As the DM content of silage juice was not updated to the automated feeding system, it may have led to unplanned reduction of DM and thus, energy and protein content of silage juice containing feed. At farm scale, variation of DM content of silage juice would be very likely so it should be tolerated if silage juice becomes a common ingredient in pig feeds.

Meat pH and colour were similar in all groups (P>0.05), but meat oxidation (mg malondialdehyde kg^{-1} meat) increased in MA packaged meat originating from silage juice fed pigs (P<0.05). This is worth considering before large scale production of silage juice fed pigs.

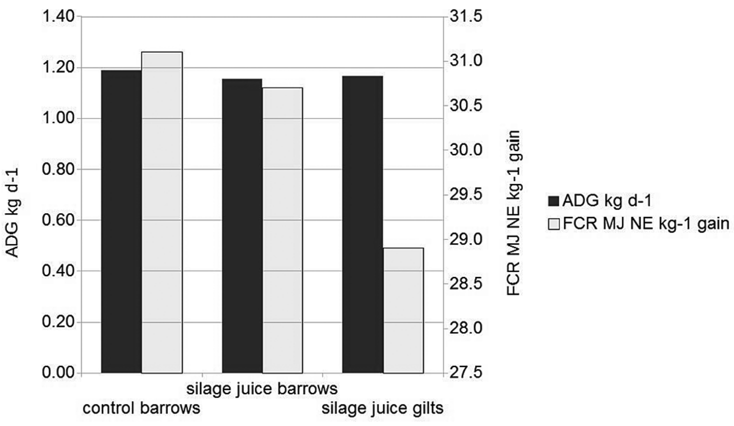

Figure 1. Average daily gain (ADG) and feed conversion ratio (FCR) of pigs fed either with control or silage juice containing feed.

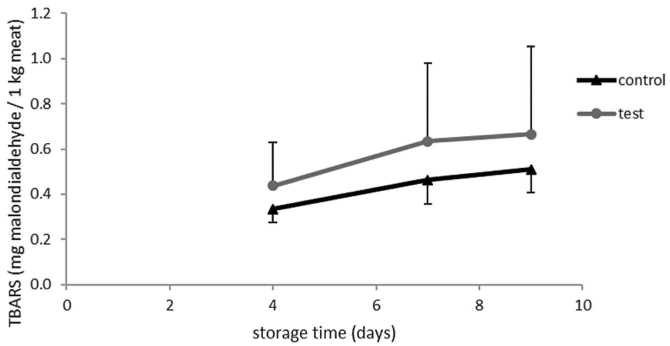

Figure 2. Thiobarbituric acid-reactive substances (TBARS) of modified atmosphere packaged (80% O_2, 20% CO_2) pork *M. longissimus thoracis* steaks (n=71) at different storage times (4, 7, 9 days) at +5 °C when control diet (conventional) and test diet (with silage juice) were fed. Error bars represent standard deviations.

Conclusions

Mechanically extracted juice from grass silage can be included successfully in the diets of growing pigs. However, due to variability of DM content of silage and silage juice included in the liquid feed, the energy and CP intake of the pigs may vary if the DM content of the silage juice is not regularly updated in the automated feeding system of the pigs. Meat pH and colour from silage juice fed pigs was similar to meat from conventionally fed pigs. However, the modified atmosphere packaged meat from silage juice fed pigs was slightly more prone to lipid oxidation during refrigerated storage than meat from conventionally fed pigs.

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References

Adler S.A., Johansen A., Ingvolstad A.K., Eltun R. and Gjerlaug-Enger E.J. (2018) Forages – a local protein source for growing pigs. In: Udén P., Eriksson T., Spörndly R., Rustas B.-O. and Liljeholm M. (eds.) *Proceedings of the 9th Nordic Feed Science Conference*, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden, pp. 61-65.

Patterson D.C. (1990) Feeding value for finishing pigs of silage effluent with a high initial pH. *Journal of Agricultural Science* 115, 129-133.

Patterson D.C. and Walker N. (1979a) The use of effluent from grass silage in the diet of finishing pigs I. Variation in composition of effluents. *Animal Feed Science and Technology* 4, 263-274.

Patterson D.C. and Walker N. (1979b) The use of effluent from grass silage in the diet of finishing pigs. II. Assessment of nutritive value of fresh and stored effluent. *Animal Feed Science and Technology* 4, 275-293.

Savonen O., Franco M., Stefanski T., Mäntysaari P., Kuoppala K. and Rinne M. (2020) Grass silage pulp as a dietary component for high yielding dairy cows. *Animal*, in press. https://doi.org/10.1017/S1751731119002970

Tampio E., Winquist E., Luostarinen S. and Rinne M. (2019) A farm-scale grass biorefinery concept for a combined pig feed and biogas production. *Water Science and Technology* wst2019356. https://doi.org/10.2166/wst.2019.356

Fractionation of forage legumes using a screw press

Adler S.A.[1], Micke B.[2], Steinshamn H.[1] and Parsons D.[2]
[1]NIBIO, Norwegian Institute of Bioeconomy Research, Department of Grassland and Livestock, Gunnars veg 6, 6630 Tingvoll, Norway; [2]SLU, Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, SLU, NJV, 901 83 Umeå, Sweden

Abstract

Technical advances in fractionation and biorefining of grassland crops may allow farmers to produce feed for monogastrics and ruminants. The objective of the present work was to quantify yields of juice and pulp from different forage legumes. We established field trials with plots of monocultures of lucerne, red clover and alsike clover in Röbäcksdalen, Sweden and Tingvoll, Norway in 2018. We harvested the crops three times in Röbäcksdalen and four times in Tingvoll in 2019 and used a twin-gear screw press to separate juice and pulp. Red clover had the highest total yields, with higher yields in Sweden than Norway. In Norway, the average dry matter (DM) content in juice was 10.7% and in pulp 33.0%. Juice accounted for 43.8% of DM on average. Lucerne had lower juice DM proportion than the clover species. No effects of harvest number on juice DM proportion were found. These preliminary results indicate that clover species yield more juice and less pulp, compared with lucerne. Quality analyses will be carried out to estimate feed value of both fractions.

Keywords: biorefining, clover, lucerne, juice, pulp

Introduction

Protein supplementation is a challenge in organic livestock production. Fractionation of forage legumes, through biorefining techniques, into protein- and fibre-rich feeds for monogastrics and ruminants, respectively, can increase farm self-sufficiency in feed.

Biorefined green forages have demonstrated promising results as a source suitable for monogastrics due to high yields of forages, high protein content and a balanced amino acid composition in the biorefined material (Houseman and Jones, 1978). Damborg *et al.* (2019) reported increased milk yield from cows fed pulp silage, compared to whole plant silage from clover grass, and concluded that protein extraction increased the feed value of the fibre part. We need more knowledge about differences between species and varieties, and primary growth and regrowth on yields and quality aspects of juice and pulp. The objective of the present work was to quantify yields of whole plant, juice and pulp from different forage legumes.

Materials and methods

We established two field trials with forage legumes in spring 2018, one in Röbäcksdalen, Sweden, and one in Tingvoll, Norway. Each field had four blocks. The forage legumes were lucerne (*Medicago sativa*, cv. Ludvig and Karlu), red clover (*Trifolium pratense*, cv. Gandalf and Lars) and alsike clover (*Trifolium hybridum*, cv. Frida) and were sown as monocultures. Lucerne seeds were inoculated with rhizobium bacteria. Each plot had an area of 12 m^2. Harvesting time for the first cut was defined as early flowering of red clover and the consecutive cuts (Röbäcksdalen 2, Tingvoll 3) were distributed evenly across the rest of the season according to growing day degrees (Table 1). Before each cut, we registered stage of development, canopy height and plant height in each plot. Two squares, 0.1 m^2 each, were harvested for determination of botanical composition. We mowed the plots to a stubble height of 10 cm, weighed the yields and stored the plant material chilled until further processing. We used a press screw (Angel 7500, Angel CO., LTD., Korea) to produce plant juice and pulp. Yields of juice and pulp were weighed and DM content was determined in whole plant and fractions after oven drying at 105 °C until constant weight.

Table 1. Harvesting dates and accumulated growing day degrees (GDD) in field trials with forage legumes at Röbäcksdalen, Sweden, and Tingvoll, Norway in 2019.

Location	Röbäcksdalen		Tingvoll	
	Date	GDD[1]	Date	GDD
First cut	June 19	496	June 12-14	577
Second cut	July 26	1,012	July 22-23	1,108
Third cut	September 6	1,576	August 19 and 21	1,591
Fourth cut	-	-	September 26	2,041

[1] Accumulated daily mean temperatures (base 0 °C), starting the fifth day in a row with average daily temperature reaching 5 °C.

Calculations

The experiment was analysed statistically using the PROC MIXED procedure in SAS 9.4 (SAS Institute, 2013). For analysis of annual yields, block and variety were treated as fixed effects. For analysis of the pulp:juice ratio we also included the fixed effects of cut and interaction of variety.

Results and discussion

The yield proportions (g g^{-1}) of the sown forage legumes in the mean DM yield were on average across cuts 0.90 for red clover, 0.76 for alsike clover and 0.60 for lucerne. The annual DM yields for the red clover varieties averaged 12.9 Mg ha^{-1} in Sweden and 8.2 Mg ha^{-1} in Norway (Figure 1). Alsike clover yielded 8.2 Mg ha^{-1} and 5.9 Mg ha^{-1}, respectively. Lucerne cv. Ludvig yields were lower, 5.8 Mg ha^{-1} in Sweden and 1.6 Mg ha^{-1} in Norway. Lucerne cv. Karlu established poorly in spring 2019 and consequently was not harvested at first cut. First-cut clovers accounted for 57% of the total yield on average for both fields. In lucerne, the second cut was larger than the first. Several factors may have contributed to low yields of lucerne. In both fields the number of plants was reduced in the first winter, the number of active rhizobium nodules was low, and at Tingvoll many plants had stem rot. The fourth harvest in Tingvoll contributed an insignificant amount to the annual yield. Pulp and juice DM yields were only calculated for Tingvoll. Red clover had higher pulp DM yields than alsike clover and lucerne, and lucerne had lower yields than Frida (Figure 2). Juice yields followed the same pattern, but red clover cv. Gandalf did not differ from alsike clover. The pulp:juice ratio was affected by plant species (P=0.006), but there was only a tendency for the effect of harvest number (P=0.09). The pulp:juice ratio for Gandalf (1.32), Lars (1.14)

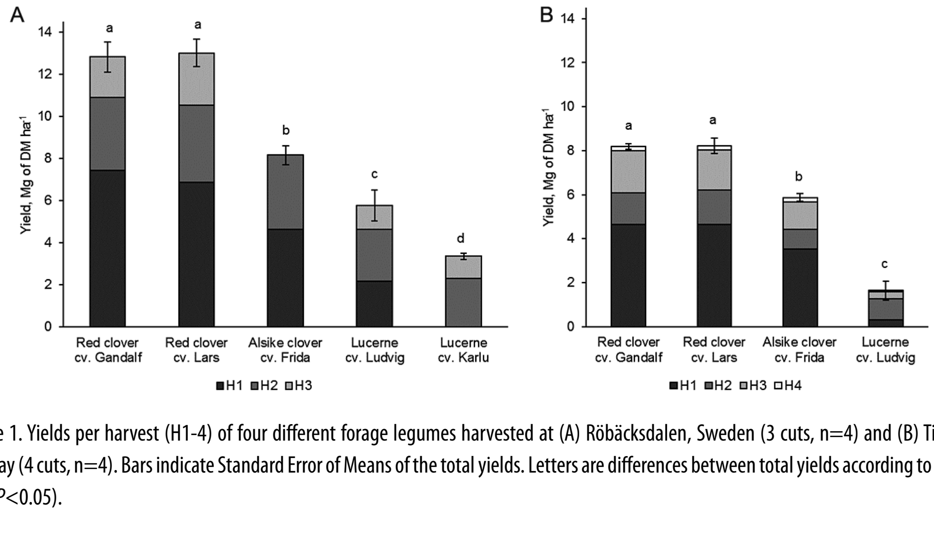

Figure 1. Yields per harvest (H1-4) of four different forage legumes harvested at (A) Röbäcksdalen, Sweden (3 cuts, n=4) and (B) Tingvoll, Norway (4 cuts, n=4). Bars indicate Standard Error of Means of the total yields. Letters are differences between total yields according to Tukey's test (P<0.05).

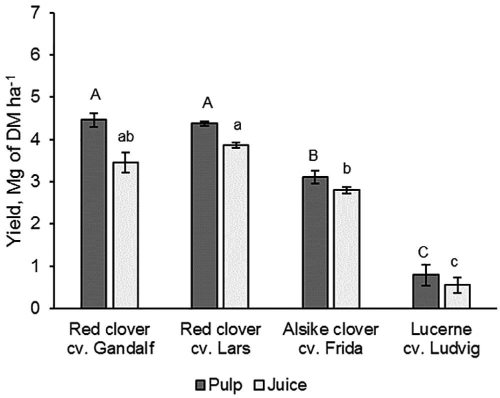

Figure 2. Annual yields of pulp and juice of four different forage legumes harvested at Tingvoll, Norway and fractionated with a screw press, (4 cuts, n=4). Bars indicate Standard Error of Means. Letters are differences according to Tukey's test (P<0.05). Capital letters to compare pulp yields, lower case for juice yields.

and Frida (1.16) did not differ. The ratio could not be calculated for Ludvig due to low yields at fourth cut, but if the fourth cut is excluded Ludvig has a higher (P<0.001) pulp:juice ratio than the other species. Quality analysis will be carried out to estimate the feed value of both pulp and juice.

Conclusions

Red clover had higher yields than alsike clover. Compared with lucerne, both clover species yielded more total plant mass and juice and less pulp.

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References

Damborg V.K., Jensen S.K., Johansen M., Ambye-Jensen M. and Weisbjerg M.R. (2019) Ensiled pulp from biorefining increased milk production in dairy cows compared with grass-clover silage. *Journal of Dairy Science* 102(10), 8883-8897. https://doi.org/10.3168/jds.2018-16096

Houseman R.A. and Jones A.S. (1978) Green crops and animal production. *Veterinary Record*. 102(15), 336-339.

SAS Institute Inc. (2013) SAS® 9.4 [Computer software]. Cary, NC, USA: SAS Institute Inc.

Effect of screw pressing and days of regrowth on grass silage characteristics and quality

Hansen N.P.[1], Bitsch J.[1], Jensen S.K.[1], Weisbjerg M.R.[1], Ambye-Jensen M.[2] and Johansen M.[1]
[1]*Department of Animal Science, Aarhus University – Foulum, Denmark.*; [2]*Department of Engineering, Aarhus University, Foulum, Denmark*

Abstract

Grass harvested at early and late maturity stages was processed using a twin-screw press to produce a fibrous pulp fraction of which half was ensiled and the other half was pressed a second time before ensiling. For comparison, grass from the same field and maturity stage was mown and wilted before chopping and ensiling. The effects of single and double screw pressing compared with chopped grass on density of the fresh material, fermentation weight loss, and fermentation pattern were investigated in lab-scale silo bags. All combinations of processing and time for regrowth resulted in well-preserved silages with variation in dry matter content, fermentation weight loss, and fermentation products.

Keywords: silage, grass, pulp, density, fermentation loss

Introduction

Biorefinery of fresh grass-clover by screw pressing for production of green protein gives a fibrous pulp as a side stream. The ensiled pulp has potential to increase energy corrected milk yield in dairy cows (Damborg *et al.,* 2019). However, as an effect of screw pressing, the concentration of soluble carbohydrates in the pulp fraction is decreased and may affect the ability of the pulp to ensile sufficiently. Processing the pulp in the screw press a second time is expected to increase the amount of extracted green protein, but also reduces concentration of soluble carbohydrates in the pulp even more. When plant material passes through the screw press, the physical structure of stems and leaves is broken and cell walls are ruptured, and therefore, a better compaction is expected. The objectives of the study were to investigate the effects of processing and days of regrowth of grass on the ability to ensile.

Materials and methods

A grass field (perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), with 1.4% clover on a dry matter (DM) basis) was harvested late summer 2019 at Aarhus University, Foulum Denmark at early (E) and late (L) maturity stage corresponding to 35 and 44 days of regrowth, respectively. The grass was either mowed and wilted before chopping and ensiling (GS), or harvested and processed immediately using a twin-screw press (5 Mg h^{-1}) yielding a pulp fraction of which half was ensiled (1×P) and the other half was pressed a second time before ensiling (2×P). Each processing was applied within each maturity stage yielding six treatments: GSE, 1×PE, 2×PE, GSL, 1×PL, and 2×PL. Within each treatment, four samples (buckets) where taken, and from each sample, two replicates were made giving eight replicates in total. Each replicate was ensiled in a plastic bag containing (mean ± standard deviation) 580±10.0 g fresh matter. Immediately after sealing and weighing the vacuum bags, silage density was determined as weight of fresh material over the amount of water displaced from each bag (n=4). Fermentation weight loss (FWL) was measured by weighing all bags 0 (n=8), 1 (n=8), 7 (n=8), 14 (n=8), 30 (n=6), and 60 (n=4) days after ensiling. Two replicates per treatment were frozen after weighing on day 14, 30, and 60 after ensiling in order to stop the ensiling process, and these bags were used to investigate the fermentation pattern over time. DM was determined by drying in a forced-air oven at 60 °C for 48 hours. Extracts of silage from each bag were used for pH measurement and analysis of NH_3-N, glucose, L-lactate, and volatile fatty acids. NH_3-N is expressed as a proportion of DM, since total N was only analysed in a pooled sample from all four buckets at day 0. Statistical analyses were performed in R (version 3.5.2) and the following model was used to analyse data for FWL:

$$Y_{pmdbs} = \mu + \alpha_p + \beta_m + \tau_d + \alpha_p \times \beta_m + \alpha_p \times \tau_d + \beta_m \times \tau_d + \alpha_p \times \beta_m \times \tau_d + A_b + B_{s(b)} + E_{pmdbs}$$

where Y is the dependent response variable, μ is the overall mean, α is the fixed effect of processing (p = GS, 1×P, 2×P), β is the fixed effect of maturity stage (m = E, L), τ is the fixed effect of day after ensiling (d = 1, 7, 14, 30, 60), A is the random effect of bucket within processing × maturity stage (b = 1 to 4), B is the random effect of silage bag within bucket (s = 1, 2), and E is the random residual error assumed to be independent and normal distributed. When analysing data from extracts, B was removed from the model, and τ only had three levels (d = 14, 30, 60). When analysing density, τ, all interactions including τ, A, and B were excluded from the model. FWL was log-transformed and the back-transformed data are shown in the results, without standard error of the mean.

Results and discussion

Processing affected all parameters except pH (Table 1). As expected, density (DM-basis) was highest in 2×P, probably caused by the intensive breakdown of the plants' physical structure due to the mechanical treatment, as shown by Samarasinghe *et al.* (2019). However, in the current experiment, treatment was partly confounded with DM concentration. Relative to total FWL, all silages had a high rate of FWL, especially in the first seven days, and reached a plateau around 30 days after ensiling. Overall, FWL was lowest for 2×P, which also had the highest content of DM. In all silage samples, pH was below 4.3 indicating that all silages were well preserved. GSL had higher concentration of fermentation products than the other silages, probably due to the high water content causing a large production of lactate and acetate. Acetate was present in relatively high concentrations in all silages except 2×P, where also propionate was low, which might reduce aerobic stability (Wilkinson and Davies, 2013). Butyric acid was only detected in three out of the total of 48 samples at concentrations ranging from 2 to 2.8 g kg^{-1} DM, but showed no pattern according to treatments. 2×P had the lowest concentration of NH_3-N, indicating reduced protein degradation during ensiling, as the CP concentration before ensiling was almost identical among type of processing, in agreement with previous studies (Damborg *et al.*, 2019). All silage samples except one had less than 1 g glucose kg^{-1} DM (data not shown). Compared with E, the L-harvested samples had lower DM contents in silage (P<0.01, data not shown), lower pH, and higher concentration of L-lactate and propionate, resulting in a smaller FWL.

Conclusions

All treatments resulted in well-preserved silages with variation in DM, FWL, and fermentation products.

Acknowledgements

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Table 1. Effect of maturity stage and processing (traditional precision chopping, and one or two processes through a screw press) of grass-clover prior to ensiling on initial density, fermentation weight loss (FWL), and fermentation characteristics.[1]

	Day[2]	Treatment[3]						SEM[4]	*P*-value[5]						
		GSE	1×PE	2×PE	GSL	1×PL	2×PL		P	M	D	P×M	P×D	M×D	P×M×D
DM[6], g kg^{-1}	0	289 (13)	286 (13)	401 (21)	207 (3)	324 (18)	401 (17)								
CP[6], g kg^{-1} DM	0	178	181	188	146	157	156								
Density, kg DM (m^3)$^{-1}$	0	278^{b}	293^{b}	370^{a}	189^{c}	316^{b}	391^{a}	11.2	<0.01	0.12		<0.01			
FWL, g kg^{-1} FM[7]	1	4.86^{a,D}	5.44^{a,C}	3.13^{b,D}	2.57^{b,C}	3.38^{b,C}	3.12^{b,C}		<0.01	0.03	<0.01	0.39	0.02	<0.01	<0.01
	7	9.52^{ab,C}	11.0^{a,B}	6.62^{c,C}	10.3^{ab,B}	9.96^{ab,B}	7.51^{bc,B}								
	14	11.2^{ab,B}	12.7^{a,AB}	8.30^{bc,B}	10.5^{ab,B}	10.7^{ab,AB}	7.68^{c,B}								
	30	12.6^{a,AB}	13.9^{a,A}	9.71^{ab,AB}	11.6^{ab,AB}	11.4^{ab,AB}	8.32^{b,AB}								
	60	13.7^{ab,A}	15.3^{a,A}	10.8^{ab,A}	13.0^{ab,A}	12.3^{ab,A}	9.51^{b,A}								
pH	14	4.21	4.18	4.13	4.08	4.11	4.12	0.05	0.57	0.01	0.33	0.12	0.42	0.49	0.05
	30	4.17	4.14	4.24	4.03	4.04	4.10	0.05							
	60	4.26^{a}	4.12^{ab}	4.18^{ab}	4.00^{b}	4.20^{ab}	4.19^{ab}	0.05							
L-Lactate, g kg^{-1} DM	14	34.7^{b,B}	35.8^{b}	20.2^{c}	50.7^{a,B}	34.4^{b}	22.8^{c}	2.45	<0.01	<0.01	<0.01	<0.01	0.09	0.87	0.93
	30	41.5^{b,AB}	38.7^{b}	21.7^{c}	61.6^{a,A}	38.1^{b}	24.0^{c}	2.45							
	60	45.9^{b,A}	39.9^{b}	24.8^{c}	63.0^{a,A}	40.6^{b}	25.9^{c}	2.45							
Acetate, g kg^{-1} DM	14	34.2^{bc,B}	34.3^{ab}	22.0^{cd}	41.2^{a}	27.9^{bc,B}	18.5^{d,B}	1.66	<0.01	0.12	<0.01	<0.01	0.79	0.47	0.48
	30	36.6^{a,AB}	37.4^{a}	27.8^{b}	42.4^{a}	28.6^{b,AB}	21.8^{b,AB}	1.66							
	60	39.6^{ab,A}	36.9^{ab}	26.9^{cd}	44.0^{a}	33.9^{bc,A}	23.8^{d,A}	1.66							
Propionate[8], g kg^{-1} DM	14	4.47^{bc}	5.00^{ab}	3.37^{c}	6.42^{a}	4.46^{bc,B}	3.37^{c}	0.423	<0.01	0.01	0.01	<0.01	0.74	0.47	0.50
	30	4.77^{bc}	5.32^{b}	3.85^{c}	6.96^{a}	4.54^{bc,B}	3.97^{c}	0.388							
	60	4.86^{bc}	5.24^{bc}	4.03^{c}	6.77^{a}	5.60^{ab,A}	4.18^{c}	0.274							
NH_3-N, g kg^{-1} DM	14	1.07^{ab,B}	1.31^{a,B}	0.662^{c,B}	1.37^{a,B}	0.901^{bc,B}	0.653^{c,B}	0.080	<0.01	0.82	<0.01	<0.01	0.28	0.60	0.37
	30	1.42^{b,A}	1.46^{b,AB}	0.927^{c,AB}	1.89^{a,A}	1.14^{bc,B}	0.966^{c,A}	0.080							
	60	1.69^{a,A}	1.74^{a,A}	1.21^{b,A}	1.85^{a,A}	1.56^{ab,A}	1.24^{b,A}	0.080							

[1] Values within same line with different lowercase superscripts differ between treatments (*P*<0.05); Values within same treatment and item with different uppercase superscripts differ over time (*P*<0.05).

[2] Days after ensiling.

[3] Chopped and ensiled at early harvest (GSE), pulp ensiled from early harvest pressed once (1×PE), pulp ensiled from early harvest pressed twice (2×PE), chopped and ensiled at late harvest (GSL), pulp ensiled from late harvest pressed once (1×PL), pulp ensiled from late harvest pressed twice (2×PL).

[4] Highest standard error of mean for LS-mean within row is given.

[5] P = processing, M = maturity stage, D = day of ensiling, P×M = interaction between P and M, P×D = interaction between P and D, M×D = interaction between M and D, P×M×D = interaction between P, M, and D.

[6] Dry matter (DM) and crude protein (CP) in material prior to ensiling. Standard deviation given in brackets for DM (n=8). For CP, n=1.

[7] FM = fresh matter; Back-transformed LSM from log-transformed data.

[8] No detection in three samples (one in GSE 14 days after ensiling and two in GSL 30 and 60 days after ensiling). Samples were not included in the statistical analysis.

References

Damborg V.K., Jensen S.K., Johansen M., Ambye-Jensen M. and Weisbjerg M.R. (2019) Ensiled pulp from biorefining increased milk production in dairy cows compared with grass-clover silage. *Journal of Dairy Science* 102, 8883-8897.

Samarasinghe M.B., Larsen M., Johansen M., Waldemar P. and Weisbjerg M.R. (2019) Effects of shredding on silage density and fermentation quality. *Grass and Forage Science* 74, 244-253.

Wilkinson J.M. and Davies D.R. (2013) The aerobic stability of silage: key findings and recent developments. *Grass and Forage Science* 68, 1-19.

Estimating grassland quality with reflectance information: how far along are we actually?

Astor T.[1], Wijesingha J.[1], Hensgen F.[2] and Wachendorf M.[1]
[1] *Grassland Science and Renewable Plant Resources, University Kassel, Germany;* [2] *Project Management Jülich, Jülich, Germany*

Abstract

Fodder quality of grassland systems depends on local environmental conditions and management strategies and thus varies greatly among different locations and time spans. While in some cases digestibility or protein content might be the most important measure, in other cases dry matter content might be of special interest for an evaluation of grassland quality. For three decades reflectance information from either biomass samples or grassland canopies has been used for estimating and monitoring grassland quality parameters on large scales. The applied sensor systems are ranging from satellites to lab-based near infrared spectroscopy. However, not all available quality parameters have been successfully predicted – either because they do not affect the reflectance signal, or because they have not yet been tested. In the present literature review 104 publications about reflectance-based estimation of grassland quality parameters were analysed regarding the studied climate zone, quality parameters, and prediction quality. We found dependencies of the applied sensor systems, as well as a strong bias towards specific geographical regions. Overall, the review reveals a knowledge gap in the field of remote sensing-based grassland quality assessment, which needs to be closed if the digitalisation of grassland management is considered to be a success.

Keywords: literature review, grassland quality, remote sensing

Introduction

Grasslands cover about 40% of the world's land surface and store approximately 10% of the global soil organic carbon (SOC) stock. From these, approximately 28 million km^2 (state: 2000) are in agricultural use for feeding animals (Ramankutty *et al.,* 2008). Local environmental conditions and management strategies among many others strongly affect the fodder quality of grassland systems and thus varies greatly among different geographic locations and time spans. Chemical composition like protein content, fibre content (i.e. neutral detergent fibre (NDF), acid detergent fibre (ADF)), digestibility, and metabolic energy content are of high relevance but also other factors like dry matter content and nutrient concentrations might be of interest for an evaluation of grassland quality. Reflectance information from either biomass samples or grassland canopies have been used for estimating and monitoring grassland quality parameters for several decades and ranging from small to large scales. However, not all quality parameters of interest have been successfully predicted – either because they do not affect the reflectance signal, or because they have simply not been tested. To our knowledge, no thorough review of literature about grassland quality estimation using spectral information exists so far. In the present literature review 104 publications about reflectance-based estimation of grassland quality parameters are analysed regarding the spatial location of the conducted studies, the estimated quality parameters, and the applied sensor systems. Specific questions this review will answer are: (1) Is there a geographical bias in the location of the studied grassland systems? Is the effect of measurement scale (i.e. field spectrometer to satellite) well understood for the selected quality parameter?

Materials and methods

For the literature search ISI Web of Science was browsed, using the search terms 'forage quality' AND 'remote sensing' as well as 'grassland forage quality estimation'. The time span of search ranged from 1980 to 2018. Overall, 104 references were found. The list of references was limited to studies working on the following parameters: nitrogen (N), phosphorous (P), potassium (K), NDF, ADF, and crude protein content (CP) as these belong to the most relevant parameters for evaluating fodder quality of grassland. Literature focusing on other quality parameters like chlorophyll concentration, leaf area index, or diversity were excluded from further analysis. The full list of all references can be provided by the author on request.

Results

In total, existing studies were found for 20 countries (Figure 1). Of these, South Africa and the USA were the countries with most of the studies (n=36). Considering the distribution of pastures worldwide (Figure 1) and the potential variation of quality parameters due to, for example, soil variability or management strategy, a strong bias can be seen.

The first study about remote sensing-based estimation of grassland quality parameter was done in 1991 but the majority of the studies were conducted after 2002, which illustrates the relatively young history of this research topic (Figure 2). Looking at the instrumentation used for the studies, most of them applied terrestrial spectral measurements (n=51) followed by satellites (n=20) and airborne missions (n=12). Interestingly, most of the terrestrial studies (n=36) were conducted using the ASD FieldSpec Fr instrument, which could imply further bias towards instrumentation. The relatively new drone-based examination of grassland quality was only used in 2018 in two studies. More than 57% of all studies (n=43) were conducted as field studies, followed by field experiments (Figure 3). Considering the complexity and variability of grassland systems, and the resulting difficulties for conducting greenhouse experiments it seems logical that studies in greenhouses were the least common ones (n=6). N was the quality parameter most frequently examined (n=40) followed by CP, NDF, ADF, P, and K (n=23, 20, 19, 12, and 7, respectively) (Figure 3).

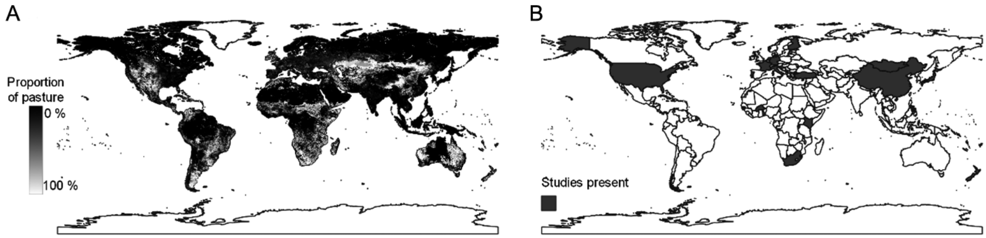

Figure 1. (A) Distribution of pastures in the world adapted from Ramankutty *et al*. 2008 in the year 2000. (B) World map showing the countries in which studies about a remote-sensing based estimation of grassland quality parameter were conducted.

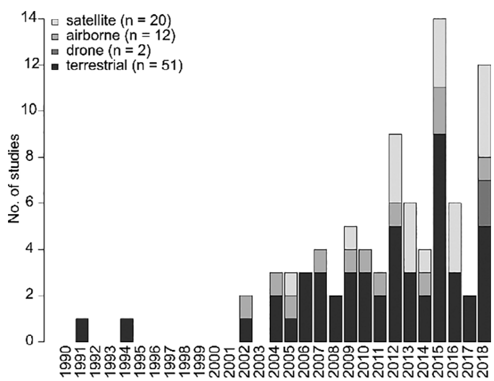

Figure 2. Number of conducted studies within each year between 1990 and 2018. Height of the bars shows the sum of the studies per year. The colours indicate the applied sensor system.

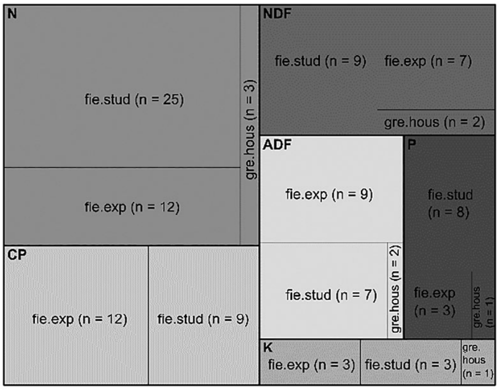

Figure 3. Proportion of study designs (fie.stud = field study, fie.exp = field experiment, gre.hous = greenhouse) used for the estimation of the quality parameter (N = nitrogen, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, P = phosphorus, and K = potassium). The number in the brackets shows the respective number of studies.

Discussion

In this review 104 published studies were analysed regarding their location, study design, applied sensor, and examined quality parameter to identify knowledge gaps. The results reveal a strong geographical bias towards two countries (USA, South Africa). Regions with a large land cover of grasslands like Ireland, the Netherlands, or New Zealand are under-represented or no study at all could be found.

The reason could, in part, be that traditional field methods like clipping and lab analysis are still the usual method for grassland quality evaluation. On the other hand, as the reliability of remote sensing-based models and the technical development (i.e. sensor systems) has only recently matured, more studies will most likely follow in other countries. unmanned aerial vehicle based sensor systems might play an important role in the further application of spectral reflectance for grassland quality estimation as they can provide high spatial and spectral resolution combined with a large spatial and temporal flexibility. The applied sensor systems, so far, are focused on terrestrial measurements, which limit the application of the resulting models on spectral point measurements. The number of airborne and satellite-based studies is very low and thus the effect of measurement scale is not well examined for any grassland quality parameter. Technical developments will enable sensor systems to become smaller and less expensive, which might allow the combined sensor usage on one airborne system (i.e. aeroplane and drone). The sensor fusion approach might increase the prediction accuracy of the grassland quality parameter (Wachendorf *et al.,* 2018). However, the need for technical knowledge for using such systems might have a dampening effect on the quick development of this research area.

Conclusions

Overall, the review reveals a knowledge gap in the field of remote sensing-based grassland quality assessment. In particular, the upscaling from local small-scale studies to a broader scale is needed. Furthermore, the great variability in quality parameter around the world has not been well examined. In the future these gaps need to be closed if the digitalisation and thus the sustainability of grassland management is considered to be a success.

References

Ramankutty N., Evan A.T., Monfreda C. and Foley J.A. (2008) Farming the planet: 1. Geographic distribution of global agricultural lands in the year 2000. *Global Biogeochemical Cycles* 22, GB1003.

Wachendorf M., Fricke T. and Möckel T. (2018) Remote sensing as a tool to assess botanical composition, structure, quantity and quality of temperate grasslands. *Grass and Forage Science* 73, 1-14.

Ground based photogrammetry to assess herbaceous biomass in Sahelian rangelands

Bossoukpe M.[1], Ndiaye O.[2], Diatta S.[1], Diatta O.[1,2], Diouf A.A.[3], Assouma M.H.[4], Faye E.[5] and Taugourdeau S.[4]
[1]UCAD, Dep Biologie végétale – PPZS, Dakar, Senegal; [2]ISRA, CRZ Dahra-PPZS, Dahra Djoloff, Senegal; [3]Centre de suivi écologique, PPZS, Dakar, Senegal; [4]Cirad, UMR SELMET-PPZS, Dakar, Senegal; [5]UPR Hortsys, CIRAD-UnivMontpellier, Montpellier 34000, France

Abstract

This study, conducted in pastureland in Senegal, aims to evaluate herbaceous biomass from ground-based photogrammetry. Photogrammetry is an image analysis that uses a set of images of an object to create a 3D model. In this study, the technique used the parallaxes obtained from images taken from different viewing points. We used the photogrammetry with a low cost RGB camera to predict herbaceous biomass values. We made a set of images taken with different positions on 35 plots (each of 1 m^2) of grass on pastureland. The images were processed using Pix4D software. Herbaceous volume was assessed using a canopy height model. Red, Green and Blue reflectance were obtained from the orthomosaics. We calculated normalised difference index for each combination of colours. We measured fresh and dry herbaceous biomass on the field on the same day of image capturing. To test the reliability of our methods for assessing biomass, a PLS analysis between field measurements and variables obtained from the camera was performed. Around 60% of the variability of herbaceous biomass was explained by camera variables, especially the volume from photogrammetry and the red reflectance. This work showed that a low-cost camera could serve as useful tool for estimation of biomass.

Keywords: photogrammetry, herbaceous biomass, pasture, Senegal, livestock pastoralism

Introduction

In the Sahel, rangelands are the main source of feed for pastoral livestock activity. The vegetation of Sahelian rangeland is composed of two types of plant communities: annual grasses and sparse tree communities. Even if vegetation of both communities is eaten by livestock, annual grasses represent the main part of their feed. Furthermore, annual grasses are strongly related to the annual variations in weather. Evaluating the available grass quantity in this region is a key aspect for the management of livestock pastoralism systems.

Many studies have used remote sensing to upscale annual grass production at a wider scale, but this upscaling requires field biomass measurements. Field biomass measurement is quite demanding undertaking work; for example, it may require specific botanical knowledge.

The possibility for using indirect measurements of grass characteristics is now a hot topic in grassland science, and there is a European Grassland Federation working group on sensing in grassland. One novel aspect of grassland sensing is the use of an unmanned aerial vehicle (UAV), including its use to produce 3D models from photogrammetry (Lussem *et al.*, 2019). Photogrammetry is an image analysis that can recreate an object in 3D using a series of images taken from different viewing points, and thus a UAV can allow estimation the grass mass (Possoch *et al.*, 2016). Photogrammetry can also be used with traditional cameras. In this study, we tested the use of photogrammetry based on field camera images to assess the biomass of Sahelian rangeland.

Materials and methods

Our study sites were in the ISRA (Institut Sénégalais de Recherches Agricoles) Zootechnical Centre of Dahra Djoloff (15.349 N; 15.445 W) in Northern Senegal. The Research Centre has an area of almost 7,000 ha including natural Sahelian rangeland.

We selected 36 plots around the Research Centre. On each plot in October 2018, we chose areas of 1 m^2 of natural grass communities, using a metal square quadrat. Around each quadrat, we cut around 10 cm of the grass surrounding the quadrat to have bare soil around the measured square. We also put a similar object (here a box) as a height reference point close to all the squares. We used a Canon Ixus 180. We made a video capitation of the grass vertically to the ground taken at around one metre from the ground. We used a grid of 5 lines to cover all the squares with the camera. After taking the video capitations we cut all the grass within each square and weighed it to obtain fresh mass. The samples were dried to obtain dry mass and so the dry matter content.

The video obtained were analysed using PIX4D software. The video was cut into different images (around 300), which are used subsequently in the 3D mapping process. In the 3D mapping processing, the first step is to identity some key points that are present on several images. Triangulation is made to evaluate the position of the key point. Thus, a densification process is made by interpolation of the different positions of the key points and so produce a 3D model of the object. We used the square and the box to set the reference of the 3D model. An example of 3D model obtained is presented in Figure 1.

We extracted for the 3D model the surface model and the orthophotography. We also used pix4D to produce a terrain model. For each plot we extracted the volume of grass from the difference between surface model and terrain model. The Terrain model was automatically obtained from the Pix4D software. We also extracted the red, blue and green values. We calculated the normalised difference index between two colours (NGRDI normalised green red difference index; NRBDI normalised red blue difference index, NGBDI normalised green blue difference index). We made a partial least squared regression between the different variables obtain from the video (volume, three colours and three normalised difference indices).

Results and discussion

Figure 2 shows the correlation circle obtained for the PLS. The R^2 for the Y of the PLS was of 0.55. The Q^2 of the two dimensions were 0.58 for fresh mass (FM), 0.60 for the dry mass and 0.10 for the dry

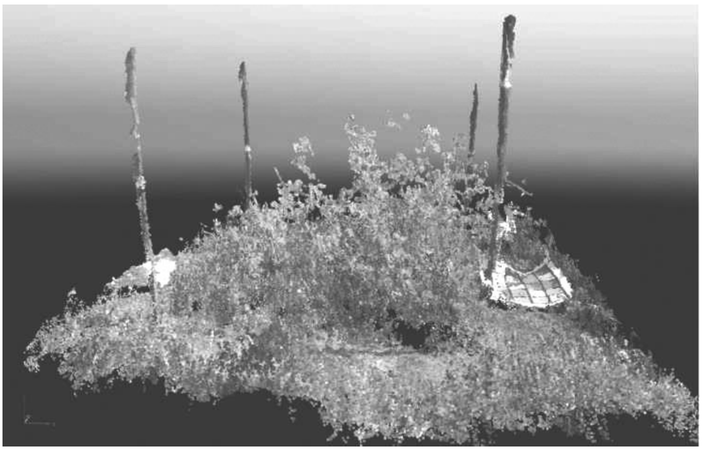

Figure 1. Example of 3D model of a Sahelian grass square obtain from PIX4D.

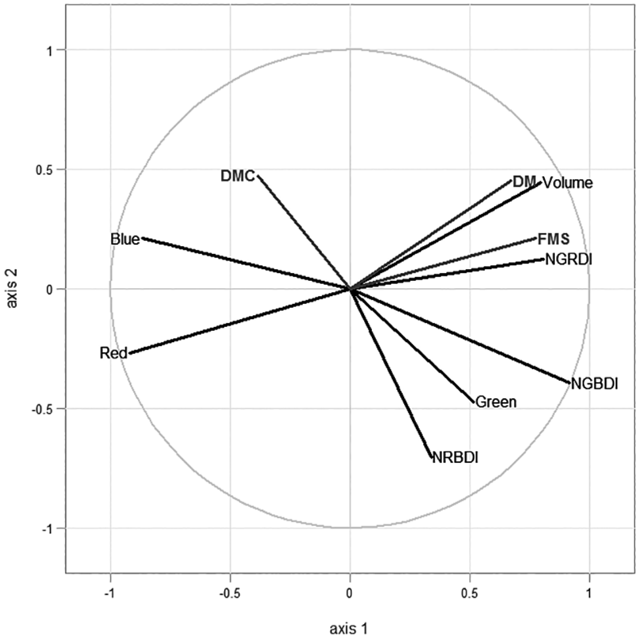

Figure 2. Circle of correlation from the PLS. X variables are volume, colours canal and normalised difference indices. Y variables are fresh mass (FM), dry mass (DM) and dry matter content (DMC). The two axes represent the two first components of the PLS.

matter content (DMC). The DMC and FM were positively correlated with the volume and the difference between green and red and negatively with the red colours. This work is a first work that shows that the interest of photogrammetry from a simple camera to assess some characteristics of the vegetation. In our test, the state and the composition of the vegetation were quite similar, although more work is required to increase the variability of the situation tested. Similar techniques could be done with a camera with more colour bands, especially in the infrared. Another perspective is to use this technique for video to obtain participatory observations. People could take video with a smartphone and send it to a server that could make grass biomass prediction.

References

Lussem U., Bolten A., Menne J., Gnyp M.L., Schellberg J. and Bareth G. (2019) Estimating biomass in temperate grassland with high-resolution canopy surface models from UAV-based RGB images and vegetation indices. *Journal of Applied Remote Sensing* 13, 034525.

Possoch M., Bieker S., Hoffmeister D., Bolten A., Schellberg J. and Bareth G. (2016) Multi-temporal crop surface models combined with the RGB vegetation index from UAV-based images for forage monitoring in grassland. *The International Archives of Photogrammetry, Remote Sensing and Spatial Information Sciences* 41, 991.

Pattern of milk production and behaviour of dairy cows according to the residence time per paddock

Delagarde R. and Robic Y.
INRAE, Agrocampus Ouest, PEGASE, 16 Le Clos, 35590 Saint-Gilles, France

Abstract

Under rotational grazing systems, the residence time per paddock may vary from 1 to 10 days according to paddock size. The farmer's decision to change a herd from one paddock to another one is not always easy. We hypothesised that recording grazing behaviour may help farmers to take decisions. The objective of this study was to determine the effect of the residence time (1, 2, 4 or 8 days per paddock) on the pattern of milk production and grazing behaviour in rotationally grazed dairy cows. Treatments were compared in a completely randomised design with 9 Holstein cows per treatment, during 72 days in spring 2018. Residence times were obtained from changing paddock size, and treatments were compared at similar levels of herbage allowance. Milk production and grazing time were recorded daily. The average milk production was similar between treatments. However, milk production and grazing time varied cyclically, with both duration and amplitude of the cycles directly proportional to the residence time. Cows never stopped grazing, even at longest residence time, with very low pasture herbage availability in the last days. It is concluded that recording of grazing behaviour is not sufficient to help farmers take grazing management decisions.

Keywords: dairy cow, grazing system, residence time, grazing time, Lifecorder

Introduction

In addition to pasture herbage allowance and length of the rotation period, the residence time (RT) per paddock (or paddock size) is one of the main grazing management factors. With grazing dairy cows, the RT per paddock may vary from less than one day to more than one week according to paddock size. The between-day variation of milk production increases with increasing RT per paddock. When RT averages 10 days per paddock, the cyclical variation of milk production is so clear that the relative drop of milk production at the end of each paddock may be used for deciding when to move the cows from one paddock to the next one (Hoden *et al.*, 1991). It is known that the decline of sward height and proportion of leaves during the grazing-down process affect grazing behaviour (Delagarde *et al.*, 2010), and it may be hypothesized that recording continuously the grazing behaviour may help to manage grazing. The objective of this study is to determine the link between milk production and cow behaviour during the grazing-down process in four grazing systems differing by the RT per paddock.

Materials and methods

The experiment took place at the INRAE experimental farm of Méjusseaume (Le Rheu, Brittany, France), from April to June 2018. Four grazing systems, differing by the RT per paddock (1 vs 2 vs 4 vs 8 days), were compared according to a completely randomized design with 9 mid-lactation Holstein dairy cows per treatment. In practice, 9 plots were grazed successively, with 8 days of RT per plot, for a total of 72 days. Each plot (of 2.4 ha on average) had been previously divided into four 0.6-ha subplots, the subplots being grazed simultaneously by the four herds of 9 cows, with one herd per treatment (T1, T2, T4, and T8). At each rotation, T1 subplots were divided in 8 paddocks, T2 subplots in 4 paddocks, T4 subplots in 2 paddocks, and T8 subplots were not divided (1 paddock, no electric fences). Thus, cows entered a new paddock after the morning milking every day in T1, on days 1, 3, 5 and 7 in T2, on days 1 and 5 in T4, and only on day 1 in T8. Treatments were compared at similar and low pasture herbage mass allowance, determined on day 0 (22 kg of dry matter (DM) cow^{-1} day^{-1} at 3 cm above ground level). As area and time per plot are fixed, some non-experimental cows could have been added in each herd, to compensate any pre-grazing herbage mass differences between subplots. Cows characteristics during the reference period in early April were: 22% of primiparous, 125 days in milk, 599 kg of live weight, 28.0 kg d^{-1} of milk production.

Pasture sward height (plate meter, 4.5 kg m^{-2}) was measured daily for each treatment. Milk production and grazing time were measured daily for each cow. Grazing activities were recorded by the Lifecorder Plus device, based on a mono-axial accelerometer (Delagarde and Lamberton, 2015). Animal data were analysed through ANOVA taking into account of the effects of RT (n=4), cow within RT (n=36), rotation number (n=8, rotation 1 considered as the adaptation period), day number in a plot (n=8), as well as the interactions between RT and rotation number, and between RT and day number in a plot.

Results and discussion

Post-grazing sward height averaged only 30% of pre-grazing sward height, which indicates as expected a severe grazing management (Table 1). Residence time had no effect on milk production, but there was a strong interaction between RT and day within plot, with a clear cyclicity (Table 1 and Figure 1). The duration of the cycles of milk production strictly followed RT, with lowest production on first and last days within a paddock, and greatest production during intermediate days. The amplitude of the variation also increased with RT, with a between-day range in milk production of 1.2, 2.8, 3.7 and 6.3 kg d^{-1}, which means 6, 14, 18 and 32% of variation for 1, 2, 4 and 8 days of RT, respectively. Daily grazing time was lower on T1 than on other treatments, mainly due to lower grazing time after evening milking (Figure 1). There was a strong interaction between RT and day within plot. On T1, grazing time increased slowly but regularly during the plot, probably due to the increase in paddock size (no back fence). In other treatments, grazing time was often high in the first day, probably because cows are hungry after the previous day in another paddock, and lowest on day 2, because there is more herbage available and probably a higher herbage intake rate (Pérez-Prieto and Delagarde, 2013). In T4 and T8, grazing time was greatest on the last days within a paddock, probably due to low sward height and a lower herbage intake rate. No cessation of grazing was observed in any treatment, even on the last day in a paddock with long RT. This shows that cows, probably hungry, still choose to graze despite very low availability of herbage. Under very severe conditions, a cessation of grazing has been observed previously only in early morning (Delagarde *et al.*, 2010). The fact that 'night' grazing (from evening to morning milking) was much greater in T8 and T4 than in T2 or T1 shows that cows seem to be more motivated to graze at night when herbage is more available.

Table 1. Effects of residence time per paddock on sward heights, milk production and grazing time in rotationally-grazing dairy cows.[1]

Variable	Residence time per paddock (d)				SE	Effects (P<)	
	1	2	4	8		RT	RT×d
Pre-grazing sward height (cm)	18.4	18.5	19.4	17.2	0.14	*	-
Post-grazing sward height (cm)	5.6	5.8	6.5	4.5	0.01	ns	-
Milk production (kg d^{-1})	20.8	20.1	21.1	19.5	1.65	ns	**
Grazing time (min d^{-1})	522	559	575	562	44	**	**
'Day' grazing time (min d^{-1})	301	293	265	267	30	**	**
'Night' grazing time (min d^{-1})	218	263	306	292	34	**	**

[1] 'Day': between morning and evening milkings; 'Night': between evening and morning milkings; SE = standard error, RT = residence time; RT×d = interaction RT by day within plot; * P<0.05; ** P<0.01; ns = not significant.

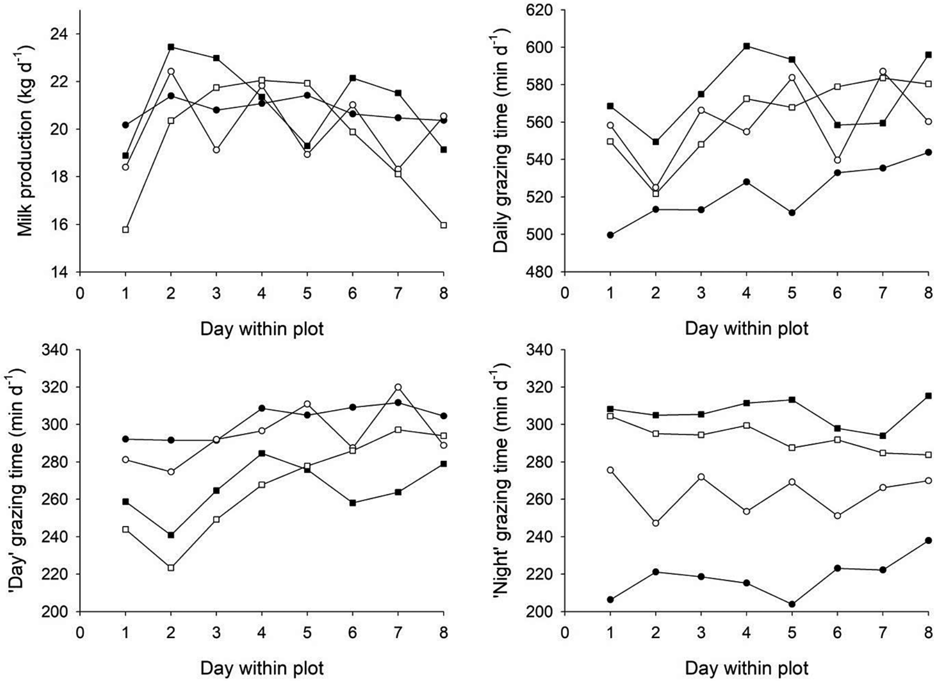

Figure 1. Pattern of milk production, on daily grazing time, on 'day' grazing time (morning to evening milkings), and on 'night' grazing time (evening to morning milkings) within paddocks according to the residence time (●, ○, ■, □: 1, 2, 4 and 8 days per paddock, respectively).

Conclusions

Residence time per paddock largely affected the between-day variation of milk production, but not the average milk production per cow. Grazing time and grazing pattern during the day also showed cyclical variations in relation to the RT per paddock, but with much less amplitude. Both duration and amplitude of the cycles were directly proportional to RT. Cows never stopped grazing, even with very low availability of herbage in the pasture at the end of the grazing-down process. Recording grazing time automatically seems to be not sufficient for helping farmers to take grazing management decisions.

References

Delagarde R. and Lamberton P. (2015) Daily grazing time of dairy cows is recorded accurately using the Lifecorder Plus device. *Applied Animal Behaviour Science* 165, 25-32.

Delagarde R., Peyraud J.L. and Wade M. (2010) Daily pattern of feeding activities of dairy cows in an 8-d rotational grazing system. *Grassland Science in Europe* 15, 931-933.

Hoden A., Peyraud J.L., Muller A., Delaby L. and Faverdin P. (1991) Simplified rotational grazing management of dairy cows: effects of rates of stocking and concentrate. *Journal of Agricultural Science, Cambridge* 116, 417-428.

Pérez Prieto L.A. and Delagarde R. (2013) Meta-analysis of the effect of pasture allowance on pasture intake, milk production, and grazing behaviour of dairy cows grazing temperate grasslands. *Journal of Dairy Science* 96, 6671-6689.

Effect of mechanical processing of ley crop silages on *in vitro* gas production and neural digestive fibre digestion

Elgemark E., Eriksson T. and Rustas B.O.
Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, Box 7024, 750 07 Uppsala, Sweden

Abstract

Reducing silage particle size can increase forage intake through enhanced rumen passage rate. Increased passage rate might depress rumen digestion and counteract the positive effect of intake on nutrient supply. Extrusion is a mechanical treatment causing particle size reduction and plant cells to break open, resulting in improved fermentation in biogas reactors. The objective of this study was to investigate if extrusion could increase *in vitro* ruminal digestion of silage. Primary growth of timothy and red clover silage, harvested at two maturity stages, was processed in an extruder. *In vitro* incubations with rumen liquid were performed over 72 h in a system with continuous measurement of gas production. Analysis of neutral detergent fibre (NDF) digestion was performed on fermented residues. Extrusion increased gas production from both timothy and clover, indicating improved digestion, and enhanced NDF degradation of late harvest timothy and early harvest red clover silage. Extrusion has the potential to increase rumen digestion of ley crop silage.

Keywords: forage, *in vitro* digestion, particle size reduction, extrusion

Introduction

Due to limited forage intake capacity, considerable amounts of concentrate feed are needed to fulfil the nutritional demands of dairy cows. Reducing forage particle size increases rumen passage rate, and hence feed intake can increase. Chopping to less than 10 mm can slightly increase forage intake by dairy cows (Nasrollahi *et al.*, 2015). Pelleting causes extensive particle size reduction of forage and can have positive effects on animal production through increased intake, despite the often-observed depressed digestibility (Minson, 1963). Extrusion is a mechanical process adopted by the biogas industry that causes extensive particle size reduction and cell rupture in lignocellulosic plant material, including silage. The result is improved biogas production (Hjorth *et al.*, 2011) which indicates that extrusion might be beneficial also for rumen degradation. The aim of this study was to investigate if extrusion increases *in vitro* rumen fermentation of grass and clover silage.

Materials and methods

Pure stands of timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.), grown at Säby in Uppsala, Sweden (N 59° 50' 20.95'; E 17° 43' 9.72') were harvested on 5-6 June (early) and 19-20 June (late) in 2018. Crops were dried indoors to 35-40% dry matter content, chopped to 2-4 cm length, packed by hand into lab-scale silos (4 l, 3 kg fresh weight) and stored for 15-17 weeks.

Silages were processed in an extruder for lignocellulosic plant material (Bio-extruder MSZ-B15e, LEHMANN Maschinenbau GmbH, Rüdersdorf, Germany). Processed and control samples were stored at -20 °C for later analysis of dry matter (103 °C), ash (550 °C), crude protein (Kjeldahl; Kjeltec 2460, Tecator, Höganäs, Sweden) and neutral detergent fibre (NDF; Chai and Udén, 1998).

An *in vitro* system for measuring gas production (Gas Endeavour, Bioprocess Control AB, Lund, Sweden) was used for incubations. Thawed feed samples (corresponding to 4 g of dry matter) were incubated fresh, without further particle size reduction, in rumen fluid and buffer (ratio 1:3; VOS buffer; Lindgren,

1979) in 500 ml bottles. Two incubations of each crop were performed. Each run included triplets of unprocessed and processed silage (a total of 12 bottles) and a blank (without sample). The residues after 72 h incubation were transferred to filter bags (pore size 12 μm) which were sealed, gently kneaded to remove liquid, placed in glass vessels and treated with ND (Neutral detergent) solution at 85 ° C overnight (Chai and Udén, 1998).

Gas production measurements and residual NDF were corrected for blank measurements before statistical analysis. Treatment, harvest and their interaction were treated as fixed effects. Crops were analysed separately with Proc GLM in SAS (SAS, 2018; version 9.4).

Fermentation rate was estimated using the following model (Oerskov and McDonald, 1979): $p = a + b[1-\exp(-ct)]$, where p is estimated gas volume at time t, a is estimated volume at time 0, b is asymptotic gas volume at infinite time and c is fermentation rate. Treatment means of data recorded at 0, 2, 4, 8, 16, 24, 48 and 72 h were used.

Results and discussion

Silage composition values are shown in Table 1. Lower levels of crude protein and NDF digestibility at late harvest reflect lower nutritional value, verified by significantly lower gas production at most time points for both crops at late harvest (Table 2).

Extrusion resulted in greater gas productions from both harvest times, for timothy and red clover silages (Table 2). After 12 hours of incubation the increases in gas production were from 35 to 49% and at 72 hours from 5 to 11%. Similar results have been obtained in biogas experiments, albeit on a different time scale where extrusion of grass material increased methane production for 62 and 9% after 28 and 90 days of fermentation, respectively (Rodriguez *et al.*, 2017).

The gas production rate of both red clover and timothy were higher for extruded silages and range from 6.2 to 6.8% and from 8.5 to 9.2% before and after extrusion, respectively, indicating increased rumen digestion rate.

The main advantage of processing forage lies in an increased passage rate, digestibility and as a consequence increased intake potential. However, increased passage rates depress rumen digestion due to the shorter retention time of feed particles in the rumen. In order not to lower the feed efficiency, the digestibility must not decrease significantly. This might be compensated for by increased digestion rate caused by

Table 1. Dry matter (DM), chemical composition and NDF digestibility for unprocessed and extruded timothy and red clover silages, harvested at two different dates.[1]

	Timothy				Red clover			
	Early		Late		Early		Late	
	E	C	E	C	E	C	E	C
DM, g kg^{-1}	37.1	35.0	39.3	39.7	35.7	33.6	34.3	34.6
NDF, g kg^{-1} DM	550	532	539	534	327	324	395	382
CP, g kg^{-1} DM	141	156	121	115	183	188	153	162
Ash, g kg^{-1} DM	74.7	79.9	71.5	72.0	92.6	92.1	79.4	79.4
NDF digestibility[2]	0.64	0.63	0.61	0.53	0.60	0.53	0.47	0.46

[1] E = extruded; C = control; CP = crude protein; for comparisons within crop, standard error of the mean = 0.017, P<0.001 for harvest, treatment and harvest × treatment of timothy and harvest of red clover, P=0.059 for harvest and P=0.014 for harvest × treatment of red clover, respectively.

[2] Digestion after 72 h *in vitro* incubation in buffered rumen liquid.

Table 2. Volume of gas produced from unprocessed and extruded timothy and red clover silages, harvested at two different dates, during *in vitro* incubation in buffered rumen liquid.[1]

	Incubation time (h)	Harvest				SEM	*P*-value		
		Early		Late			Harvest	Extrusion	H×E
		E	C	E	C				
Timothy	6	296	201	300	210	7.5	0.394	<0.001	0.742
	12	490	338	444	330	11.4	0.029	<0.001	0.117
	24	634	542	591	508	15.2	0.018	<0.001	0.767
	48	716	653	683	611	16.3	0.035	<0.001	0.770
	72	728	669	704	632	16.1	0.069	<0.001	0.686
Red Clover	6	262	200	254	187	6.8	0.151	<0.001	0.702
	12	522	351	492	345	8.8	0.053	<0.001	0.195
	24	660	591	620	564	14.0	0.026	<0.001	0.647
	48	715	681	669	633	14.5	0.004	0.024	0.957
	72	722	689	675	641	154.9	0.005	0.034	0.963

[1] E = extruded; C = control; SEM = standard error of the mean; H×E = interaction between harvest date and extrusion.

extrusion. The effect of extrusion on *in vivo* digestion might, however, be different from that *in vitro* due to the effect of mastication, which can even out differences in feed particle size after ingestion (Schadt *et al.*, 2012).

Conclusions

The results of this experiment show that extensive mechanical processing of grass and clover silage can improve rumen fermentation *in vitro*, suggesting a positive effect also *in vivo*.

References

Chai W.H. and Udén P. (1998) An alternative oven method combined with different detergent strengths in the analysis of neutral detergent fibre. *Animal Feed Science and Technology* 74, 281-288.

Hjorth M., Granitz K., Adamsen A.P.S. and Moller H.B. (2011) Extrusion as a pretreatment to increase biogas production. *Bioresource Technology* 102, 4989-4994.

Lindgren E. (1979) Vallfodrets näringsvärde bestämt *in vivo* och med olika laboratoriemetoder. Rapport 45. Institutionen för husdjurens utfodring och vård, SLU, Uppsala.

Minson D.J. (1963) The effect of pelleting and wafering on the feeding value of roughage – a review. *Journal of Brittish Grassland Society* 18, 39-44.

Nasrollahi S.M., Imani M. and Zebeli Q. (2015) A meta-analysis and meta-regression of the effect of forage particle size, level, source, and preservation method on feed intake, nutrient digestibility, and performance in dairy cows. *Journal of Dairy Science* 98, 8926-8939.

Oerskov E.R. and McDonald I. (1979) The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage [sheep]. *Journal of Agricultural Science,* 499-503.

Rodriguez C., Alaswad A., Benyounis K.Y. and Olabi A.G. (2017) Pretreatment techniques used in biogas production from grass. *Renewable and Sustainable Energy Reviews* 68, 1193-1204.

Schadt I.J., Ferguson D., Azzaro G., Petriglieri R., Caccamo M., Van Soest P and Licitra G. (2012) How do dairy cows chew? – Particle size analysis of selected feeds with different particle length distributions and of respective ingested bolus particles. *Journal of Dairy Science* 95, 4707-4720.

Remotely sensed grass sward parameters as a basis for variable rate nitrogen fertilisation

Gnyp M.L., Portz G. and Jasper J.
Research Centre Hanninghof, Yara International ASA, 48249 Dülmen, Germany

Abstract

Real time variable rate nitrogen (VRN) fertilisation based on spectral crop sensing is a well-established technology in arable farming, while still at an early stage in grassland management. This study aims to evaluate remotely sensed grass sward parameters derived from spectral data and how this information can be turned into in-field spatially variable nitrogen (N) application. For the latter, site-specific N-response and nitrogen use efficiency (NUE) were investigated, using a chessboard trial design. The trial was set up with 39 sets of four plots with increasing N-rates, which allowed the calculation of 125 N response functions across the field. Handheld N-Sensor measurements and grass samples (analysed for fresh and dry matter yield and N content) were taken during two subsequent cuts in 2017 and 2018. It was found that spectral crop canopy sensing enables reliable estimates of the N uptake in grass swards with high spatial resolution. N uptake, in turn, is an indicator of NUE and closely related to optimum site-specific N-rates. This enables the development of VRN fertilisation algorithms for more efficient and environmentally sound fertiliser use.

Keywords: N-Sensor, chessboard trial, grassland, crop canopy sensor, N management

Introduction

Grassland is receiving more attention as a resource for cost-effective production of high value feed for ruminants with a minimum of losses, and with this an increasing focus on VRN on grassland. Analogous to arable farming, VRN on grassland addresses in-field spatial variation in N response, primarily driven by variations of soil parameters, nutrient availability and uptake. Compared to arable cropping systems, grassland is diverse in many aspects (Schellberg *et al.*, 2008), which can make the introduction of standardised precision farming applications more challenging. Diversity in this respect means that, apart from various intensity levels of grassland use, spatial and temporal heterogeneity of soil characteristics, interacting with weather and crop management, can result in highly variable grassland sward composition, making it more difficult to measure relevant crop characteristics and to derive appropriate decisions (Jasper, 2017). To explore the suitability of proximal measurements of grass sward parameters and how to make use of those parameters for VRN algorithm, a field experiment was set up in a hilly area with varying soil conditions, using a chessboard trial design that allows the calculation of multiple N response functions across the experiment.

Materials and methods

A nitrogen fertiliser experiment was carried out on a ryegrass-dominated and intensively managed grass sward on a loamy soil situated in Neunkirchen-Seelscheid, Germany (50°51'32"N, 7°18'46"E), in 2017 and 2018. A chessboard trial was set up with 39 replications of sets of four plots (each 6 m by 6 m) with increasing N-rates (Figure 1) of 0 (N1), 50 (N2), 100 (N3) and 150 (N4) kg N ha^{-1}, applied as calcium ammonium nitrate (27% N) to each of the first two cuts in both years. The trial was fertilised using a spreader with a defined working width, applying alternating fertiliser rates of 0 and 50 kg N ha^{-1} in crosswise direction, and 0 and 100 kg N ha^{-1} lengthwise in order to create the chessboard design (Berry *et al.*, 2017). All 156 plots were measured with a handheld version of the Yara N-Sensor® in the spectral range of 400-1000 nm at 10 nm spectral resolution. A spectral vegetation index (VI), using the simple ratio of near infrared and red-edge, named S1, was calculated (Link and Jasper, 2003). The harvest was

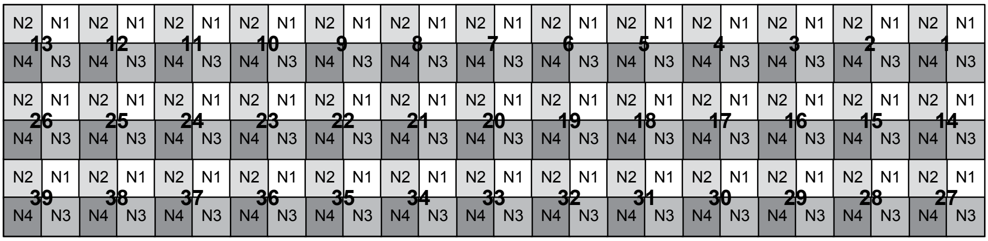

Figure 1. Chessboard trial with 39 replicated sets of four plots with increasing N-rates.

done with a mower by cutting a harvest area of 0.47 m × 5.53 m, i.e. 2.6 m^2 per plot. Fresh grass yields were weighed directly in the field and dry matter yields were determined in the experimental station after drying the samples in an oven at 75 °C to constant weight. N content was analysed in the laboratory using the Kjeldahl method and N-uptake calculated by multiplying N content by dry matter yield. This trial setup enables the calculation of in total 125 N response curves (2nd order polynomial; for details see Link and Jasper, 2003) and the derivation of as many economic optimum N rates (N_{opt}, calculated with a price ratio between N fertiliser and grass dry matter yield of 6.5) across the trial area for each cut. Nitrogen use efficiency (NUE) was calculated from the yield response to fertiliser N as follows (Equation 1):

$$\text{NUE (kg dry matter kg N}^{-1}) = (\text{Yield at } N_{opt} - \text{Yield at N0}) / N_{opt} \qquad (1)$$

Results and discussion

In line with Portz *et al.* (2017) the N-Sensor value S1 was found to be a good estimator for N uptake (Figure 2), although affected by the severe drought that occurred during the growth of the second cut in 2018 (Figure 2B). N_{opt} varied widely across the field with mean values of approx. 100 kg N ha^{-1} cut^{-1} due to varying soil conditions and topography (Table 1). NUE also varied widely (Table 1) and was negatively correlated to N uptake at N0 in 2017 (Figure 3A) and also, though to a lesser extent, in the dry year 2018 (data not shown). This confirms the results of Berry *et al.* (2017) and indicates that NUE and N supply from the soil are negatively related. As a consequence, recommended optimum N rates were also negatively correlated to N uptake across all plots of the trial (Figure 3B).

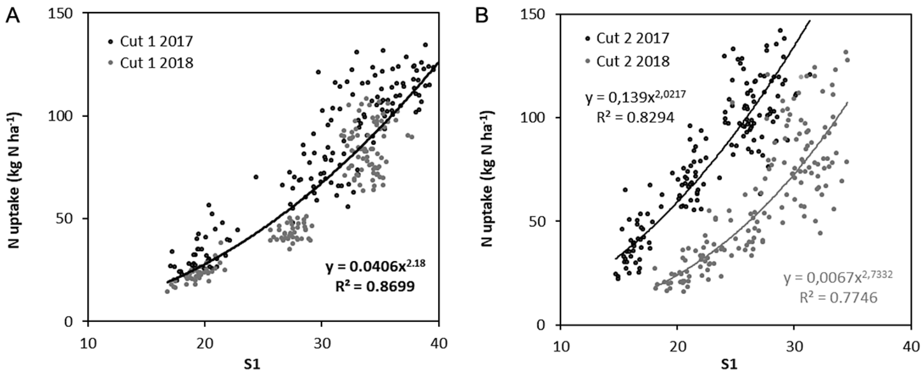

Figure 2. Relationship between S1 and N uptake for (A) cut 1 and (B) cut 2.

Table 1. Variability of N_{opt} (kg N ha^{-1}) and nitrogen use efficiency (NUE) (kg dry matter kg N^{-1}) in 2017 and 2018.

	Cut 1 2017		Cut 2 2017		Cut 1 2018		Cut 2 2018	
	N_{opt}	NUE	N_{opt}	NUE	N_{opt}	NUE	N_{opt}	NUE
Min	68	8	60	7	74	3	49	3
Max	129	21	132	29	140	15	151	17
Median	111	14	98	15	110	10	109	10

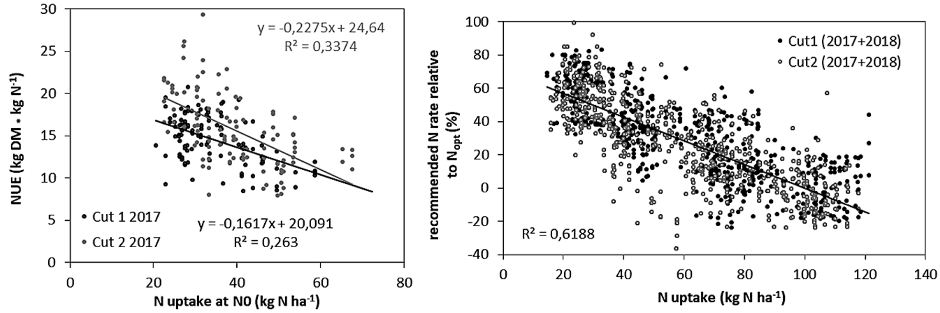

Figure 3. (A) N uptake in N0 plots as nitrogen use efficiency (NUE) indicator and (B) VRN algorithm for cut grass systems.

Conclusions

This study reveals that N uptake is a suitable indicator for NUE and closely related to optimum site-specific N-rates. As N uptake estimates can be derived from remote sensing, this enables the development of VRN fertilisation algorithms based on these findings.

Acknowledgements

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References

Berry P.M., Holmes H.F. and Blacker, C. (2017) Developments of methods for remotely sensing grass growth to enable precision application of nitrogen fertilizer. In: *Advances in Animal Biosciences*, 8 (2), 11th ECPA 2017 in Edinburgh, Cambridge University Press, Cambridge, UK, pp. 758-763.

Jasper, J. (2017) Precision farming in grass silage production – possibilities and current status. In: *Swedish University of Agricultural Sciences, Proceedings of Forage Conference* 2017, February 7-8, 2017, Uppsala, Sweden pp. 63-66.

Link A. and Jasper J. (2003) Site-specific N fertilization based on remote sensing – is it necessary to take yield variability into account. In: Stafford, J. and Werner A. (eds.) *Precision agriculture.*, Proceedings of the 4th ECPA, Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 353-359.

Portz G., Gnyp M.L. and Jasper J. (2017) Capability of crop canopy sensing to predict crop parameters of cut grass swards aiming at early season variable rate nitrogen topdressing. In: *Advances in Animal Biosciences*, 8 (2), Papers presented at the 11th ECPA 2017 in Edinburgh, Cambridge University Press, Cambridge, UK, pp. 792-795.

Schellberg J., Hill M., Gerhards R., Rothmund M. and Braun M. (2008) Precision agriculture on grassland: applications, perspectives and constraints. *European Journal of Agronomy* 29, 59-71.

Biological protection of red clover against root fungal diseases

Hakl J. and Pisarčik M.
Department of Agroecology and Crop Production, Czech University of Life Sciences, Prague, Czech Republic

Abstract

Root diseases of red clover (*Trifolium pratense* L.) contribute to reduced persistence and production of sown red clover stands; however, the potential of biological control of these diseases has not yet been investigated. Our objectives were to summarise the effect of application of *Pythium oligandrum* on plant density, root morphology and disease score and forage yield and yield components of red clover in the field experiments. Results demonstrated a potential for biological control for lower root disease score and enhancement of red clover yield via the plant growth stimulation, but optimisation of application timing is necessary for determination of practical recommendations and economic efficiency.

Keywords: forage crops, root morphology, *Pythium oligandrum*, biological control

Introduction

Red clover is a perennial forage legume of limited persistence, mainly used for cutting in grass-clover leys of 2-4 years of duration, but also occurring naturally in permanent grassland (Boller *et al.*, 2010). Root diseases cause significantly reduced red clover persistence (Wallenhammar *et al.*, 2006). Available options for crop protection were summarized by Pisarčik *et al.* (2020). According to Benhamou *et al.* (2012), the mycoparasitic fungus *Pythium oligandrum* has ability to colonise the rhizosphere of many crop plants' root system; it can directly attack soil-borne fungal pathogens, promote plant growth and increase crop protection against fungal disease via the activation of the plant immune system. Pisarčik *et al.* (2020) reported positive impact of *P. oligandrum* on red clover forage yield in relation to changes in crop root morphology. Testing of plant stimulator (poly-beta-hydroxy butyric acid) application did not show any positive effect over this three-year experiment, therefore the main aim of this paper is to summarised effects of application of Polyversum on forage yield and stand height of particular cuts in the second post-seeding year. Another aim is to present the first results of the next field experiment verifying the positive effects obtained of biological control in red clover.

Materials and methods

The field experiments were established with four replications at the Plant Breeding Station Větrov in the Czech Republic. Experiment 1 was seeded In April 2016 and comprised two varieties of red clover varieties (Start and Callisto), which were established and managed under two treatments of biological control for disease (untreated control and spraying *P. oligandrum* at two intensities). Preparation Polyversum containing 1,000,000 active oospores of *P. oligandrum* M1 per gram (Biopreparáty spol. s r.o., Czech Republic) was applied in a dose of 100 g ha^{-1} after the last cut (P1) or after each cut (P2) over three years. The three-cut management was used in the two post-seeding years. Compressed height (CH, cm) was measured before each harvest by a rising plate meter with six measurements per plot. Fresh matter yield was measured after cutting with a Haldrup plot harvester, and dry matter yield was calculated from the average dry matter content.

In autumn, plant root systems were dug from each plot. Plant density (PD, plant m^{-2}) was calculated from number of plants per sample and size of the root sampling area. For each plant, the tap-root diameter below the crown (TD, mm) and lateral root number per plant tap-root (LRN, when larger than 1 mm) were evaluated. Plant root disease score (PRDS) was scored subjectively, based on discoloration on a cross-cut section of the tap-root, in line with Hakl *et al.* (2017). Ratio of infected plant (IP) was assessed as a proportion of plants with visible root discoloration, and PRDS values were averaged for infected plants. The percentage of branch-rooted plants (RB) was calculated for each sample. More details about experimental design and statistical analyses were described by Pisarčik *et al.* (2020).

Field Experiment 2, including variety Start, used the same treatments with identical design and was established in April 2018. The same root and forage traits were measured in the establishment year. Due to drought, yield data are not reported from this experiment but analysis of root traits in 2019 is shown in Table 1. Data from both experiments were analysed by ANOVA where all factors were considered as fixed, and significant differences between means were reported using the Tukey HSD test at α=0.05.

Results and discussion

In Experiment 1, application of Polyversum stimulated higher TD and root branching of red clover plants (Table 1). This is in line with general statements about *P. oligandrum* promoting plant growth (Benhamou *et al.*, 2012) but these effects have seldom been studied on plant roots, and for field studies. Effect of biological control on root disease occurrence was only visible in the last year of the experiment (Pisačik *et al.*, 2020) where significantly lower PRDS was detected for both Polyversum treatments (Table 1). Similarly, a positive effect of *P. oligandrum* was also reported showing reduction in tomato root damage by *Fusarium* (Pharand *et al.*, 2002). Percentage of infected plants reached a value around 95% and was not significantly different between tested treatments.

Polyversum application provided greater compressed height than the untreated control in almost all cuts but this did not result in significantly higher yield at the P1 treatment (Figure 1). Intensive Polyversum application reached significantly higher yield in the first and third cut (P2 treatment in Figure 1) which is in line with improved annual forage yield for this treatment (Pisačik *et al.*, 2020).

Results of Experiment 2 confirmed positive effects of Polyversum on enhancement of plant TD and a positive trend for higher root branching. Autumn application significantly improved ratio of infected plants compared with untreated control and P2 treatment. It seems that effect of Polyversum application in field conditions can be variable because of complex interactions between plant, biological preparation, and environment.

Table 1. Effect of biological control on red clover plant density (PD), tap root diameter (TD), percentage of branch-rooted plants (RB), lateral root number (LRN), percentage of infected plants (IP) and plant root disease score (PRDS).[1]

	Var	PD	TD	RB	LRN	IP	PRDS	n
Experiment 1;	Untreated control	198	8.52^{b}	47.9^{a}	2.04	93.7	4.94^{b}	218
established 2016,	Polyversum 1	202	9.39^{a}	54.8^{a}	2.18	95.6	4.53^{a}	231
data from 2018	Polyversum 2	222	8.92^{a}	60.9^{b}	2.35	94.9	4.63^{a}	226
	P treatment	0.604	0.001	0.021	0.253	0.591	0.013	
	Density (covariate)	-	<0.001	0.001	0.007	-	-	
Experiment 2;	Untreated control	268	3.28^{a}	8.2	0.91	54.2^{c}	4.43	72
established 2018,	Polyversum 1	509	4.02^{b}	19.9	1.33	23.1^{a}	4.77	97
data from 2019	Polyversum 2	299	3.94^{b}	14.9	1.61	36.0^{b}	3.75	57
	P treatment	0.274	0.010	0.330	0.194	<0.001	0.416	
	Density (covariate)	-	<0.001	0.105	0.647	-	-	

[1] *P* = probability of ANOVA, different letters indicate statistical differences between treatments.

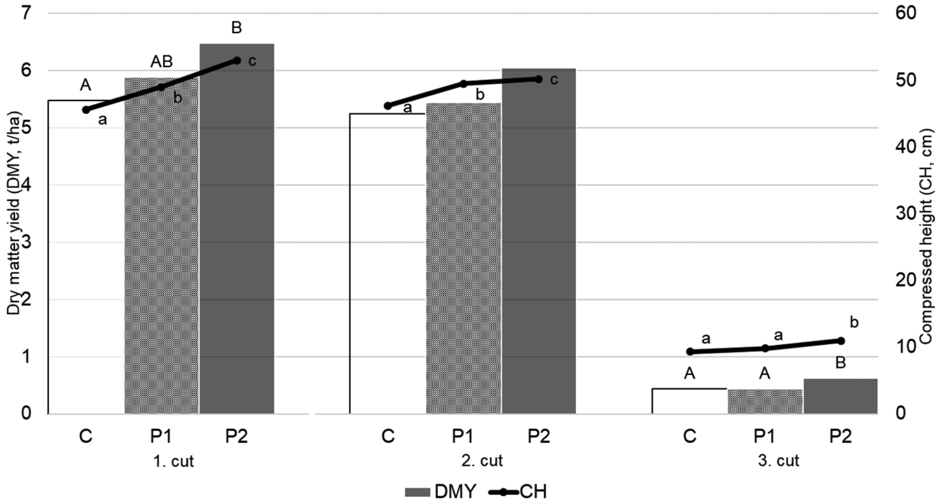

Figure 1. Forage yield and compressed height of red clover in three cuts in 2018 (C = untreated control, P1 = autumn application, P2 = application after each cut). Letters indicates differences between treatments within cut for yield (capital) and stand height (lowercase).

In conclusion, in both experiments Polyversum was able to provide positive responses on reduced PRDS and it stimulated red clover root branching. Improved forage yield was visible only under intensive application in the second post-seeding year and this was associated more with plant growth stimulation than protection against fungal disease. A dry matter yield enhancement of about 18% demonstrated a potential for biological control for improving red clover yield but optimisation of application timing is necessary for practical recommendations and economic efficiency. Association with root morphology highlights the need for evaluation of root disease occurrence together with root traits and forage yield.

Acknowledgements

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References

Benhamou N., leFloch G., Vallance J., Gerbore J., Grizard D. and Rey P. (2012) *Pythium oligandrum*: an example of opportunistic access. Microbiology 158, 2679-2694.

Boller B., Schubiger F.X. and Kölliker R. (2010) Red clover. In: Boller B., Posselt U.K. and Veronesi F. (eds) *Fodder crops and amenity grasses. Handbook of Plant Breeding.* 5. Springer, New York, NY, USA, pp. 439-455.

Hakl J., Pisarčik M., Hrevušová Z. and Šantrůček J. (2017) In-field lucerne root morphology traits over time in relation to forage yield, plant density, and root disease under two cutting managements. *Field Crops Research* 213, 109-117.

Pharand B., Carisse O. and Benhamou N. (2002) Cytological aspects of compost-mediated induced resistance against *Fusarium* crown and root rot in tomato. *Phytopathology* 92, 424-438.

Pisarčik M., Hakl J. and Hrevušová Z. (2020) Effect of *Pythium oligandrum* and poly-beta-hydroxy butyric acid application on root growth, forage yield and root diseases of red clover under field conditions. *Crop Protection* 127, in press.

Wallenhammar A.C., Adolfsson E., Henriksson M., Lundmark S. and Roempke G. (2006) Field surveys of *Fusarium* root rot in organic red clover leys. *Grassland Science in Europe* 11, 369-371.

Reliable biomass estimates of multispecies grassland using the rising plate meter

Hart L.[1,2], Werner J.[2], Velasco E.[2], Perdana-Decker S.[2], Weber J.[3], Dickhoefer U.[2] and Umstaetter C.[1]
[1] Competitiveness and System Evaluation, Agroscope, Tänikon, Switzerland; [2] Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Stuttgart, Germany; [3] Grassland Division, Agricultural Centre for Cattle Production, Grassland Management, Dairy Management, Wildlife and Fisheries Baden Wuerttemberg, Aulendorf, Germany

Abstract

Rising plate meters are a powerful and easy-to-use tool for quantifying the available forage on pastures. Today, semi-automated systems convert compressed sward height measurements into a biomass estimate in real-time and even georeferenced. However, species-rich pastures can contain very heterogeneous biomass and we hypothesised that one standard conversion equation is not suitable for different swards. Therefore, we studied the effect of the botanical composition of different swards in various field experiments in southern Germany and Switzerland throughout the vegetation period in 2019. Sampled swards were classified into ryegrass-rich, grass-rich, herb-clover-rich or balanced between grass, herb and clover. A first analysis verified the hypothesis that sward type affects the adequate estimation of available biomass. In order to achieve reliable biomass estimations based on compressed sward height, we propose different conversions for rising plate meter users based on sward composition.

Keywords: multispecies pasture, rising plate meter, sward specific biomass calibration

Introduction

Due to the expected environmental and societal challenges, pasture-based feed is a valuable resource for meat and dairy production. In comparison with pure stands, multispecies swards have the potential to stabilise and increase grassland yield as well as biodiversity, and are more heterogeneous in terms of plant material. Therefore, estimating available biomass on pasture is difficult, but it is a key requirement for farmers seeking to optimise allocation of herbage for their grazing animals. One fairly new tool to aid this purpose is the Grasshopper® (TrueNorth Technologies, Shannon, Ireland), a semi-automated rising plate meter (RPM) that measures the compressed sward height (CSH) and thus it estimates the available biomass with a standard calibration that was established for homogeneous grasslands in Ireland. Previous research has shown that the accuracy of RPM calibrations not only depend on the region and the season but also on the flexibility of the plant material against compression and the tiller density (Klootwijk *et al.*, 2019). The Grasshopper® has been found to estimate biomass more accurately in homogeneous clover-ryegrass based swards in Denmark and less reliable in multispecies swards in Switzerland (Hart *et al.*, 2019). Therefore, we hypothesise that specified conversion equations can be determined leading to an improved performance of semi-automated RPMs. For this reason, we studied the conversion of CSH into biomass for sward compositions of permanent multispecies swards. We aimed at identifying sward types that need customized calibrations.

Materials and methods

The experimental data were gathered from five different observers at fifteen sites in eastern Switzerland and southwest Germany within the regions of Blackforest, Neckar-Schwaebische Alb, Schwaebische Alb-Donau and Oberschwaben during the vegetation period of 2019. The sampled swards were chosen in order to represent permanent and multispecies pastures. The number of plant species within the sampled pastures ranged from three to twenty-eight. The sampled swards were located at altitudes from 300 m to 1,300 m above sea level. All of them were grazed intensively during the experimental year, except for the

Blackforest sites, which were protected Natura 2000 habitats and managed according to the directive. In total, 1,142 herbage samples were taken from thirty-eight swards ranging from three to 257 samples each. In order to determine the available herbage biomass, frames of 0.25 m^2 and 1 m^2 were placed randomly within a representative area of the pastures avoiding dung patches. The plant biomass therein was cut to 42 mm above soil surface on average, and weighed. Afterwards, the samples were oven-dried at 60 and 100 °C until mass was stable; the dry matter (DM) concentration was calculated and used to estimate the above-ground plant biomass per hectare (kg DM ha^{-1}) for each frame. Furthermore, the mean CSH was measured before and after cutting with three or five measurements per sampling area depending on the size of the frame. This represented the cut difference (CSH_{cut}). For that, we used a semi-automated RPM (Grasshopper®, G2 Sensor, TrueNorth Technologies, Shannon, Ireland). Before cutting, the foliar cover was estimated visually within each frame and later categorized into swards with ≥80% and <80% foliar cover. Additionally, we determined the sward type. To do so, the sampled areas were classified visually according to the proportion of grass within the total fresh foliar biomass. Swards with >70% grasses of total foliar biomass were considered as grass-rich and divided into ryegrass-based swards (RG) and other grasses (G). If the swards contained 50 to 70% grasses, they were considered to be balanced (B), whereas they were considered rich in clover and herbs (H), if the proportion of grasses was <50%. As the plate lift is also dependent on the plant material, we assessed visually if class H contained mostly flexible ('flex', i.e. clover) or more rigid ('rig', i.e. forbs) materials. In order to create equations for converting CSH into biomass, we excluded data with less than 80% foliar cover (n=100), because they caused an overestimation of biomass. As we focused on grazing conditions, we also excluded CSH above 200 mm (n=11). Additionally, we tested for plausibility and excluded data where CSH_{cut} was ≤0 mm (n=6). The relationship between CSH_{cut} and above-ground plant biomass was analysed using linear regression.

Results and discussion

We analysed the combined data from all regions with the aim of studying the different sward types that have been identified at the various locations. As shown in Figure 1, the largest differences in the regression lines were found between clover-rich swards (H-flex), ryegrass-rich swards (RG) and herb-rich swards with rigid plant material (H-rig). The regression line found by Murphy *et al.* (2019) was based on sown perennial ryegrass and is in agreement with the regression line for the RG sward type in our study. Figure 1 clearly shows that H-rig is less dense (kg DM ha^{-1} per CSH unit) compared with other swards and cannot be converted in the same way, and especially not like H-flex. After a first analysis, we

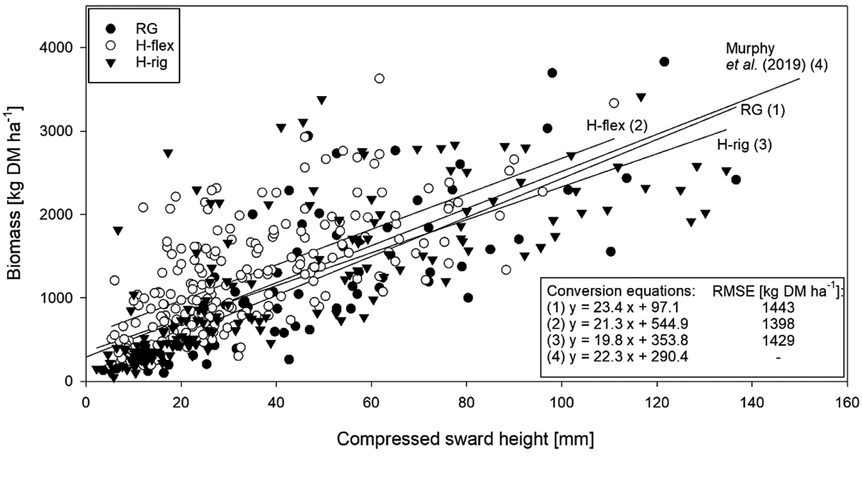

Figure 1. Linear regressions between cut compressed sward height (x) and herbaceous biomass (y). Symbols differentiate between ryegrass-based (RG), clover-rich (H-flex) and herb-rich swards with rigid plant material (H-rig).

identified conversion equations for multispecies swards of permanent grassland for three sward types (Equation 1-3 in Figure 1), where y is the biomass in kg DM ha^{-1} and x is the CSH_{cut} in mm. For a large number of samples (n=594), which were classified as other grasses or balanced sward (G & B, not shown in the graph), the identified regression line was very similar to the one derived for the clover-rich swards. However, as the root mean squared errors (RMSE) for the individual regression lines are high, the next step will be to determine other factors that can explain the large variance, such as the season or the detailed botanical composition.

Conclusions

A standard calibration for estimating above-ground plant biomass from CSH that had been developed for homogeneous ryegrass-based swards was not suitable for clover- and herb-rich swards on permanent multispecies grassland. Herb-rich swards with rigid plant material would be highly overestimated by considering only this standard calibration, compared with clover-rich swards with their more flexible biomass. In the next step, data should be analysed further to identify conversion equations that account for differences in sward structure and composition between seasons. Additionally, the identified equations should be validated.

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References

Hart L, Oudshoorn F., Latsch R. and Umstatter C. (2019) How accurate is the Grasshopper system in measuring dry matter quantity of Swiss and Danish grassland? In: O`Brien B., Hennessy D. and Shalloo L. (eds.) *Precision Livestock Farming '19.* The 9th European Conference on Precision Livestock Farming, The Organising Committee, Teagasc and Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Ireland, pp. 188-193.

Klootwijk C.W., Holshof G., Van den Pol-van Dasselaar A., Van Helvoort K.L.M., Engel B., De Boer I.J.M. and Van Middelaar C.E. (2019) The effect of intensive grazing systems on the rising plate meter calibration for perennial ryegrass pastures. *Journal of Dairy Science* 102, 10439-10450.

Murphy D., O`Brien B., Askari M.S., McCarthy T., Magee A., Burke R. and Murphy M. (2019) GrassQ – a holistic precision grass measurement and analysis system to optimize pasture based livestock production. In: ASABE Paper No. 1900769 *An ASABE Meeting Presentation. ASABE 2019 Annual International Meeting*. ASABE, St. Joseph, USA, pp. 2-9.

Estimating daily grass intake: first experience on five commercial dairy farms

Holshof G., Philipsen A.P. and Van Dixhoorn I.D.E.
Wageningen University and Research Centre, Wageningen Livestock Research, De Elst 1, 6708 WD Wageningen, the Netherlands

Abstract

As part of the 'Amazing Grazing' project, a model was developed to estimate grass intake at the herd level. The model uses data from the Nedap neck sensor (grazing activity) and specific cow-related parameters to calculate daily grass intake. The model was developed with data collected in 2015 to 2017 and was evaluated in 2018 on the experimental farm Dairy Campus, Leeuwarden, the Netherlands. In 2019 the application of the model was tested on five commercial dairy farms in the Netherlands. The pilot had three objectives: (1) to automatically estimate the daily grass intake, (2) to develop a way to (semi-) automatically update the farmer daily with the previous day's grass intake features, and (3) to evaluate how the grass intake results were used by the farmers. Automatic data flow acquisition could be established and a daily updated web-site was developed that showed graphically the grass intake at herd level. Some farmers used the daily results directly in their grazing management strategy. Other farmers found that daily grass intake estimates revealed extra valuable information that might be used in management and a good extra opportunity of making use of the sensor.

Keywords: grazing, grass intake, sensor data, farmer evaluation, data flow, advisory

Introduction

As part of the 'Amazing Grazing' project, a grass intake model at herd level was developed, based on data collected by Nedap neck and leg sensors, combined with cow parameters and herd management data from 2015-2017 on the experimental farm Dairy Campus in the Netherlands. The model was tested in 2018 on the same farm and results of testimated grass intake based on the grass intake model looked promising (Zom *et al.*, 2016). In 2019, a pilot was performed where the practical use and utility of the model was tested on five commercial dairy farms in the Netherlands. Objectives of this pilot were to test (1) whether daily acquisition of necessary data could be established automatically followed by automatic calculation of daily average fresh grass intake (kg dry matter (DM) cow^{-1} day^{-1}) of a grazing herd, (2) whether the estimated daily grass intake could be presented graphically to the participating farmer automatically and (3) how the added value of this rather new key figure was appreciated by the farmer in terms of potential influence on management decisions.

Materials and methods

In spring of 2019, five dairy farmers who grazed their herds daily during the grazing season were recruited to participate in this pilot. The cows were equipped with at least a neck sensor (Nedap smarttag Neck, Groenlo, the Netherlands) and cows of three of the five farms also were equipped with leg sensor (Nedap smarttag leg). Because the organisation of automatic daily data acquisition and processing was part of this pilot, farms were selected with fixed grazing times per herd (three farms with a milking parlour and a fixed milking and paddock access time) and herds with individual varying grazing times per cow (two farms with automatic milking system (AMS)). At the farms with an AMS, individual grazing time was determined by automatic detection of cows entering and leaving the paddock/stable. At all farms supplemental silage was fed and expected daily grass intake was between 3 and 8 kg DM cow^{-1} day^{-1}. Data acquisition, processing and graphical presentation of the calculated daily fresh grass intake at the three farms with milking parlour (Dairy Farms I in Figure 1) was arranged on a special platform (site)

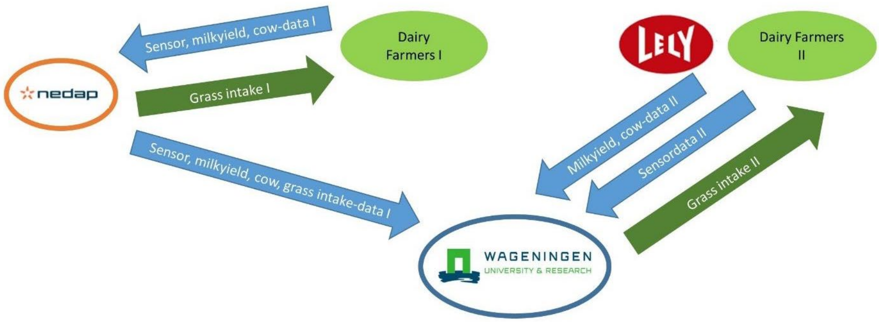

Figure 1. Visualisation of two different ways of data processing to generate daily estimation of grass intake.

provided by Nedap. Data acquisition from the farmers with an AMS (Dairy Framers II) was performed by Lely (Maasluis). Individual grazing times were calculated by Lely based on data of the AMS and Grazeway system (Lely). After this first step, the data files were sent to Wageningen Research where the daily fresh grass intake was estimated and reported to the farmers. The two different data processes are visualised in Figure 1.

At the end of the grazing season all five farmers were asked to fill in a questionnaire about their findings during the pilot. Questions were related to the following topics:

- accessibility of the figures;
- quality/reliability of the estimated grass intake;
- the application /use in farm management;
- availability of the data;
- overall opinion on this new feature.

Results and discussion

At the three farms with fixed grazing times per herd, acquisition and processing of sensor data, cow and farm data could be performed by Nedap using the Velos platform system. This facilitated automatic calculation of the daily grass intake. Nedap provided an extra service by building a platform, where farmers could login, to present the estimated daily grass intake graphically. In a figure, Nedap presented one graph of the grass intake of the last week and another graph with grass intake for the last 60 days. It was highlighted if grass intake was much lower or higher than the day before. Besides the grass intake, the average real grazing time of the herd was also presented. The estimated grass intake over the five pilot farms was on average 3.5 kg DM cow^{-1} day^{-1} which varied between from 2.6 to 3.9. The average estimated daily grass intake differed significantly between farms (P<0.001, standard error =0.091). The highest intake was estimated for April and May (across the total herd, the overall highest intake of fresh grass was on 7.4 kg DM cow^{-1} day^{-1}).

Processing data from the two AMS farms was more complex. Data came from different sources with different owners. Calculation of the estimated daily grass intake was performed by Wageningen Livestock Research. Therefore, it was first necessary to assess and connect the farm data from the management system (cow-related data) to the sensor data. Sensor data needed a first processing step, to select cows that had been in the paddock and calculate their individual grazing time. Within the selected farms with AMS, cows had free access to the paddock. So it was very important to know where each individual cow was located or, more precisely, whether a cow was in the paddock or in the stable. Cows were detected in the AMS and in a Grazeway (selection port). An algorithm to select cows and calculate time spent in the paddock based on data acquired by the AMS and Grazeway system was developed and performed by Lely. These data, sensor data and individual cow data (days in lactation, lactation number, milk yield) was automatically sent to Wageningen on a weekly basis. The next step was to combine semi-automatically these data sources and calculate the daily fresh grass intake. The last step was to make a report with a graph of the estimated daily grass intake of the previous week accompanied by some comments, which was sent by e-mail to the farmers (Farmer II). In the first two weeks of September this process was performed daily so that the farmers could also experience the potential added value of daily estimates of fresh grass intake. It is possible to develop a fully automatic data processing system also for farmers with AMS.

After one season with the key figure 'Fresh grass intake' participating farmers were asked to fill in a questionnaire about their findings. Farmers found the accessibility of the figures reasonable to good. Reasonable was argued by the fact that some farmers preferred to match directly the supplemental feeding ration during the night. All farmers found the reliability in the estimated grass intake good, or good enough to use in practical management. Farmers in this pilot used the data in very different ways, but mainly to finetune the feeding strategy. One farmer used this figure to look more often at the paddock and grassland management and one farmer suggested using this figure to benchmark the individual paddocks. Most farmers think there are far more possibilities to use these data and figures, for example to select typical grazing cows (or even use it in breeding programmes). All farmers indicated the added value of the use of the (neck) sensor.

Conclusions

The first step of the pilot was the acquisition and processing of data automatically. On farms with a milking parlour, this appeared to be relatively easy. On farms with an AMS, this will cost some extra effort, because more data owners are involved, but there are also good possibilities to develop a similar system. This pilot showed that the technical difficulties can easily be solved. All participating farmers found added value in having the new key figure of daily grass intake. They thought of it as the beginning of the introduction of a relatively new figure. There are likely to be more possibilities in the application of daily estimated grass intake that should be explored further by farmers, researchers and advisers.

Acknowledgements

We thank the participating famers, Nedap and Lely for providing data and Dutch Ministry of Agriculture, Nature and Food Quality and Zuivel NL for financial support.

References

Zom R., Van Der Werf J., Timmer B., Hoeksma D. and Van Reenen C (2016) Grazing behaviour in dairy cows as a predictor of grass intake. In: *Proceedings of the Abstracts of the 67th Annual Meeting of the European Association for Animal Production*, Belfast, UK, 29 August – 1 September 2016; p. 498.

Grazing time of a grazing dairy herd recorded by sensors on practical farms

Holshof G., Schils R.L.M., Philipsen A.P. and Van Dixhoorn I.D.E.
Wageningen University and Research Centre, Wageningen Livestock Research, De Elst 1, 6708 WD Wageningen, the Netherlands

Abstract

During the grazing season of 2019, the grazing activity of the herds on five farms was recorded. All farms had a high level of supplementary feed, resulting in a restricted residence time (and therefore grazing time). Nedap neck tags collected data from grazing cows, which were used to estimate daily grass intake per herd. Besides the estimated grass intake it was possible to calculate the actual eating time during the period cows were in the paddock. In this experiment cows spent on average (over 5 farms) 31% of their total time in the paddock as actual eating time, varying between individual farms from 18.4 to 38.5%. Combining actual eating time and residence time will give an estimation of the efficiency of grazing during the period a herd is in the paddock. Farmers may be better informed about the real grass intake, and residence time can be tuned better to grass intake, to avoid unnecessary losses. With these experiments we show that increasing availability of information about grazing can have a positive effect on the efficiency of grassland management in future.

Keywords: grazing, grass intake, recording intake, sensors

Introduction

Grazing is a low-cost strategy (De Klein, 2001) and improves consumer perceptions of the dairy sector (Boogaard *et al.*, 2011). However, Dutch farmers are often reluctant to graze dairy cows because grass intake is an uncertain and unknown factor. Within the 'Amazing Grazing' project a model was developed to estimate the daily grass intake of a grazing herd, based on behavioural data collected with neck and leg tags (Zom *et al.*, 2016). Furthermore, these sensor data can also provide information about actual eating time. We expect that actual eating time will provide farmers with more insight about grazing efficiency in comparison to the much-used residence time. In 2019, sensor data from grazing herds with a high level of supplementary feed were collected on five Dutch dairy farms, to discover the relation between residence time, actual grazing time and grass intake. This information will lead possibly to a more efficient way of grazing in future.

Materials and methods

In 2016 and 2017, a grazing experiment was carried out at the 'Dairy Campus' research farm, Leeuwarden, where data were collected to develop a model to estimate daily grass intake. This model was used in 2019 on five commercial dairy farms in the Netherlands. The herd was equipped with a Nedap Neck sensor and on two farms also with a Nedap leg sensor. Sensor data were recorded daily and used to estimate a daily herd grass intake. The five farms differ in herd size, grazing time, grazing system and amount of supplementary feed. Table 1 gives a brief overview of the farms in this project. On three farms, cows had restricted access to the paddock during the daytime, between milkings in the milking parlour. Therefore, information at the beginning and end of a daily grazing period was easy to collect.

On two farms, an automatic milking system (AMS) was used and the daily start and end time of grazing was collected for each cow by the Grazeway (automatic fence) combined with the AMS selection port. On the other farms, start and end time was collected by the leg sensor or provided by the farmer. As the required recording technology was not available on the same date for every farm, the recording period

Table 1. Farm characteristics in the monitoring period.[1]

Farm	Cows (n)	Supplementary forage (kg DM cow^{-1} day^{-1})	Start date of recording	End date recording	Grazing system	Milk production (kg cow^{-1} day^{-1})	Grass intake (kg DM cow^{-1} day^{-1})
1	123	17.6	08/04	06/10	CCG	30.4	3.6
2	131	9.9	26/04	07/10	RG	29.6	3.9
3	175	9.2	20/04	02/11	RG	26.5	3.6
4	178	11.6	19/06	01/09	CCG	30.7	2.6
5	95	9.4	21/05	01/09	CCG	26.6	3.3

[1] CCG = continuous compartment grazing; RG = rotational grazing; DM = dry matter.

was not the same for all five farms. Data were analysed with the restricted maximum likelihood (REML) method (Harville, 1977), using Genstat (18th edition).

Results and discussion

The average daily residence time in a paddock for the five farms was 6.6 h, varying from 5.9 to 10.7 h. The 10.7 h was on the organic farm. Due to a drought period, none of the farms could graze continuously throughout the grazing season. As result of a shortage of fresh grass, cows were kept inside for some periods on all five farms. No data were collected during those periods. The actual eating time per day, collected by the neck tag, was on average 2.25 h, varying from 1.45 h to 2.69 h. The overall average estimated daily grass intake was 3.5 kg dry matter (DM) cow^{-1} day^{-1}, varying over the five farms from 2.6 to 3.9 kg DM cow^{-1} day^{-1}. The relatively low grass intake fits well with the high level of supplementary feeding of around 10 kg DM cow^{-1} day^{-1} (Table 1). Farm 1 showed a strongly deviating amount of supplementary feeding but also a relatively high calculated grass intake, which cannot be explained. With both key figures (daily residence time and daily actual eating time) the percentage of time that cows really graze can be calculated by:

Percentage actual eating time = (daily actual eating time / daily residence time) × 100.

This can be seen as a measure for grazing efficiency. Figure 1A shows there was a large variation in the relation between residence (access) time and actual eating time. The grazing efficiency varied from almost zero to around 50%. Farms differ in actual eating time (P<0.001, standard error (se)=0.058) as well the number of residence hours (P<0.001, standard error (SE) = 0.1447)). Grazing activity of the herd varies between farms, within the season and even from day to day (Delagarde and Lamberton, 2015). Figure 1B shows how efficient the herd is grazing during their stay in the paddock. Overall, the efficiency declines during the growing season ($P<0.001$, se=0.0076), which means that in autumn the cows are spending

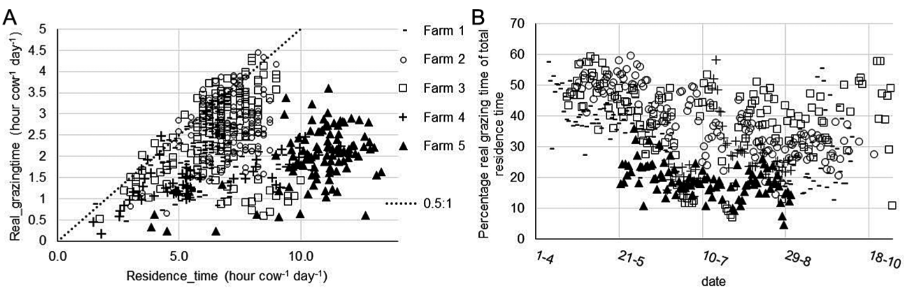

Figure 1. (A) Relation between actual eating time and residence time and (B) percentage actual eating time of residence time throughout the grazing season.

less time at actual eating, but this also varies between farms. The dry season also had effect on the grass allowance. In this pilot study, the farm with largest residence time had the lowest grazing efficiency.

As a practical rule of thumb, residence time is often used as a rough proxy for fresh grass intake This pilot study shows that the relation between actual eating time and residence time is not so straightforward; therefore, actual eating time seems to be a better predictor for estimating fresh grass intake than residence time in a paddock. In this pilot study an average intake of 1.58 kg DM of grass cow^{-1} actual eating h^{-1} was calculated, using actual eating time as one of the estimate factors. Combining this with the real residence time, this means 0.5 kg DM of grass intake cow^{-1} residence h^{-1}. But there are a lot of differences between farms, days and within the season. This pilot study clearly showed that the time a herd has access to a paddock is not directly correlated to the actual eating time. Our conclusions might be affected by some uncertainties in the study. There were some data with high leverage. In the case of the long residence time in relation to a short period of actual eating on farm 5, the automatic detection of the Grazeway was probably not always accurate; this would affect the calculated actual eating time. In this pilot no additional measurements, such as grass height, feeding value of the fresh grass, exact allowance, had been carried out on the farms, so no clear explanations for the large differences can be formulated.

Conclusions

For grazing dairy cows, actual eating time differs strongly from day to day and between farms. Recording actual eating time presents a better insight of grazing behaviour and is a more accurate factor to calculate daily grass intake than residence time of a herd in a paddock.

Acknowledgements

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References

Boogaard B.K., Bock B.B., Oosting S.J., Wiskerke J.S.C. and Van Der Zijpp A.J. (2011) Social acceptance of dairy farming: the ambivalence between the two faces of modernity. *Journal of Agricultural and Environmental Ethics* 24, 259-282.

De Klein C.A.M. (2010) An analysis of environmental and economic implications of nil and restricted grazing systems designed to reduce nitrate leaching from New Zealand dairy farms. II. Pasture production and cost/benefit analysis. *New Zealand Journal of Agricultural Research* 44, 217-235.

Delagarde R. and Lamberton P. (2015) Daily grazing time of dairy cows is recorded accurately using the Lifecorder Plus device. *Applied Animal Behaviour Science* 165, 25-32.

Harville D.A. (1977) Maximum likelihood approaches to variance component estimation and to related problems. *Journal of the American Statistical Association* 72, 320-338.

Zom R., Van Der Werf J., Timmer B., Hoeksma D. and Van Reenen C (2016) Grazing behaviour in dairy cows as a predictor of grass intake. In *Abstracts of the 67th Annual Meeting of the European Association for Animal Production*, Belfast, UK, 29 August – 1 September 2016; p. 498.

How to get cows back to pasture? Current trends in livestock systems and perspectives for improving grazing system with an holistic framework

Horn J.[1] and Isselstein J.[1,2]

[1]Grassland Science, Department for Crop Sciences, University of Goettingen, Von-Siebold-Str. 8, 37075 Goettingen, Germany; [2]Centre of Biodiversity and Sustainable Land Use, University of Goettingen, Büsgenweg 1, 37077 Goettingen, Germany

Abstract

Nowadays, livestock production depends heavily on feed grown on arable land. In this study evidence from literature was compiled on current trends. Pork production needs about 128.2 m^2 of arable land to produce one kilogram of human-edible protein, followed by 36 m^2 of arable land for chicken, 30 m^2 for beef production and 17.5 m^2 for dairy milk. Feed grown on grassland contributes to 89.4 $m^2$of grassland for beef production and 25 m^2 for milk production. In many European countries there has been a sharp decline in livestock grazing. There is an ongoing stagnation in the development of precision farming technologies for grazing systems. To achieve the shift towards a sustainable and economically attractive grazing livestock system, an integrated framework combing innovative technologies and concepts is required. The inter-disciplinary project GreenGrass focuses on the development of innovative grazing systems by using novel technologies such as virtual fences and remote sensing in order to bring cows back to pasture.

Keywords: livestock systems, land-use, precise spatiotemporal pasture management, precision farming

Introduction

Nowadays we are confronted with an immense and emotionally charged debate about food production. Livestock production competes for arable land, emits greenhouse gases and increases the risk of biodiversity losses (Taube *et al.*, 2014). In recent decades, the keeping of monogastric species (pork, poultry) has overtaken the role of ruminants (cattle, sheep). However, in many European countries dairy systems are mostly detached from grazing land. Today, feed intake of dairy cows depends heavily on silage and concentrated feed (FAO *et al.*, 2014; Schingoethe, 2017) from intensively managed grassland and arable land. To counteract the dependence on arable crops and to exploit the potential of grasslands, we recommend bringing cows back to pastures in a sustainable manner. The shift to viable and sustainable grazing livestock systems needs a holistic, multi- and interdisciplinary approach using innovative herd management and remote sensing technologies for an timely precise pasture management.

Materials and methods

We evaluated scientific articles (reviews and original research) in Web of Science and databases of livestock production systems and the dairy sector to indicate the current trends. In addition, we searched for scientific articles on innovative precision farming technologies for pasture management. Overall, the literature research resulted in a dataset containing information of feed intake and efficiency, land-use, production, and emissions for European countries and ten regions around the world, for four livestock species categories (cattle, small ruminants, pork and poultry), and three products (milk, meat, eggs).

Results and discussion

For pork production, the area of arable land required to obtain one kilogram of human-edible protein is about 128.2 m^2. This is followed by 36 m^2 of arable land for chicken, 30 m^2 for beef production and

17.5 m^2 for dairy milk. The feed intake of pork and chicken depends mainly on maize (30 and 34%), wheat (for both 18%) and soy meal (13 and 21%) all grown on arable land. Pork and chicken production rely on only very small amounts of conserved forage. Feed grown on grassland contributes to 89.4 m^2 of grassland for 1 kg of beef production and 25 m^2 for 1 kg milk production (Figure 1). Globally, the feeding basket of the dairy cows mainly consists of roughage (67%), whereas cereals (9%), compounds (12%) and by-products such as soy meal (11%) make up a smaller feeding fraction. However, in many European countries the role of pastures for cattle grazing has decreased dramatically. For instance, in the Netherlands, Hungary, Spain, Austria, France and Germany the pasture area has decreased by up to 22% over the past decades. In these countries, zero-grazing and housing in tie-stalls are common practices for dairy cows, and exceeding 50% of the dairy systems in Germany, Denmark, Italy and Spain. In Denmark, Germany and Austria fresh forage from pasture contributes only up to 7% of total feed intake, with maize and grass silage completing the feeding basket (up to 70% in Germany), followed by cereals, soymeal and compounds (FAO, 2018; FAO *et al.*, 2014; Flachowsky *et al.*, 2017; Steinfeld *et al.*, 2006).

Perspectives for grassland management

In order to reduce the environmental risks from further expansion and intensification of fodder production on arable land for feeding cows, sustainable systems based on grazing landf with ruminants offers a good chance. Precise and efficient pasture management throughout the grazing season has the potential to meet most of the nutritional needs of cattle through fresh grass while reducing the amount of supplementary feed. This requires the farmer to have precise spatiotemporal information on the available biomass and quality on the pasture to feed the cows sufficiently and to optimise herbage utilisation through precise animal distribution. However, precision farming for managing grazing livestock is rare and developments of novel technologies have stagnated over recent years. Our literature research indicates that precision technologies for measuring and improving inter- and intra-paddock efficiency of pasture systems as well as for management of grazing cows are rare. Cow management is restricted to automatic milking and walk over weighing systems, herd management software, and electronic cow identification systems (French *et al.*, 2018; Gargiulo *et al.*, 2017). However, new technologies are now becoming more available, and advances are being made in automated technologies of virtual fencing and remote sensing. In total, we made a preliminary evaluation of 89 studies of current developments and advances in virtual fencing and sensory or GPS-based systems for controlling and monitoring animal movement and behaviour, and advances in remote sensing technologies for monitoring and evaluation of grasslands biomass and habitat structures. Concerted development, combination and integration of these two techniques embedded in a holistic framework integrating the needs of different stakeholders,

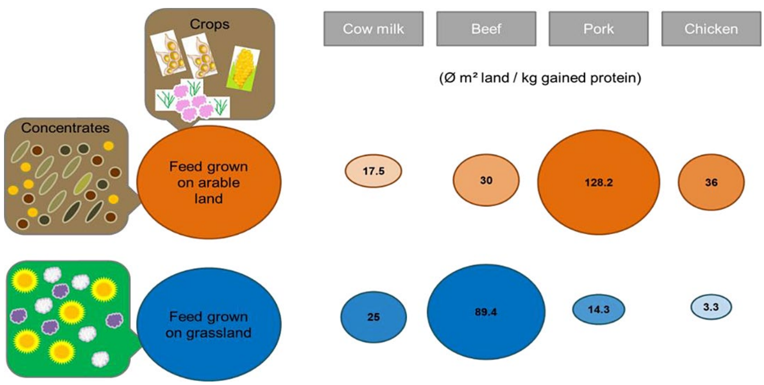

Figure 1. Land use of arable land and grassland for feeding dairy cows, beef cattle, pigs, and poultry (m^2) needed to gain one kilogram of protein (data derived from FAO, 2018; FAO *et al.*, 2014; Flachowsky *et al.*, 2017).

the economic evaluation of production costs and outcomes of premium products and public goods as well as on-time information about the pasture state for a targeted management is particularly promising for innovative and sustainable grazing systems. Virtual fences can control the accessibility of the area for the grazing animals. The information required for setting and changing the fences comes from remote sensing. Recent advances in remote sensing with constantly improving sensor technology allow the spatial distribution of habitats and plant communities as well as the biomass and quality of forage crops to be recorded in high spatial and temporal resolution. Depending on the purpose of the land use, the fences can be set variably; biodiversity can be maintained and promoted, and at the same time, adequate quality feed can be offered in appropriate quantities. The BMBF-project GreenGrass develops and integrates forward-looking technologies to design and test new sustainable grazing systems in a holistic way.

Conclusions

The grazing of ruminants is socially desirable and has a high potential to ensure the nutritional supply of animals. A knowledge-based grazing regime might be able to reduce conflicts between agricultural production and environmental protection. Modern techniques of 'smart precision farming' must be used to support the development of economically and environmentally sustainable pasture systems in a multi- and interdisciplinary approach. The project GreenGrass has recently started to develop novel and sustainable pasture concepts with ruminants under the development of innovative technologies in a transformative and integrative approach and evaluation of their practical potential.

Acknowledgements

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References

FAO, IDF, IFCN (2014) World mapping of animal feeding systems in the dairy sector. Food and Agriculture Organization of the United Nations (FAO), International Dairy Federation (FIL-IDF; Brussels, Belgium), IFCN Dairy Research Network (Kiel, Germany), FAO, Rome, Italy.

FAO (2018) Food and Agriculture Organization of the United Nations (FAO). FAOSTAT Database.

Flachowsky G., Meyer U. and Südekum K.-H. (2017) Land use for edible protein of animal origin – a review. *Animal 7,* 25.

French P., O'Brien B. and Shalloo L. (2015) Development and adoption of new technologies to increase the efficiency and sustainability of pasture-based systems. *Animal Production Science* 55, 931-935.

Gargiulo J.I. *et al.* (2018) Dairy farmers with larger herd sizes adopt more precision dairy technologies. *Journal of Dairy Science* 101, 5466-5473.

Schingoethe D.J. (2017) A 100-year review: total mixed ration feeding of dairy cows. *Journal of Dairy Science* 100, 10143-10150.

Steinfeld H., *et al.* (2006) Livestock's long shadow. *The Livestock, Environment and Development Initiative (LEAD).* Rome, FAO.

Taube F., Gierus M., Herrmann A., Loges R. and Schönbach P. (2014) Grassland and globalization – challenges for northwest European grass and forage research. *Grass and Forage Science* 69, 2-16.

A comparison of once and twice a day milking in a high grass input, low concentrate input grazing system

O'Donovan M.[1], Murphy J.P.[1], Delaby L.[2], Casey T.[1] and Kennedy E.[1]
[1]*Teagasc, Animal and Grassland Research Centre, Moorepark, Fermoy, Co.Cork, Ireland;* [2]*INRA, ArgoCampus Ouest, UMR 1348, Physiologie, Environement et Genetique pour L'Animal et les Systemes d'Elevage, 35590 Saint Gilles, France*

Abstract

Once a day (OAD) milking is growing as a strategy to manage dairy cows in Ireland. While twice a day milking is the standard milking frequency, many farmers have grown herd size, but the lack of skilled labour and increased seasonal workload has made OAD milking a viable option for a proportion of dairy farmers in Ireland. A study was set up in spring 2019 at Teagasc Moorepark, 85 spring calving cows were blocked on calving date, lactation number, previous lactation milk yield, bodyweight and pre calving body condition score. They were assigned to one of five treatments (n=17), twice a day milking (TAD), once a day for 2, 4 and 6 weeks directly post calving and full time OAD. The comparative milk production differences for 210 days into lactation show the TAD cows produced 5,198 kg milk with milk fat 45.3 g kg^{-1} and milk protein 34.7 g kg^{-1}, the OAD herd produced 3,960 kg milk, milk fat 52.7 g kg^{-1} and milk protein 37.3 g kg^{-1}, respectively. The level of concentrate offered to the herd was approximately 360 kg per cow. The results of this study show that OAD milking frequency is a realistic option for many farmers providing that grazing and livestock management levels are maintained at a high level.

Keywords: milking frequency, grass, spring calving

Introduction

Recent Ireland CSO figures show the number of dairy cows in Ireland has increased to 1.4 million. ICBF have also released six-week calving rates for 2018 showing a 6% increase since 2016. A greater number of cows calving more compactly mean increased workload for the farmer during the spring period. A number of farmers are now actively investigating the option of once-a-day (OAD) milking during spring or during the entire lactation. A survey conducted on 50 farms in the Southern region of Ireland in 2017 showed that almost 10% of the herds are OAD in the early part of lactation.

While twice-a-day (TAD) milking is accepted as the standard milking frequency, more dairy farmers are challenging this, as there is increased workload due to cows calving (including numerous night calvings), calf feeding and difficulties employing suitable staff. Although OAD milking offers numerous benefits such as improved energy balance due to lower bodyweight and body condition score (BCS) loss which can result in improved fertility performance, there are potential drawbacks. These include a reduction in milk production and increased somatic cell count (SCC), which may result in decreased farm profitability.

To date, experiments examining the effect of milking frequency on dairy cow production have reported average milk production losses of approximately 22%; however, this ranges from 7 to 40% in short-term experiments and up to 50% in full-lactation studies (Stelwagen *et al.*, 2013). Reductions in milk production tend to be greater when OAD milking frequency is practised in early lactation.

The objective of this study was to investigate the effects on milk production of OAD milking frequency for two, four or six weeks in early lactation, or OAD milking for the entire lactation compared to a fulltime twice-a-day milking frequency.

Materials and methods

In spring 2019, during the dry period, a group of dairy cows were pre-assigned to 5 milking treatments pre-calving. The treatments were twice a day milking (TAD), once a day milking for the first 2 weeks of lactation (OAD2), once a day milking for the first four weeks of lactation (OAD4), once a day milking for the first 6 weeks of lactation (OAD6) and once a day for the full lactation (OAD). Calving started on 30 January. Mean calving date of the individual herds was 20 February. Each herd had 23% first lactation animals; herd size was 17 cows per treatment. Average herd EBI (economic breeding index) was €164. For the purposes of this paper, the first 30 weeks of lactation are reported. As cows calved they were assigned to their respective treatments. TAD cows were milked at 07:00 and 16:00, OAD cows were milked in the morning only (07:00). All OAD cows were milked OAD until their respective date to commence TAD milking was reached. Cows were managed as two herds – one herd was milked TAD and the other herd was milked OAD; as cows moved from OAD to TAD they switched between herds. Both herds grazed individually but adjacent to one another to ensure swards of similar pasture composition were offered. Herbage mass (HM; >4.0 cm) was measured twice weekly by cutting six strips (120 m^2) per grazing area. Pre- and post-grazing heights were measured daily. Equal quantities of fresh pasture were allocated to both herds twice daily. Cows were offered high levels of fresh grass from calving, and remained on low concentrate levels, concentrate input never increased >4 kg per cow day. Grass herbage allowance was targeted at 18 kg DM per cow day throughout the main grazing season. The OAD cows received their concentrate at morning milking only. They remained in their respective paddock each day, while the rest of the herd were milked.

Data were analysed using covariate analysis and mixed models in SAS v9.4. Terms for parity and treatment were included in the model. Pre-experimental values and days in milk were used as covariates in the model.

Results and discussion

There was a significant effect of milking frequency on milk yield ($P<0.001$), milk fat ($P<0.05$) and milk solids yield ($P<0.001$) (Table 1). Fulltime OAD milking significantly reduced milk yield and milk solids yield, but significantly increased milk fat content. There was no difference between treatments in milk protein and lactose content. OAD in early lactation did not affect milk production performance over the first 30 weeks of lactation, and cows exposed to OAD in early lactation tended to respond positively in milk output subsequent to returning to TAD milking (Figure 1). Management of the lactation curve in OAD would seem to be very important. Once-a-day milking requires a high level of management in terms of ensuring cows are offered correct pre-grazing yield (~1,500 kg DM ha^{-1} >4 cm) and graze paddocks to a target post grazing residual of 4 cm. During this 30-week period mean concentrate input was 340 kg per cow.

Table 1. Milk production performance up to 30 weeks in lactation.[1]

	Twice a day milking	OAD – 2 Weeks	OAD – 4 Weeks	OAD – 6 Weeks	Full time OAD	SED	Significance
Milk yield, kg cow^{-1} day^{-1}	22.2	21.5	20.2	20.4	16.8	0.036	***
Milk fat, %	4.50	4.94	4.69	4.80	5.01	0.006	*
Milk protein, %	3.49	3.63	3.52	3.62	3.71	0.003	NS
Milk lactose, %	4.73	4.73	4.73	4.74	4.56	0.001	NS
Milk solids, kg cow^{-1} day^{-1}	1.76	1.83	1.65	1.71	1.46	0.003	***

[1] OAD = once-a-day milking; SED = standard error of the difference; NS = not significant; * $P<0.05$; *** $P<0.001$.

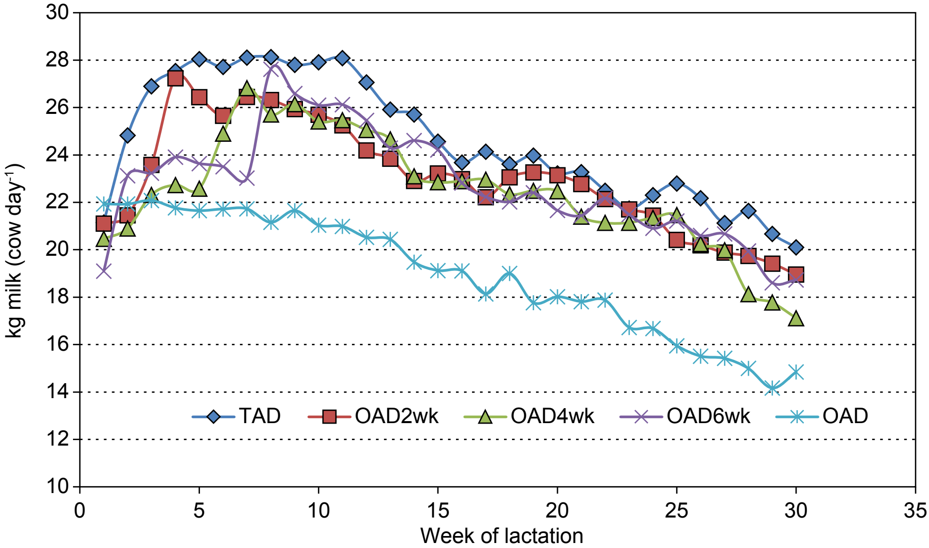

Figure 1. Milk production profile of spring calving cows milked twice a day, once a day for 2, 4 and 6 weeks and once a day full time (first 30 weeks of lactation illustrated).

Conclusions

The results of this study indicate that full time OAD milking reduces milk yield (-24%) and milk solids production (17%) during the first 30 weeks of lactation. OAD may suit some farmers as a strategy to reduce farm workload, however, high levels of grazing and milking management are required in order to successfully achieve reasonable levels of milk output from OAD milking.

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References

Central Statistics Office (2019) Livestock survey 2018. Available at: https://www.cso.ie/en/releasesandpublications/er/lsd/livestocksurveydecember2018/

Stelwagen K., Phyn C.V.C., Davis S.R., Guinard-Flament J., Pomiès D., Roche J.R. and Kay J.K. (2013) Reduced milking frequency: milk production and management implications. *Journal of Dairy Science* 96, 3401-3413.

Predicting whole-crop cereal yield and nutritive value in Northern Sweden

Persson T.[1], Höglind M.[1], Wallsten J.[2], Nadeau E.[3], Huang X.[1] and Rustas B.-O.[4]

[1]Norwegian Institute of Bioeconomy Research (NIBIO), 4353 Klepp Stasjon, Norway; [2]Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, SLU, NJV, 901 83 Umeå, Sweden; [3]Swedish University of Agricultural Sciences, Department of Animal Environment and Health, P.O. Box 234, 532 23 Skara, Sweden; [4]Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, P.O. Box 7024, 750 07 Uppsala, Sweden

Abstract

Harvesting of cereals for whole-crop silage is an alternative to conventional grain harvest at full maturity. Harvest of whole-crop cereals can reduce the risk of crop losses due to unfavourable conditions later in the growing season, such as drought reducing grain filling or wet conditions making the harvest difficult. Whole-crop cereals can also be a buffer feed in years with grass shortage due to winter kill or drought. The developmental stage at cutting affects the yield and nutritive value of whole-crop cereals. We adapt a weather-driven simulation model for forage grass to predict whole-crop cereal yield and nutritive value. Fibre content (neutral detergent fibre), digestibility, and crude protein content from samples of spring barley (cv. Anneli and cv. Judit) harvested at Zadoks growth stage 59, 75-77, 83-85 and 89 in field variety trials at Ås (63°15'N; 14°34'E), Lännäs (63°10'N; 17°38'E) and Röbäcksdalen (63°48'N; 20°12'E), Sweden are being analysed in 2019 and 2020. Data from these experiments and from previous experiments are used to adapt, calibrate and test the whole-crop model. The model is planned to be incorporated into the prognosis system for forage crops in Sweden (www.vallprognos.se).

Keywords: barley, harvest security, silage, simulation model, weather variability

Introduction

In regions with short growing seasons, harvesting of cereal crops for whole-crop silage is an alternative to conventional grain harvest at full maturity. Harvest of whole-crop cereal can reduce the risk of crop losses due to unfavourable conditions later during the growing season, such as drought reducing grain filling, or wet conditions that make harvesting difficult. Process-based simulation models, which are driven by weather, soil and management variables, are used to predict growth, yield (Ewert *et al.*, 2015) and composition of cereal crops (Nuttall *et al.*, 2017) and forage grasses (Kipling *et al.*, 2016).

The sensitivity of the characteristics of whole-crop cereals, which include vegetative and non-mature grain biomass, to weather and soil conditions, and to management practices call for simulation models, which are specifically developed for this crop. Such models could be used to adapt the whole-crop management, including cutting time and fertilisation, to reach biomass yield and quality goals, which in turn can facilitate the planning of forage crop growth and livestock feeding management.

The Basic Grassland (BASGRA) model was developed to simulate the growth (Höglind *et al.*, 2016) and crude protein (CP) concentration, neutral detergent fibre (NDF) concentration and NDF digestibility of timothy (Höglind *et al.*, 2020), a major forage grass in high latitude regions. Here, we adapt this model to simulate whole-crop barley, the most commonly grown cereal species in high latitude regions with short growing seasons. Calibration and evaluation of the model is being carried out against yield and nutritive value data from variety trial fields in northern Sweden, and data from previously published studies of whole-crop characteristics and feeding value.

Materials and methods

Samples from barley (cv. Anneli and cv. Judit) were taken at Zadoks growth stage 59, 75-77, 83-85 and 89 in field variety trials at Ås (63°15'N; 14°34'E), Lännäs (63°10'N; 17°38'E) and Röbäcksdalen (63°48'N; 20°12'E) in northern Sweden in 2019. Samples were dried at 60°C and milled through a Kamas Slagy 200 B hammer mill (Kamas, Malmö, Sweden) with a 1 mm screen for further analysis. Dry matter was determined after drying at 103 °C, and ash after heating the samples to 550 °C for 3 h. Total N was analysed following the Kjeldahl procedure with digestion in a Kjeltec 2460 device (Tecator, Höganäs, Sweden). CP was calculated as N content ×6.25. NDF was analysed using an undiluted detergent solution, α-amylase, and sodium sulphite (Chai and Udén, 1998). *In vitro* organic matter digestibility was determined after 96 h of incubation in a buffered rumen fluid (Lindgren, 1979). The same sampling and analyses are planned for 2020.

The BASGRA model for timothy grass is being modified to simulate whole-crop cereals. In this model, growth and development are simulated as a function of the weather, soil and crop management. The plant carbon and nitrogen are divided into pools for leaves, stems, roots and reserves. The dynamics between these plant parts are affected by outer environmental factors as well as plant phenological stage, and water and nitrogen status. Whole-crop cereals are harvested at a developmental stage when a part of the carbon and nitrogen is allocated to grains. To simulate the growth and development of grains and their nitrogen content, a grain pool was introduced along with equations for direct allocation of carbon in carbohydrates that have been newly photosynthesized, and for translocation of carbon and nitrogen from other pools to the grain pool. Leaf senescence during the grain filling stage, which triggers translocation of carbon and nitrogen from leaves to grains, is also being added.

Probability distributions of the new parameters that regulate these processes as well as for parameters regulating other processes that are not modified from the previous version of BASGRA are calibrated against data from the variety trial samples in 2019 and 2020 applying Bayesian calibration techniques (Van Oijen *et al.*, 2005). CP, NDF and digestibility data from previous whole-crop barley experiments (Nadeau *et al.*, 2007; Rustas *et al.*, 2011; Wallsten *et al.*, 2010) are also included in the calibration. Part of the set of whole-crop barley data was saved for testing of the prediction accuracy of the model.

Daily weather input data including maximum and minimum temperature, precipitation, solar radiation, relative air humidity and wind speed for the simulations are obtained from the Swedish Meteorological and Hydrological Institute (SMHI) through the Field Research Unit (Fältforsk) of SLU. Input on soil water holding characteristics for the experimental sites that are needed in the BASGRA model is either taken from direct measurements or derived from information about soil texture fraction distribution, bulk density and organic matter content.

Results and discussion

This paper presents an ongoing project where a grassland model is adapted to predict the yield and nutritive value of whole-crop barley. In 2019, the measured crude protein and fibre content decreased with increased developmental stage for both cultivars at all three locations. We plan to show preliminary results from model calibrations and tests at the General Meeting of EGF in 2020. Weather driven models are already used in prognosis system for forage yield and nutritive value of forage crops in Sweden and other countries. Whole-crop cereals are a complement to traditional forage crops, especially perennial grass and legume mixtures. More variable weather conditions with increased heat and drought stress could trigger a larger fraction of whole-crop silage in animal feeding programmes. Inclusion of whole-crop cereal models in prognosis systems could improve prediction accuracy and hence also the feeding standard.

Conclusions

This paper presents a study in which the BASGRA model is adapted to predict whole-crop cereal yield and nutritive value under high-latitude climate conditions. Given that adequate model prediction accuracy can be achieved, the adapted model could be implemented in the prognosis system for forage crops.

Acknowledgements

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References

Chai W.H. and Udén P. (1998) An alternative oven method combined with different detergent strengths in the analysis of neutral detergent fibre. *Animal Feed Science Technology* 74, 281-288.

Ewert F., Rötter R.P., Bindi M., Webber H., Trnka M., Kersebaum K.C., Olesen J.E., Van Ittersum M.K., Janssen S., Rivington M., Semenov M.A., Wallach D., Porter J.R., Stewart D.,Verhagen J., Gaiser T., Palosuo T., Tao F., Nendel C., Roggero P.P., Bartošová L. and Asseng S. (2015) Crop modelling for integrated assessment of risk to food production from climate change. *Environmental Modelling & Software* 72, 287-303.

Höglind M., Cameron D., Persson T., Huang X. and Van Oijen M. (2020) BASGRA_N: a model for grassland productivity, quality and greenhouse gas balance. *Ecological Modelling* 417, 108925.

Höglind M., Van Oijen M., Cameron D. and Persson T. (2016) Process-based simulation of growth and overwintering of grassland using the BASGRA model. *Ecological Modelling* 335, 1-15.

Kipling R.P., Virkajärvi P., Breitsameter L., Curnel Y., De Swaef T., Gustavsson A-M., Hennart S., Höglind M., Järvenranta K., Minet J., Nendel C., Persson T., Picon-Cochard C., Rolinski S., Sandars D.L., Scollan N.D., Sebek L., Seddaiu G., Topp C.F.E., Twardy S., Van Middelkoop J., Wu L. and Bellocchi G. (2016) Key challenges and priorities for modelling European grasslands under climate change. *Science of the Total Environment* 566-567, 851-864.

Lindgren E. (1979) *The nutritional value of roughages estimated in vivo and by laboratory methods*. Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Nadeau E. (2007) Effects of plant species, stage of maturity and additive on the feeding value of whole-crop cereal silage. *Journal of the Science of Food and Agriculture* 87: 789-801.

Nuttall J., O'Leary G., Panozzo J., Walker C., Barlow K. and Fitzgerald G. (2017) Models of grain quality in wheat – a review. *Field Crops Research* 202, 136-145.

Rustas B.-O., Bertilsson J., Martinsson K., Elverstedt T. and Nadeau E. (2011) Intake and digestion of whole-crop barley and wheat silages by dairy heifers. *Journal of Animal Science* 89, 4134-4141.

Van Oijen M., Rougier J. and Smith R. (2005) Bayesian calibration of process-based forest models: bridging the gap between models and data. *Tree Physiology* 25, 915-927.

Wallsten J., Bertilsson J., Nadeau E. and Martinsson K. (2010) Digestibility of whole-crop barley and oat silages in dairy heifers. *Animal* 4, 432-438.

Evaluation of a decision support tool for the *in situ* prediction of grass wilting time

Pickert J.[1], Hecker M.[2], Brüning D.[3], Hoffmann T.[4], Frühauf C.[5], Weise G.[6] and Wellenbrock K.-H.[6]
[1]Leibniz Centre for Agricultural Landscape Research, Eberswalder Str. 84, 15374 Müncheberg, Germany; [2]agrathaer GmbH, Eberswalder Str. 84, 15374 Müncheberg, Germany; [3]Landeslabor Berlin-Brandenburg, Rudower Chaussee 39, 12489 Berlin, Germany; [4]Leibniz Institute for Agricultural Engineering and Bioeconomy Potsdam-Bornim, Max-Eyth-Allee 100, 14469 Potsdam, Germany; [5]German Meteorological Service. ZAMF, Bundesallee 33, 38116 Braunschweig, Germany; [6]Paulinenauer Arbeitskreis Grünland und Futterwirtschaft e.V., Gutshof 7, 14641 Paulinenaue, Germany

Abstract

In Germany, many farmers still fail to reach the optimum dry matter (DM) content in their grass silages. In 2007-2018, only 34% of all grass silages in the state of Brandenburg had DM contents in accordance with the main recommendations for grass silage production. The model 'WILT-EXPERT' was developed to support farmers in predicting the optimal wilting time for the recommended DM *in situ* in real time. This paper presents the results of a study which evaluated the success of 'WILT-EXPERT' during 64 wilting periods on 9 farms in northeast Germany between 2015 and 2019. At a Mean Absolute Error of 74 minutes, the results reveal that the model could be used satisfactorily by the farmers as a decision support tool during practical grass silage production. We recommend the practical application of the tool via a smartphone app to support flexible decision-making in the field. Additional work is recommended for better coverage of very hot and dry situations.

Keywords: grass silage, prediction model, decision support tool, smartphone app

Introduction

In Land Brandenburg (Germany), the dry matter (DM) content of grass silages has mainly varied between 25 and 60% during the last 12 years. Complying with the general recommendation to wilt cut grass in the field up to a DM content of about 30 to 45% (BAF 2013, PAGF 2012) is still a problem of grass silage production. In some countries, e.g. Norway and Denmark, farmers and advisers can make use of models for predicting the optimum wilting time during grass silage production (SEGES, 2019; VIPS, 2019). A similar model, 'WILT-EXPERT', was developed in northeast Germany to support farmers in predicting the adequate wilting time necessary for the recommended DM content on each single field under the actual weather situation on the farm. During an experimental phase in 2015, 'WILT-EXPERT' predicted the wilting time from mowing to ensiling with a Mean Absolute Error (MAE) of 108 minutes at a Modelling Efficiency (EF) of 0.99 (Pickert *et al.,* 2016). In the subsequent years till 2019, model testing was extended and comprehensive data were collected on farm under various conditions as a basis for a decision support tool. Since 2019 the tool is in field trial in a smartphone app and will be introduced into farming practice in 2020. This paper presents the results of the wilting experiment conducted under various weather conditions.

Materials and methods

In northeast Germany, a grass wilting experiment was conducted on 9 farms during different grassland cuts from 2015 to 2019. The experiment covered 64 grass-wilting periods, which started at mowing and ended at ensiling. To describe the applied harvest process, the farmers reported fresh matter yield, type and use of mower, conditioner and tedder, mowing and swath width, mowing and ensiling date and time, and also rainfall. Grass samples were taken at the same locations of the field at mowing and at ensiling for DM content determination by oven drying. Weather data (evapotranspiration and precipitation) for the different locations were provided by Germany's National Meteorological Service. The observed DM content at mowing, the time of mowing, and the observed DM content at ensiling (serving as the target DM content) were used as model inputs. The predicted time required for wilting from observed DM content at mowing till observed DM content at ensiling was compared with the observed wilting time. In order to characterize the predictive ability of the model, MAE, Root Mean Square Error (RMSE) and EF (Nash and Sutcliffe, 1970) were calculated. In 15 out of 64 trials, grass had more than 30% DM and almost reached the target DM content for grass silage without wilting already at mowing. Those wilting periods were not involved in the calculations.

Results and discussion

The weather during the wilting experiments varied between cool temperatures in 2015 and 2016, very high rainfall in summer 2017 and very high temperatures combined with severe drought in 2018 and 2019. The average daily evapotranspiration ET_{pTW} (Turc-Wendling; Wendling *et al.,* 1991) during all wilting periods amounted to 3.8 mm per day. The number of days with ET_{pTW} ≥5 mm and ET_{pTW} ≥6 mm increased particularly in the hot and dry years. This corresponds well to very high DM content ≥25 or even ≥30% at mowing in several wilting periods particularly in 2018 and 2019 (Table 1). The model was able to reliably predict the wilting time at an EF of 0.95 with MAE of 74 and RMSE of 89 minutes compared to the observed wilting time (Figure 1).

Table 1. Mean air temperature (TA) and rainfall (RF) for the period from April to June, wilting periods with very high dry matter (DM) content at mowing and days with very high daily TURC-Wendling evapotranspiration (ET_{pTW}) (day 1 and 2 after mowing).

	TA	RF	DM ≥25% at mowing		DM ≥30% at mowing		ET_{pTW} ≥5 mm	ET_{pTW} ≥6 mm
	°C	mm	No. of periods	% of all periods	No. of periods	% of all periods	No. of days	No. of days
2015-19	-	-	27	42.2	15	23.4	35	4
2015	12.4	86	5	31.2	1	6.2	2	0
2016	14.1	90	0	-	0	-	2	0
2017	13.7	266	5	38.5	1	7.6	8	0
2018	16.3	44	11	55.0	8	40.0	18	2
2019	14.7	82	6	54.5	5	45.4	5	2

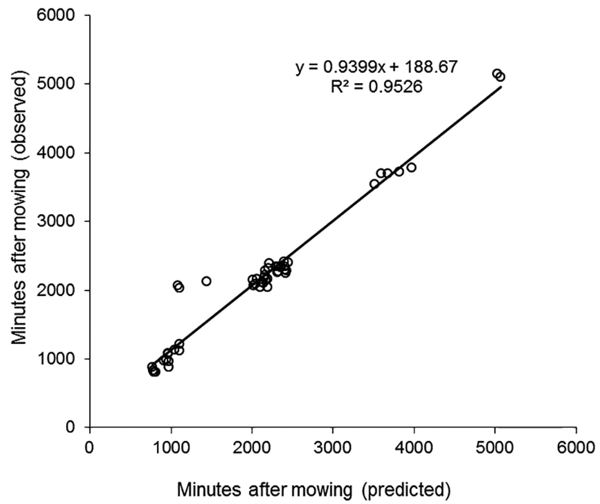

Figure 1. Comparison between observed and predicted wilting time from mowing to ensiling, for dry matter content.

Conclusions

The optimal wilting time of grass for silage production under various site and weather conditions in Northern Germany can be reliably predicted using the 'WILT-EXPERT' model. The experimental results have supplied a comprehensive data basis for a decision support tool implemented in a smart phone app, which was entering the final test phase 2019. The app will enable farmers in Germany to respond quickly to higher frequencies of both wet and dry conditions, which is crucial for a sustainable grassland management in the future. The current developments of automated DM determination on harvesting machines (DLG, 2019) have the potential to increase the accuracy of wilting models and could be implemented into the smartphone app. Against the background of increasing climatic variability associated with climate change, further effort should be made on better involvement of weather extremes in the model.

Acknowledgements

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References

BAF (2013) *Handbuch Futterkonservierung*. Bundesarbeitskreis Futterkonservierung. DLG-Verlag, Frankfurt/Main, 353 pp.

DLG (2019) *CLAAS NIR Sensor (FW: 1.30.7; Kalibrationsmodell: Gras V4.1.1) Feuchtemessung in Gras*. Prüfbericht 7020. Groß-Umstadt, 8 pp. Available at: www.DLG-Test.de.

Nash J.E. and Sutcliffe J.V. (1970) River flow forecasting through conceptual models part I – a discussion of principles, *Journal of Hydrology* 10 (3) 282-290.

PAGF (2012) *Grünland-Tabellenwerk*. Paulinenauer Arbeitskreis Grünland und Futterwirtschaft. Paulinenaue, 48 pp.

Pickert J., Hoffmann T., Herrmann A., Thaysen J., Weise G. and Wellenbrock K.-H. (2016) 'WiltExpert' – a model for on-farm prediction of grass wilting time from mowing to ensiling dry matter content. *Grassland Science in Europe* 21, 200-202.

SEGES (2019) *Fortørringsprognose for slætgræs*. Available at: https://www.landbrugsinfo.dk/.

VIPS (2019) *Grovfôrmodellen*. NIBIO – Norsk institutt for bioøkonomi. Available at: https://www.vips-landbruk.no/roughage/nutrition/.

Wendling U., Schellin H.-G. and Thoma M. (1991) Bereitstellung von täglichen Informationen zum Wasserhaushalt des Bodens für Zwecke der agrarmeteorologischen Beratung. *Zeitung für Meteorologie* 34, 82-85.

Grass silage for biorefinery – *in vitro* digestion of processed grass silage fibre

Piou L., Stefański T., Franco M., Kuoppala K. and Rinne M.
Natural Resources Institute Finland (Luke), Finland

Abstract

An *in vitro* gas production study was conducted to estimate the degradation rate of grass silage, fibre fraction and reconstituted silages processed by laboratory (Angel) and farm-scale (Haarslev) twin-screw presses, and red clover silage, fibre, and reconstituted silage processed by Angel. Samples were incubated as fresh, but the weights were adjusted to 1 g of dry matter. Samples were incubated in two replicates within run and there were three separate runs that lasted for 72 h each. Red clover silage had a greater digestion rate than grass silage, while reconstituted red clover silage from Angel press produced less gas than intact red clover silage during the incubation time. The reconstitution of the silage after processing with the Angel press, for both grass and red clover, did not result in any differences in digestion rate compared with the intact silages. We hypothesised that processing of fibre would improve the rate of its digestion, but we could not confirm this hypothesis.

Keywords: digestion rate, fractionation, gas production, red clover, screw press

Introduction

North-West European countries have a humid temperate climate which provides good conditions for grass growth. These areas have a capacity for higher grass and forage legume biomass production compared with annual crops. Furthermore, high protein yields, with a low impact on environment can be achieved with these crops (Damborg *et al.*, 2019).

The concept of the green biorefinery is based on the processing of green biomass into a range of innovative products. The purpose of a biorefinery is to use green biomass in the most optimal way to produce energy and marketable products. The development of biorefinery allows the utilisation of plants to feed both non-ruminants and ruminants due to the separation of liquid and solid fractions. The aim of this study was to compare the gas production, the digested fraction of the potentially digestible dry matter, and degradation rate of intact and biorefined grass and red clover silages, using an *in vitro* gas production method. We hypothesised that processing of the fibre during screw-pressing will aid its microbial digestion and result in a faster rate of digestion *in vitro*.

Materials and methods

An *in vitro* experiment was carried out using a standard method based on gas production (Rinne *et al.*, 2016). We used the same grass (mixed timothy and meadow fescue) silage and separated fibre fraction as used by Savonen *et al.* (2020), where silage was fractionated using a farm-scale twin-screw press (Haarslev Indistries A/S, Søndersø, Denmark). We also included a red clover silage, and fractionated both silages using a laboratory scale twin-screw press (Angel Juicer Ltd., Busan, South Korea). Liquid and solid separation was carried out by steady feeding of the press with the raw materials.

Eight treatments were included in the study:
1. grass silage;
2. grass silage fibre from Haarslev press (solid fraction);
3. reconstituted grass silage processed by Haarslev press (solid + liquid fractions);
4. grass silage fibre from Angel press (solid fraction);

5. reconstituted grass silage processed by Angel press (solid + liquid fractions);
6. red clover silage;
7. red clover silage fibre from the Angel press (solid fraction);
8. reconstituted red clover silage processed by Angel press (solid + liquid fractions).

Inoculums for the anaerobic *in vitro* incubation were collected from three Nordic Red milking cows equipped with rumen cannula. The donor cows were in different stages of lactation to increase the versatility of inoculum and were fed a diet based on grass silage and concentrates according to the stage of lactation. Rumen fluid was collected 2 h after morning feeding and samples were incubated in glass bottles (250 ml). All samples were incubated in two replicates within the runs and there were three separate runs. The *in vitro* incubation lasted for 72 h and gas measurement was recorded every 1 min. Each experimental vessel contained 1 g of sample dry matter. The samples were incubated as fresh matter to enable detection of the effects of feed physical structure on the microbial digestion, but the amount of samples was calculated based on the dry matter concentration.

Laboratory analyses were conducted according to Savonen *et al.* (2020) and are presented in Table 1. The mean particle size of silages and solid fractions was determined by wet-sieving. A 2-pool Gompertz model was used to estimate the degradation rate of the feeds (Rinne *et al.*, 2016). The data were analysed using the GLM procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS.

Results and discussion

The biorefinery process greatly affected fibre particle size in comparison with intact silages (Table 1). The Angel press resulted in the smallest particle size relative to the intact silage and the Haarslev press.

Total gas production, digested fraction of the potentially digestible dry matter and digestion rate are presented in Figure 1. Fibre fraction, regardless of press type or plant species, resulted in lower gas production ($P<0.05$) than silage and reconstituted silage. Furthermore, grass silages produced higher total gas than red clover silages ($P<0.05$; Table 2). Reconstituted red clover silage from the Angel press produced less gas during the incubation time ($P<0.05$; Figure 1A and Table 2).

There were no statistical differences for the digested fraction of the potentially digestible dry matter ($P>0.05$; Figure 1B and Table 2) according to the different plant species, press method and biorefined silage fractions. Highest digestion rate ($P<0.05$) was found for the fibre fraction of red clover pressed by

Table 1. Chemical composition of original silages, fibre and liquid fractions.

	Grass					Red clover		
	Silage	Haarslev press[1]		Angel press[2]		Silage	Angel press[3]	
		Fibre	Liquid	Fibre	Liquid		Fibre	Liquid
Dry matter (DM), g kg^{-1}	221	449	70	492	84	258	418	117
Ash, g kg^{-1} DM	70	43	180	45	148	106	74	230
Crude protein, g kg^{-1} DM	136	105	277	97	255	173	160	190
Neutral detergent fibre, g kg^{-1} DM	573	699	-	691	-	460	546	-
Acid detergent fibre, g kg^{-1} DM	319	388	-	384	-	299	354	-
Acid detergent lignin, g kg^{-1} DM	29	34	-	34	-	63	79	-
Mean particle size, mm^{-1}	6.0	4.5	-	0.4	-	12.7	0.3	-

[1] Farm scale twin screw press, liquid yield 0.605.

[2] Laboratory scale twin screw press, liquid yield 0.587.

[3] Laboratory scale twin screw press, liquid yield 0.421.

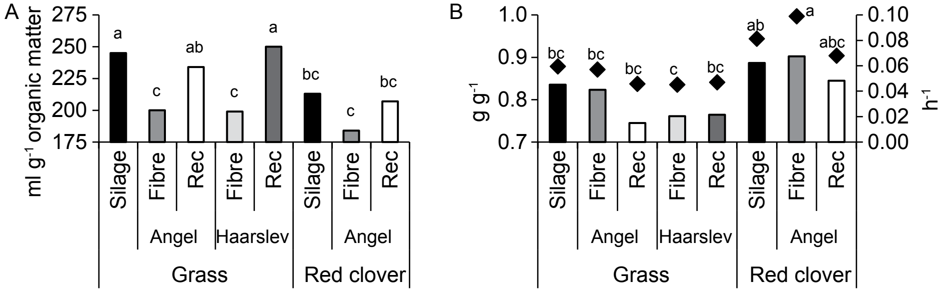


Figure 1. Total gas production (ml g^{-1} organic matter; A), and digested fraction of the potentially digestible dry matter (bars, g g^{-1}; B, no statistically significant differences) and digestion rate (dots, k_d, h^{-1}; B) of grass and red clover silages, fibre and reconstituted (Rec) silages processed by Angel or Haarslev presses. Values with same letter are not significantly different at 5% Tukey test.

Table 2. *P*-values for the contrasts in Figure 1.[1]

	Contrasts							SEM
	G vs RC	G vs GRA	G vs GRH	GFA vs GFH	RC vs RCRA	GFA vs RCFA	GRA vs RCRA	
Total gas	<0.01	0.28	0.64	0.93	0.50	0.11	<0.01	6.9
Digested	0.36	0.09	0.15	0.26	0.57	0.24	0.19	0.068
k_d	0.04	0.16	0.18	0.26	0.35	<0.01	0.12	0.012

[1] G = grass silage; GRA = grass silage reconstituted from Angel press; GRH = grass silage reconstituted from Haarslev press; GFA = grass silage fibre processed by Angel press; GFH = grass silage fibre processed by Haarslev press; RC= red clover silage; RCRA = red clover silage reconstituted from Angel press; RCFA = red clover silage fibre processed by Angel press; SEM = standard error of the mean.

the Angel press, followed by red clover silage and reconstituted red clover silage (Figure 1B). Grass fibre fraction resulted in lower digestion rate than red clover fibre processed by the Angel press. Red clover silage had greater digestion rate than grass silage (Table 2). Reconstituted silages from the Angel press for both grass and red clover did not differ in digestion rate compared with the original silages.

Conclusions

The press methods did not affect the fermentation dynamics of silage, fibre fraction and reconstituted silage, but the plant species behaved differently with greater digestion rate for red clover than grass silage. We could not confirm the hypothesis that processing of forage fibre during screw-pressing would result in faster rate of digestion *in vitro*.

References

Damborg V. (2019) *Green biorefinery – characterisation and utilization of the pulp fraction from extraction of green protein from grassland plants*. PhD Thesis – Science and Technology. Aarhus University.

Rinne M., Kautto O., Kuoppala K., Ahvenjärvi S. and Kitunen V. (2016) Digestion of wood-based hemicellulose extracts as screened by *in vitro* gas production method and verified *in vivo* using sheep. *Agricultural and Food Sciences* 25, 13-21.

Savonen O., Franco M., Stefanski T., Mäntysaari P., Kuoppala K. and Rinne M. (2020) Grass silage pulp as a dietary component for high yielding dairy cows. *Animal,* in press. https://doi.org/10.1017/S1751731119002970

Innovations in grassland-based farms: focusing on the sources of inspiration for a better dissemination

Porqueddu C., Melis R.A.M., Sanna F. and Franca A.
CNR – Institute for Animal Production System in Mediterranean Environment (CNR-ISPAAM), Traversa La Crucca 3, 07100, Sassari, Italy

Abstract

Inno4Grass is the acronym for 'Shared Innovation Space for Sustainable Productivity of Grasslands in Europe'. This international and multi-actor project gathers farmers' organisations, extension services, and education and research institutions from eight EU countries with the aims of closing the gap between practice and science communities, and facilitating the adoption of innovations on grassland-based farms. In this paper, we describe the outcome of a survey carried out in Sardinia (Italy). Twenty innovative dairy farmers were interviewed face-to-face. A standardised questionnaire was used, based on both semi-open and open questions concerning the farm structure and management and the innovations adopted by farmers. The farm innovations covered different domains: production techniques, product processing and marketing. The most used source of inspiration for the adoption of innovations was the Internet. The knowledge about these potential farmers could be useful for researchers to build a more effective communication strategy to disseminate innovations and increase the number of potential innovators.

Keywords: dairy farms, grasslands, innovation, survey

Introduction

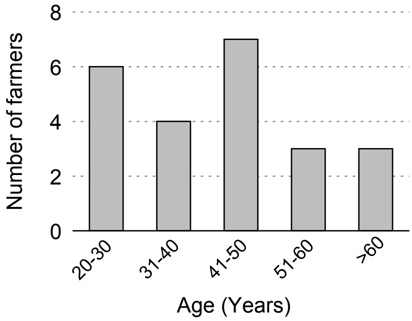
Filling the gap between practice and science is pivotal for improving sustainability and resilience of European farms and farmers. This is the aim of the H2020 thematic network 'Shared Innovation Space for Sustainable Productivity of Grasslands in Europe' (Inno4Grass, www.inno4grass.eu), which focuses on grassland-based farming systems, their innovations and methodologies to bridge the knowledge gap. This multi-actor project involves 20 Partners from 8 European Countries. Both practice and science actors are represented in the Consortium to facilitate the capture of innovations emerging from several sources, including farmers running livestock farms in the partner countries. In this paper, the outcome of the survey carried out in Sardinia (Italy) is described. The main aim of the survey was to capture innovations from farmers, but also to investigate the main motivations that brought them to change the management of their farms, the added value obtained and the sources of inspiration for the adoption of the innovations. This latter information is particularly interesting to set the most efficient methodology for exchanging information with farmers.

Materials and methods

Twenty innovative farmers were identified in Sardinia (Italy) with the help of local stakeholders (extension services, consortia, local action groups) and other farmers of the CNR-ISPAAM network. All farmers run dairy farms, among them one dairy-cow farm, three sheep-goat farms and the rest sheep farms. Interviews were carried out from September 2017 to April 2018. A questionnaire composed of two sections was set up to carry out face-to-face interviews, with the aim of drawing an overall picture of the farm characteristics, its management and the innovations introduced by the farmer. In detail, the questions in section one focused on farmers' age, climate and soil traits, the use of utilised agricultural area of the farm, the incidence of grasslands and their management, the stocking rates and the animal production. In section two, the innovation, the sources of inspiration, the goal pursued by the farmer with its adoption, the obtained added-value, and farmers' further needs were inquired. In the latter section, more answers were allowed for each question. Data are shown as numbers or percentages.

Results and discussion

The distribution of the number of farmers per group, based on age, showed that the farmers younger than 50 years prevailed in the group of local innovators (Figure 1, left). The classes show an inverted trend than reported in regional statistics (LAORE, 2013), where farmers over 60 are the most represented. The average age of the innovative farmers was 42 years. The total average area of their farms is 136 ha. Temporary grasslands cover 56% of the total average farm area. The LSU per sheep farm ranges from 22 to 183 (66.6 on average), with a mean stocking rate of 0.71 LSU per ha of total forage area (range 0.17-1.50). Forage areas are used mainly for both grazing and hay production in 10 out of 20 farms, and 2 farms out of 20 carry out only grazing. In these farming systems, the average milk production ranges from 130 to 400 l head^{-1} year^{-1} for sheep (average 258), 365 to 885 for goats (average 603) and 9,500 for dairy cows. The average production figures are higher than the regional averages for sheep (Sarda breed) in intensive farming systems (170 l head^{-1} year^{-1}). The innovations adopted by farmers belong to a range of domains, from machinery and tools to marketing and the whole farming system (Figure 1, right). The three most adopted innovations (forage mixtures, D_2; grazing management system, D_4; product processing, D_7) represented 44% of the total answers (41 answers). The farmers usually have finely adapted the innovations introduced to the specific farm conditions. For instance, they modified the rate of seeds in forage mixtures and the species on the basis of the soil and meteorological conditions; modified some machinery to reduce the effort required to soil tillage; tried non-traditional milk processing to obtain innovative cheese (use of thistle rennet); used new management of legumes to increase the protein self-sufficiency on farm. The sources of information for the adoption of innovations were several (Figure 2). Direct search on the Internet was the most popular option, but its main limit was related to the general information that can be obtained in the national language, which required subsequent trials by farmers or deeper information from other sources. From this point of view, the direct relationships with advisers and researchers were considered the most reliable sources to adopt innovations. Field days, for instance, are well participated in by farmers, although they are rare events. Some farmers have also been inspired by participating in meetings and seminars or travelling abroad and visiting foreign innovative farms on their own or receiving stimuli from private companies (other sources, S_{12}). Electronic discussion groups were mainly referred to as Facebook pages moderated by farmers, but most farmers considered them inappropriate for improving knowledge and implementation of innovations on farm. Finally, discussion groups were not chosen by farmers because they are not commonly carried out in the region. Nonetheless, we used them to foster peer-to-peer learning with the methodology described in Peratoner *et al.* (2019). In fact, a common feature of the innovative farmers is that they are open-minded and willing to share their approach to innovations, their success, their failures and their knowledge with the other farmers.



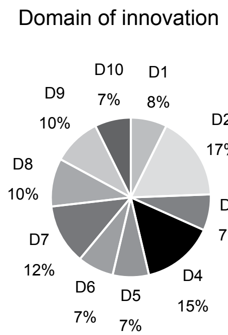


Figure 1. Number of interviewed farmers per age (left) and domain of innovation adopted in Sardinian livestock farms (right). D_1 = machinery, tools; D_2 = forage mixture; D_3 = forage conservation; D_4 = grazing management system; D_5 = legume management; D_6 = animal breed or type; D_7 = product or product processing; D_8= product selling on-farm; D_9 = farming system; D_{10} = other.

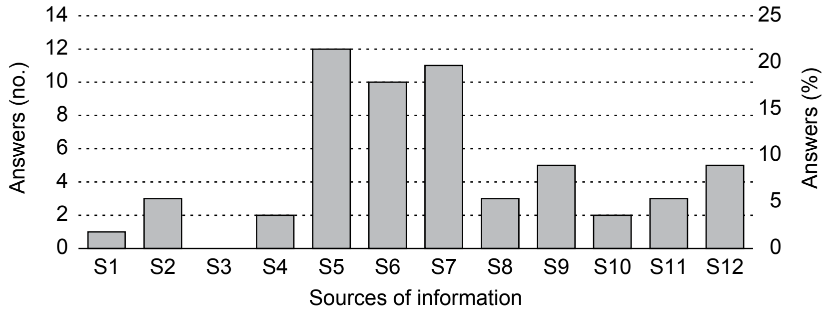


Figure 2. Sources of information for innovations in grassland-based Sardinian farms. S_1 = neighbours; S_2 = family; S_3 = discussion groups; S_4 = electronic discussion groups; S_5 = internet search; S_6 = researchers; S_7 = advisors; S_8 = agricultural journals; S_9 = conference, seminar; S_{10} = books; S_{11} = other farmers; S_{12} = other sources.

Conclusions

The face-to face interviews allowed us to meet innovative farmers and better understand their preferences concerning communication. The solutions that they found to solve specific problems can serve as a guide for other farmers. A winner approach to stimulate the adoption of innovation could be discussion groups where innovative farmers are the main actors, but other stakeholders (i.e. research, extension service) actively contributed to foster knowledge sharing.

Acknowledgements

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References

LAORE Sardegna (2013) *Comparto ovi-caprino e zootecnia regionale, dati strutturali*. Report. www.sardegnaagricoltura, 72 pp.

Peratoner G., Florian C., Mairhofer F., … and Van den Pol-van den Dasselaar A. (2019) Effect of training and methodology development on the effectiveness of discussion groups on grassland innovation. *Grassland Science in Europe* 24, 509-511.

Feasibility of cattle slurry acidified with sulphuric acid and pyrolysis liquid in grass production

Räty M.[1], Hagner M.[2], Rasa K.[2], Nikama J.[2], Peltonen S.[3] and Keskinen R.[2]
[1]Natural Resources Institute Finland (Luke), Halolantie 31 A, 71750 Maaninka, Finland; [2]Natural Resources Institute Finland (Luke), Tietotie 4, 31600 Jokioinen, Finland; [3]Association of ProAgria Centres, Urheilutie 6 D, PL 251, 01301 Vantaa, Finland

Abstract

Acidification of slurry is a known approach for minimizing gaseous emissions of ammonia, thereby reducing environmental hazards and loss of fertiliser value. Sulphuric acid (SA) is the most common agent used for this purpose but handling of strong acids involves safety risks at the farm. Replacing the synthetic SA with bio-based weak acids produced in a pyrolysis process has been suggested to improve the safety and sustainability of the treatment. However, more knowledge is needed on the suitability of pyrolysis liquids (PL) for this application. In this study, we examined the effects of SA- and PL-acidified slurry on yields and apparent nitrogen (N) recovery in herbage of a grass mixture in a field experiment. The obtained dry mass yield decreased in the order SA acidified, untreated and PL acidified slurry as the N source, although only the difference between the extremes was significant. Apparent N recovery was higher from the SA-acidified slurry (19.2±2.0%) than from PL (9.7±1.7%) and untreated slurry (10.6±1.7%). The current results support slurry acidification as a feasible measure for improving N use efficiency in grass production but the weak performance of PL needs to be clarified in future research.

Keywords: nitrogen, ammonia, animal slurry, acidification, pyrolysis liquid, sulphuric acid

Introduction

Animal slurries are susceptible to major gaseous emissions of ammonia (NH_3) due to their high pH and ammonium nitrogen (NH_4-N) content (Sommer and Hutchings, 2001). The conversion of NH_4 to NH_3 can be prevented by decreasing the slurry pH to below 6, and slurry acidification has thus become an increasingly popular approach in both preserving the slurry fertiliser value and reducing the environmental load of NH_3 during storage and spreading (e.g. Ndegwa *et al.*, 2008). Currently, strong sulphuric acid (H_2SO_4) is the most commonly used agent for the acidification treatment but safety factors complicate its use at the farm level (Fangueiro *et al.*, 2015). The safety and sustainability of the treatment could potentially be improved by replacing synthetic H_2SO_4 with bio-based weaker acids produced in a pyrolysis process, which is used to convert various biomasses to energy (gas) and biochar (Keskinen *et al.*, 2018). However, not enough knowledge exists on the impacts of pyrolysis liquid amendment on the physical and chemical characteristics and fertilising value of the receiving slurry.

In this study, we examined the agronomic effects of differently acidified (pH<5.5) surface-applied cattle slurry in grass mixture production. In a field experiment, the performance of slurry acidified by birch-derived pyrolysis liquid (PL; pH 3.0, 14% as acetic acid equivalent) was compared with conventionally (SA; 93% H_2SO_4) acidified and untreated slurry and, further, with mineral fertilisation. In the current paper, first results on the effects of the treatments on grass dry mass yields and apparent N recovery are presented.

Materials and methods

An experimental field with 1.5×8 m plots was established in Maaninka, central-eastern Finland, on a sandy loam soil in May 2018 by sowing a grass mixture (*Phleum pratense* L. 'Nuutti' and *Festuca pratensis* Huds. 'Valtteri' in 70:30 ratio) under barley. The field was managed according to conventional practices including mineral fertilisation until the first harvest in June 2019, after which the experimental treatments were conducted according to Table 1, each in four replicates arranged in a completely randomised block design. The slurries were spread onto the soil surface imitating trail hose application at a rate of 45 Mg ha^{-1} for untreated and SA-acidified slurry, and 49 Mg ha^{-1} for PL-acidified slurry. To reach a target pH, SA and PL were added 4.9 and 83 l Mg^{-1} slurry, respectively. In August, the plots were harvested by a Haldrup plot harvester and the dry matter yield of each plot determined. Total N concentration of the plant material was analysed by the Kjeldahl method. Finally, apparent N recovery was calculated as the proportion of total added N recovered in the harvested plant biomass after subtracting the amount of N supplied by the unfertilised soil. An analysis of variance was used to evaluate differences in the dry mass yields between treatments. Pairwise comparisons were conducted using the Tukey's honest significance test.

Results and discussion

Of the compared slurry treatments, SA-acidified slurry produced the highest grass dry mass yield, though the difference relative to untreated slurry was not statistically significant (Figure 1). The yield in these two slurry treatments was equal in size to that obtained with 40 kg mineral N ha^{-1}. The grass yield obtained with PL-acidified slurry was significantly (roughly 30%) lower than that of the SA-treated slurry and even lower than that produced with the untreated slurry, but the latter difference was not significant. As for the crop N uptake, the highest apparent N recoveries, 50.0±7.0% and 50.5±5.2% were recorded in

Table 1. Experimental treatments applied in the field experiment.

Treatment	pH	Added total / easily available[1] N (kg ha^{-1})
Unfertilised control (N 0)		0 / 0
Mineral fertilisation low (N 40)		40 / 40
Mineral fertilisation high (N 70)		70 / 70
Untreated slurry	6.9	164 / 62
Slurry with sulphuric acid (SA)	5.1	164 / 68
Slurry with pyrolysis liquid (PL)	5.3	144 / 58

[1]For slurries water-extractable (1:5) NH_4-N.

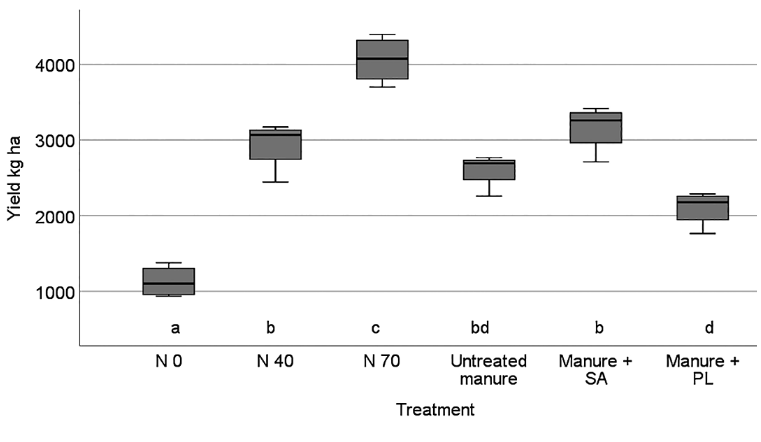

Figure 1. Dry mass yield in the field plots treated with either mineral fertiliser (0, 40 or 70 kg N ha^{-1}) or with variously treated manure slurry (45/49 t ha^{-1}). SA = sulphuric acid, PL = pyrolysis liquid (minimum, 1. quartil, median, 3. quartil and maximum, n=4). Statistically significant differences (P<0.05) are marked with different letters (a d).

the mineral fertiliser N 40 and N 70 treatments, respectively. Of the slurry treatments, the SA-acidified slurry exhibited clearly higher apparent N recovery (19.2±2.0%) in comparison with the PL acidified (9.7±1.7%) and untreated slurry (10.6±1.7%).

The acidification treatment of slurry has been shown to improve the yield level and N use efficiency in comparison with untreated slurry due to reduced gaseous losses of NH_3 (Fangueiro *et al.*, 2015). In the current study, these effects were observed only for the SA-treated slurry even though the desired decrease in pH was achieved also with the PL. Pyrolysis liquids are known to contain phenolic compounds, e.g. guaiacol and catechol that can negatively affect plant growth either directly (Chakroun *et al.*, 2012) or via microbial activities. The causes of the weak performance of PL in comparison with SA as an acidifying agent in slurry need to be clarified with further research.

Conclusions

The current results support acidification of slurry as a feasible measure for improving the slurry N use efficiency in grass production. However, on herbage yield basis, the performance of PL was not satisfactory. The causes for growth reduction in comparison with SA as the acidifying agent will be investigated via data on NH_3 emission rates, plant composition, soil biological and chemical factors and further laboratory tests.

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References

Chakroun H., Bouaziz M., Dhouib A. and Sayadi S.S. (2012) Enzymatic oxidative transformation of phenols by Trametes trogii laccases. *Environmental Technology* 33, 1977-85.

Fangueiro D., Hjorth M. and Gioelli F. (2015) Acidification of animal slurry - a review. *Journal of Environmental Management* 149, 46-56.

Keskinen R., Hyväluoma J., Wikberg H., Källi A., Salo T. and Rasa K. (2018) Possibilities of using liquids from slow pyrolysis and hyrdothermal carbonization in acidification of animal slurry. *Waste and Biomass Valorization* 9, 1429-1433.

Ndegwa P.M., Hristov A.N., Arogo J. and Sheffield R.E. (2008) A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Biosystems Engineering* 100, 453-469.

Sommer S.G. and Hutchings N.J. (2001) Ammonia emission from field applied manure and its reduction – invited paper. *European Journal of Agronomy* 15, 1-15.

Pastures of the future: prospects for virtual fencing to promote grazing in European dairy farming

Riesch F.[1,2], Komainda M.[1], Horn J.[1] and Isselstein J.[1,2]

[1]Grassland Science, Department for Crop Sciences, University of Goettingen, Von-Siebold-Str. 8, 37075 Goettingen, Germany; [2]Centre of Biodiversity and Sustainable Land Use, University of Goettingen, Büsgenweg 1, 37077 Goettingen, Germany

Abstract

Most of the dairy milk currently produced in Central Europe originates from cows kept indoors throughout the year and fed with concentrates and conserved forage. Grazing has largely been abandoned because of practical and logistical constraints, economic considerations and the challenge of meeting the animals' nutritional requirements with a grass-based diet. Returning cows to the pasture, however, could mitigate the ecological impact of dairy farming, increase animal welfare and comply with the societal demands on agricultural production. We reviewed the literature on technological innovations, i.e. virtual fencing technologies, which could lower the cost and labour for fencing and, thus, promote grazing in European intensive dairy production systems. We present the state-of-the-art of virtual fencing, weigh the potential benefits and practical challenges, and identify the most pressing research and development gaps. Recent studies showed that individual animals' responses to the stimuli of electronic collars used for virtual fencing are highly variable. Long-term observations of animal behaviour and welfare do not exist. Farm-scale experiments are required to test the potential of virtual fencing technologies under the diverse ecological and socio-economic real-life conditions of Central European dairy production.

Keywords: animal welfare, associative learning, controlled grazing, environmental impact, grassland conservation, precision livestock farming

Introduction

Dairy cows in Central Europe are predominantly housed indoors (Läpple and Sirr, 2019) and supplied with a total mixed ration (Schingoethe, 2017) composed of concentrates and conserved forage. Aiming at maximising the total annual output of milk per cow under intensive farming conditions, herd sizes have increased, while access to pasture has decreased (Knaus, 2016). To date, a sophisticated grazing regime appropriately fulfilling the nutritional requirements of high-yielding dairy cows with grazed grass, for instance as is widely practised in Ireland (Humphreys *et al.*, 2009), depends on physical fences. These fences are labour-intensive, as they need to be installed, maintained and frequently relocated to regularly provide dairy cows with fresh forage. While there are, hence, valid economic and practical reasons for not grazing cows, research has recently highlighted that cows with access to pasture are healthier and have better welfare (Burow *et al.*, 2013), which is increasingly recognised and demanded by consumers (Weinrich *et al.*, 2014). At the local scale, if managed in an environmentally friendly way, grazing can enhance the structural, functional and biological diversity and ecosystem services in grasslands (Enri *et al.*, 2017; Peyraud *et al.*, 2010; Tallowin *et al.*, 2005). Novel technologies overcoming practical obstacles to grazing could create an incentive for farmers to return dairy cows to the pasture, thereby presenting an opportunity to mitigate the ecological impact of dairy farming, increase animal welfare and comply with societal demands on agricultural production. We reviewed the literature on technological solutions that could promote grazing by circumventing the need for conventional physical fences. We assessed the prospects of virtual fencing to function as a tool to adjust the movement and grazing of livestock in accordance with the spatio-temporal distribution, quantity and quality of forage and valuable habitat structures in the pasture.

Materials and methods

To investigate the state-of-the-art of virtual fencing, we searched for scientific articles (reviews and original research) in Web of Science and Google Scholar using combinations of the following key words: virtual/wireless/fenceless fencing; dairy cows, cattle, sheep, goat, ruminants; control/movement control, animal management; pasture/grazing system/management; animal welfare, ethically acceptable; animal/cattle/cow responses/behaviour; nature conservation, landscape/habitat restauration; grazing/pasture efficiency/utilisation, agronomic outcome/performance. In total, our literature research resulted in forty-seven studies.

Results and discussion

At present, developments for virtual fencing of livestock combine remote positioning techniques with a conditioned pre-warning signal and an aversive stimulus in order to prevent animals from crossing a virtually defined border (e.g. Umstatter, 2011). The animals receive the stimuli at either the throat or neck from technical devices mounted on a collar. If an animal does not respond to the pre-warning signal, an aversive stimulus is given in the form of an electric pulse. Some advanced developments use smartphone apps to set and move virtual fences within the grazing landscape. Techniques to control animal movement without visible physical barriers were developed in the early 1970s (Anderson, 2007). Nevertheless, most virtual fencing systems are still in the prototype phase and a widespread market release has not yet been realized. Published research on virtual fencing has mainly focused on designing and reviewing technical solutions for controlling and monitoring animal movement patterns (seventeen studies). These technical studies addressed the performance of the algorithms to set virtual fences and the associated pre-warning and aversive signals, the accuracy of the positioning system and battery life. Real experimental trials were only carried out in ten studies with sheep, three studies with goats and seventeen studies with cattle, mostly on heifers (eight studies), followed by non-lactating cows (five studies) and steers (four studies). Most of these studies evaluated the effectiveness of virtual fencing in preventing animals from entering an exclusion zone (Table 1). Only two studies performed a short-term assessment of animal welfare using heart rate and cortisol as stress indicators. No long-term observations of behaviour and welfare or group dynamics were reported.

Experiments addressing cattle learning ability of the virtual fencing system and animal behaviour, i.e. spatial distribution and activity patterns, included only few individuals (six to twelve animals) and short periods of three to twenty-three days. These studies indicate that individuals differ in their responses and behaviour in relation to virtual fencing, as well as in the number of interactions required for associative learning of the pre-warning signal. Generally, the number of aversive stimuli decreased over the learning time (e.g. Lee *et al.*, 2009). Animals regularly approached virtual fence lines and started moving to a newly offered sub-paddock area rapidly when the former virtual fence boundary was removed (e.g. Lomax *et al.*, 2019). Although only two studies actually simulated grazing allocation by moving the virtual fence line to another paddock location, this implies that virtual fencing might be suitable for rotational or

Table 1. Objectives of published studies on controlling cattle by virtual fencing systems.

Study objective	No. of studies
Effectiveness of virtual fencing system (learning ability, learning time)	15
Animal behaviour (spatial distribution and activity patterns, group behaviour)	15
Short-term stress indicators of welfare	2
Grazing allocation (moving virtual fence lines)	2
Environmental protection (fencing out)	2
Alternative cues to electric pulse	3

strip grazing. In a nature conservation context, virtual fences could be useful as they facilitate the flexible exclusion of environmentally sensitive areas (Campbell *et al.*, 2018), e.g. during bird breeding time.

Conclusions

Virtual fencing has been shown to be an effective tool for controlling grazing livestock. Our investigations, however, indicate current research and development gaps that need to be addressed in order to apply virtual fencing in a sustainable modern grazing management. Multidisciplinary studies need to focus on long-term animal welfare consequences of virtual fencing, animal behaviour and group dynamics, the potential for rotational grazing, the effectiveness for optimising pasture utilisation and agronomic performance, as well as the effectiveness for conservation management. The agronomic and ecological benefits of integrating virtual fencing into grazing concepts remain to be evaluated under the varying ecological and socio-economic real-life conditions of dairy farms in Central Europe.

References

Anderson D.M. (2007) Virtual fencing – past, present and future. *The Rangeland Journal* 29, 65-78.

Burow E., Rousing T., Thomsen P.T., Otten N.D. and Sørensen J.T. (2013) Effect of grazing on the cow welfare of dairy herds evaluated by a multidimensional welfare index. *Animal* 7, 834-842.

Campbell D.L.M., Haynes S.J., Lea J.M., Farrer W.J. and Lee C. (2018) Temporary exclusion of cattle from a riparian zone using virtual fencing technology. *Animal,* 9, 1-12.

Enri S.R., Probo M., Farruggia A., Lanore L., Blanchetete A. and Dumont B. (2017) A biodiversity-friendly rotational grazing system enhancing flower-visiting insect assemblages while maintaining animal and grassland productivity. *Agriculture, Ecosystems & Environment* 241, 1-10.

Humphreys J., Aarts H.F.M., Watson C.J., Wachendorf M., Gall A.L., Taube F. and Pflimlin A. (2009) Sustainable options for grassland-based dairy production in the northwest of Europe. *Tearmann: Irish Journal of Agri-environmental Research* 7, 175-194.

Knaus W. (2016) Perspectives on pasture versus indoor feeding of dairy cows. *Journal of the Science of Food and Agriculture* 96, 9-17.

Läpple D. and Sirr G. (2019) Dairy intensification and quota abolition: a comparative study of production in Ireland and the Netherlands. *EuroChoices* 0, 1-7.

Lee C., Henshall J.M., Wark T.J., Crossman C.C., Reed M.T., Brewer H.G., O'Grady J. and Fisher A.D. (2009) Associative learning by cattle to enable effective and ethical virtual fences. *Applied Animal Behaviour Science* 119, 15-22.

Lomax S., Colusso P. and Clark C.E.F. (2019) Does virtual fencing work for grazing dairy cattle? *Animal* 9, 1-11.

Peyraud J.L., Van den Pol A., Dillon P. and Delaby L. (2010) Producing milk from grazing to reconcile economic and environmental performances. *Grassland Science in Europe* 15, 163-164.

Schingoethe D.J. (2017) A 100-year review: total mixed ration feeding of dairy cows. *Journal of Dairy Science* 100, 10143-10150.

Tallowin J.R.B., Smith R.E.N., Goodyear J. and Vickery J.A. (2005) Spatial and structural uniformity of lowland agricultural grassland in England: a context for low biodiversity. *Grass and Forage Science* 60, 225-236.

Umstatter C. (2011) The evolution of virtual fences: a review. *Computers and Electronics in Agriculture* 75, 10-22.

Weinrich R., Kühl S., Zühlsdorf A. and Spiller A. (2014) Consumer attitudes in Germany towards different dairy housing systems and their implications for the marketing of pasture raised milk. *International Food and Agribusiness Management Review* 17, 1-18.

Grass silage for biorefinery – aerobic stability affected by additive treatment and liquid removal

Rinne M.[1], Stefanski T.[1], Kuoppala K.[1], Seppälä A.[2] and Jalava T.[1]
[1]Natural Resources Institute Finland, FI-31600 Jokioinen, Finland; [2]Eastman, Typpitie 1, FI-90650 Oulu, Finland

Abstract

Green biorefineries potentially offer many benefits in the new bioeconomy by providing sustainably produced feedstock for feed, food, bioenergy and materials. Separation of the grass biomass into liquid and solid fractions is typically the first step of a biorefinery process. Grass silage is stable until it is removed from the silo and exposed to air. The length of the aerobic stability is an important factor affecting the timeliness costs and losses of the biorefinery. In the current experiment we wanted to evaluate how liquid removal by screw-pressing affects the aerobic stability of grass silage ensiled without additive or treated with fibrolytic enzymes or a formic acid based additive. The aerobic stability of intact silage was longer than that of the solid fraction (90 vs 73 h). The stability of formic acid treated silages and solid fractions (104 h) was longer than that of control and enzyme-treated silages (70 h), which did not differ from each other. There were no interactions between feed type and additive. It seems that the same factors affecting grass silage stability also apply to the separated solid fraction, and care should be taken to rapidly utilise the separated solid fraction to prevent aerobic spoilage.

Keywords: fermentation, fibrolytic enzyme, formic acid, spoilage, round bale silage, liquid solid separation

Introduction

Green biorefineries have potential to provide new biomass-based products for feed, food, bioenergy and materials. Furthermore, increased use of grasslands can provide ecosystem services such as improved soil structure, soil carbon sequestration, nitrogen fixation in the case forage legumes, and increased biodiversity. Separation of the grass biomass into liquid and solid fractions is typically the first step of a biorefinery process (Franco *et al.*, 2019). Grass silage is stable until it is exposed to air. The length of the aerobic stability is an important factor affecting the timeliness costs and losses of the biorefinery. It is well established that silage fermentation type and additives used prior to storage affect the aerobic stability of grass silage (Seppälä *et al.*, 2016). In the current experiment we wanted to evaluate how liquid removal by screw-pressing affects the aerobic stability of grass silage ensiled without additive or treated with fibrolytic enzymes or a formic acid based additive.

Materials and methods

A mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward was harvested into round bales from the third cut in Jokioinen, Finland on 19 September 2016. The dry matter (DM) content of the raw material was 253 g kg^{-1} and it contained 105 g ash, 152 g crude protein and 446 g neutral detergent fibre per kg DM. Three bales were prepared for each additive treatment, which were (1) no additive (Control); (2) a fibrolytic enzyme (E; liquid Flashzyme Plus with cellulase and hemicellulase activities, Roal Ltd., Rajamäki, Finland) at a rate of 1.1 ml kg^{-1} DM; or (3) a formic acid based additive (FA; AIV2 Plus, Eastman, Oulu, Finland) at a rate of 6.8 ml kg^{-1}. The acid application rate was somewhat higher than the commercially recommended rate (5 ml kg^{-1}) due to difficulties in adjusting the pump. The bales were stored outdoors and the average temperature was -0.5 °C during the ensiling period of 161 days. After opening, the bales were mixed in a feed mixer wagon for 10 min and representative samples were taken for routine laboratory analyses for chemical composition and fermentation quality (for analytical methods, see Seppälä *et al.*, 2016). After sampling, liquid-solid separation was conducted using a farm-scale twin-screw press using a 100 kg batch from each bale (Haarslev Industries A/S, Søndersø, Denmark), and again, representative samples were taken and analysed similarly to those of the intact silages.

The aerobic stability of both intact silages and solid fractions after liquid removal was determined by monitoring temperature changes during air exposure, as described by Seppälä *et al.* (2016). Aerobic stability was defined as the time taken to increase the temperature of the feed by 2 or 3 °C above the ambient temperature. Results for a 2 °C difference are discussed while those of 3 °C are given for comparison. The data were statistically analysed using SAS GLM procedure. Some of the parameters were analysed only for silage, and Tukey's pairwise comparison were conducted to separate the treatment means. For those parameters that were analysed for both the intact silages and the solid fractions, contrasts were used to detect differences between the treatments.

Results and discussion

On average, 56.1% of the original silage fresh matter and 26.6% of DM was removed in the separation process but the additives used did not significantly affect the yields (Rinne *et al.*, 2018). We could not detect practically any responses to treatment with fibrolytic enzyme E in the fermentation quality (Table 1) nor in the chemical composition (Table 2) of the silages when compared to the Control. The aerobic stability of the intact silage was longer than that of the solid fraction (Table 2). The stability of FA silages and solid fractions was longer (104 h) than that of Control and E silages (70 h), that did not differ from each other. There were no interactions between feed type and additive.

Ensiling grass is an efficient way to stabilize grass to be used later for a biorefinery process. The aerobic stability of silage after exposing it to oxygen is an important factor affecting the effective logistics of a biorefinery. Further, the stability of the solid fraction after liquid-solid separation dictates the possibilities for utilising it in further use. Based on the current experiment, the stability of both intact silage and the solid fraction of it after mechanical removal of liquid could be improved significantly by using a formic acid based additive, whereas no benefits were obtained from the fibrolytic enzyme application. Further, the stability of intact silages was somewhat longer than that of the solid fraction, which can be explained by the aeration during the extraction process as well as increased DM and decreased organic acid concentrations of it. The responses to additive treatments were similar in intact silages and in the

Table 1. Fermentation quality of the baled grass silages ensiled using different additives.[1]

	Silage additive treatment			SEM	*P*-value
	Control	Enzyme	Formic acid		
pH	4.76^a	4.82^a	4.51^b	0.057	0.018
In dry matter, g kg^{-1}					
Water soluble carbohydrates	83^a	89^a	161^b	5.3	<0.001
Ethanol	29.0^a	28.7^a	7.7^b	1.85	<0.001
Lactic acid	65.6^a	60.5^a	8.2^b	2.50	<0.001
Acetic acid	13.5^a	12.4^a	4.8^b	0.44	<0.001
Butyric acid	0.41	0.39	0.33	0.021	0.068
In nitrogen (N), g kg^{-1}					
Soluble N	673^a	644^b	506^c	5.0	<0.001
NH_3-N	84^a	82^a	50^b	4.1	0.002
In vitro OM digestibility, g kg^{-1} OM	800	796	803	5.0	NS

[1] SEM = standard error of the mean; OM = organic matter; means within the same row without same superscript differ significantly (*P*<0.05, Tukey test).

Table 2. Chemical composition and aerobic stability of intact silages ensiled without any additive (C) or by using fibrolytic enzyme (E) or formic acid based additive (FA), and solid fractions of them.[1]

	Silage (S)			Solid fraction (F)			SEM	Contrast *P*-values	
	C	E	FA	C	E	FA		S vs F	C+E vs FA
Dry matter, g kg^{-1}	235	243	265	402	394	426	5.4	<0.001	<0.001
In dry matter, g kg^{-1}									
Ash	109	112	113	85	93	92	2.61	<0.001	NS
Crude protein	168	161	142	143	128	136	3.8	<0.001	0.006
NDF	445	448	460	581	583	588	5.8	<0.001	0.073
ADF	252	256	251	330	336	323	3.8	<0.001	0.071
ADL	29	34	32	38	38	36	1.93	0.003	NS
Aerobic stability, h									
2 °C	82	68	120	67	63	89	9.8	0.054	0.001
3 °C	91	79	132	73	69	93	10.0	0.017	0.001

[1] SEM = standard error of the mean; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin.

solid fractions. It is noteworthy that the aerobic stability of silages and solid fraction are both relatively short, indicating that the biorefinery process should be planned so that the materials are processed quickly after aerobic exposure to prevent unnecessary losses in product quantity and quality.

Conclusions

It seems that the same factors affecting grass silage stability also apply to the separated solid fraction. In a biorefinery process, the logistics need to be developed to allow rapid utilisation of both the silage and, particularly, the solid fraction to prevent aerobic spoilage.

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References

Franco M., Hurme T., Winquist E. and Rinne M. (2019) Grass silage for biorefinery – a meta-analysis of silage factors affecting liquid-solid separation. *Grass and Forage Science* 74, 218-230. https://doi.org/10.1111/gfs.12421

Rinne M., Timonen P., Stefanski T., Franco M., Vainio M., Winquist E. and Siika-aho M. (2018) Grass silage for biorefinery – effects of type of additive and separation method. In: Gerlach K. and Südekum K.-H. (eds.) *Proceedings of XVIII International Silage Conference*, 24-26 July 2018, Bonn, Germany. pp. 182-183.

Seppälä A., Heikkilä T., Mäki M. and Rinne M. (2016) Effects of additives on the fermentation and aerobic stability of grass silages and total mixed rations. *Grass and Forage Science* 71, 458-471. https://doi.org/10.1111/gfs.12221.

Sensor fusion as a tool for biomass estimation in extensive, heterogenous grasslands

Schulze-Brüninghoff D., Astor T., Hensgen F. and Wachendorf M.
Grassland Science and Renewable Plant Resources, University Kassel, Germany

Abstract

Extensive grasslands provide multiple ecosystem services and habitats for endangered species. Quantification of heterogenous grasslands is both crucial and challenging. Remote sensing systems can increase the spatial and temporal resolution and can unlock new potentials for management strategies. A combination of complementary sensor systems can further increase the ability to estimate extensive grassland yields. In 2018, multiple measurements were made by a terrestrial 3D-laser scanner and a drone-based hyperspectral camera in extensively managed, extremely heterogenous grasslands. Additionally, dry matter ground samples were taken. From 3D-point clouds multiple parameters (Mean Canopy Surface Height, Sum of Voxel and Surface Structure) were extracted and combined with hyperspectral data to develop an optimised biomass model. Several machine learning algorithms were trained and tested. Best performance was derived from the Support Vector Machine Regression (SVMR). The model containing only hyperspectral data delivered an R^2 of 0.62 (nRMSE 4.5%). Parameters from 3D-laser data rendered an R^2 of 0.74 (nRMSE 3.9%). The combination of both sensor systems improved the performance up to R^2 0.82 (nRMSE 3.2%). The combination of complementary sensor systems can increase the power to predict biomass yields of heterogenous and extensive grasslands. Therefore, it is a promising alternative to labour-intensive, traditional biomass estimation methods.

Keywords: above-ground biomass, LIDAR, hyperspectral, sensor fusion

Introduction

At a low mountain range in central Germany, the biosphere reserve Rhön is a historically grown landscape characterised by extensively managed open grasslands. These heterogenous grasslands provide a habitat for multiple endangered species but are challenged by climatically and non-climatically driven transformations. One perceptible change is driven by the invasive species *Lupinus polyphyllus*, which was introduced to the region in the 1940s to reduce soil erosion, fix nitrogen and enrich the upper soil with phosphorus from deeper soil levels. Over decades, it has spread throughout the grassland sites and thereby reduced and eliminated native species which were adapted to sites with poor nitrogen pools (Klinger *et al.* 2019).

As species composition changed on sites dominated by *L. polyphyllus*, heterogeneity of the sites has also changed. Therefore, it is challenging to gain information about qualitative and quantitative parameters. As remote sensing methods allow surveying of larger areas and, thereby, accessing information on biomass yield in large spatial dimension, these methods provide potentially high value for heterogenous grassland sites, where traditional yield estimation methods would be highly time consuming.

To improve the performance of remote sensing methods towards enhanced biomass models, sensor fusion is considered. Therefore, 3D-laser data derived by a terrestrial laser scanner (TLS) and hyperspectral drone-based data were combined.

Materials and methods

Data collection took place at four sites. A *Nardus stricta* grassland (NS), a *Trisetum flavescens* grassland (TF) and two sites invaded by *L. polyphyllus* (NSL, TFL). Each site had 15 plots and 3 cutting dates

(15 June, 27 June and 11 July) were randomly assigned to plots. At each cutting date drone-based hyperspectral data were collected with a Firefly S185 SE (Cubert GmbH, www.cubert-gmbh.com) at a spectral range from 450 to 998 nm (138 bands) and a spatial resolution of 50 by 50 pixels per image. Flight altitude was at 20 m above ground level with a pixel size of 20 cm and images were taken with 80% overlap for image stitching.

Afterwards, 3D data were collected with a terrestrial laser scanner (Leica Scan Station P30) with a spatial resolution of 3.1 mm at 10 m distance. To reduce shadow effects each plot was scanned from two opposite directions. Both scans were afterwards merged to a single point cloud. Destructive ground reference samples were cut for each plot in three randomly selected subplots of 1 m^2 with a stubble height of 5 cm, which represents the height measured by 3D-laser scanner in early spring as a digital elevation model (DEM). Fresh matter was weighed and dry matter was measured after 48 h at 105 °C. GPS coordinates of the subplot-corners were measured with a RTK GNSS (2 cm accuracy) and used to cut corresponding 1 m^2 point clouds and hyperspectral orthomosaics.

Point cloud data were processed with R software (R Core Team 2019) to extract parameters of Mean Canopy Surface Height, Sum of Voxels and Surface Structure (Figure 1). For Mean Canopy Surface Height, point height was calculated by subtracting the z coordinate from the DEM collected in spring. Mean point heights were calculated over different height regions. The Sum of Voxels subdivides a point cloud into voxels (volumetric pixels) with an arbitrary defined size. Every voxel with a minimum of one point inside was added to the sum of voxels. Sum of Voxels were calculated for multiple Voxel edge lengths. Surface Structure was calculated in terms of slope, elevation and curvature. Therefore, a 3×3 submatrix was implemented into the point cloud and from z coordinates of these nine points, and slope, elevation and curvature were calculated for the centre of the matrix.

Hyperspectral data for each spectral band were averaged for each 1 m^2 subplot and the spectral curves were afterwards normalised by vector normalisation. Four different multivariate machine learning models were tested to develop biomass estimation models. Laser and hyperspectral parameters were used as independent variables, each separately and in combination. Training and test data selection (80 to 20%) was done randomly and repeated one hundred times to reduce the impact of biased sample distribution for model training and testing. Model performance was evaluated by the coefficient of determination (R^2) and the normalised root mean square error (nRMSE) normalised by range of observations.

Results and discussion

Best model performance to predict dry matter yield was derived by SVMR, both from 3D-laser variables (R^2 0.74, nRMSE 3.9) and hyperspectral sensor variables (R^2 0.62, nRMSE 4.5) A combination of hyperspectral and 3D-laser variables increased model performance (SVMR) up to R^2 0.82 and nRMSE 3.2 (Table 1).

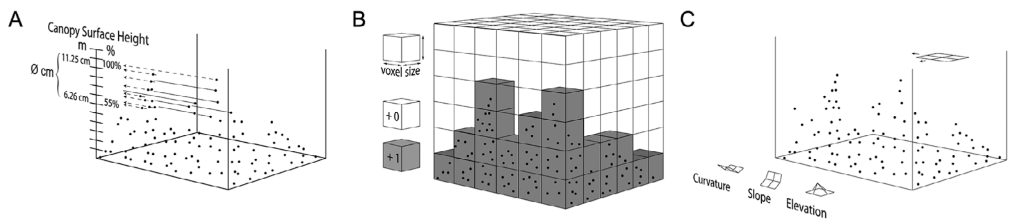

Figure 1. Scheme of parameters for biomass models derived from 3d-laser data. (A) Mean canopy surface height, (B) sum of voxel, (C) surface structure.

Table 1. Summary for best predictive algorithm models (median) for 100 different data sets.[1]

Sensor	Best Model	R^2	RMSE (kg m^{-2})	nRMSE (%)[2]
3d-laser	SVMR/(PLSR)[3]	0.74	0.07	3.9
Spectral camera	SVMR	0.62	0.08	4.5
Combination	SVMR/(RFR)[3]	0.82	0.06	3.2

[1] SVMR = support vector machine regression, PLSR = partial least square regression, RFR = random forest regression.

[2] Normalised by range of observations.

[3] Methods in brackets gained similar prediction performance.

The most important variables for the best models of separate 3D-laser data came from Mean Canopy Surface Height, especially those mean values that were calculated over a broad canopy height region. For the best model derived from hyperspectral data solely, the most important variables were those of near infrared spectral range between 714-926 nm. Biomass model derived from sensor combination had similar important variables from laser data, while the most important hyperspectral variables (again in near infrared region) had lower importance. Normalised deviation between predicted and measured biomass showed no systematic under- or overestimation comparing the different harvesting dates and the four different sites.

As 3D-point cloud variables were primarily important for model performance, cost and time saving alternatives for 3D-point cloud measurement methods should be investigated. Drone-based RGB and multispectral imaging utilising the Structure from Motion method may be a promising methodology (Wijesingha *et al.* 2019).

Conclusions

The combination of complementary sensor systems can increase the performance of biomass estimation on extremely heterogenous, extensive grasslands. Such methods may allow replacement of labour-intensive, traditional biomass estimation in the future.

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References

Klinger Y.P., Harvolk-Schöning S., Eckstein R.L., Hansen W., Otte A. and Ludewig K. (2019) Applying landscape structure analysis to assess the spatio-temporal distribution of an invasive legume in the Rhön UNESCO Biosphere Reserve. *Biological Invasions* 21, 2735-2749.

R Core Team (2019) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing.

Wijesingha J., Moeckel T., Hensgen F. and Wachendorf M. (2019) Evaluation of 3D point cloud-based models for the prediction of grassland biomass. *International Journal of Applied Earth Observation and Geoinformation* 78, 352-359.

Grip on grass with a dashboard to support continuous grazing

Stienezen M.W.J.[1], Philipsen A.P.[1], Holshof G.[1], Honkoop W.[2], Van Noord T.[2] and Schils R.L.M.[1]
[1] *Wageningen University and Research, Droevendaalsesteeg 4, 6708 PB Wageningen, the Netherlands;*
[2] *PPP Agro Advies, Lepelblad 7, 1452 VN Ilpendam, the Netherlands*

Abstract

Various systems of continuous grazing (CG) are applied in Netherlands dairy farming. These systems are characterised by restricted animal access, high stocking densities, high levels of supplemental feeding during grazing and the use of one or more platforms. To keep up grass production and quality in CG systems it is essential to maintain the target sward height at the grazing platform. Farmers in the Netherlands control sward height (SH) of the grazing platform under CG systems by adjusting supplemental feeding, grazing time and size and composition of the grazing platform itself. Since the grazing platform consists of the fields of a farm, the size and composition of the grazing platform can be adjusted. The dashboard was developed to monitor SH on the platforms in use. In the 'Grass-window' actual SH of the grazing platform is compared with target SH and target area during the grazing season. A prognosis for expected SH is given as well. In the 'Paddock-window' actual SH of the individual fields is compared with target SH and target area. Inputs are actual SH and the composition of the grazing platform. The dashboard was developed by farmers, advisers and researchers in an ongoing iterative process of identifying needs, adjusting and testing.

Keywords: continuous grazing, dashboard, sward height, grass utilisation

Introduction

In the Netherlands grazing is characterised by high stocking densities and high levels of feed supplementation. Even though annual grass production is approximately 15 to 20% less than under rotational grazing (Holshof *et al.*, 2018), continuous grazing (CG) is widely used as it is considered less complex by farmers. CG is executed on one or more platforms and often in combination with restricted animal access. For several reasons – for example improving farm profitability, grass growth and reducing nutrient losses – grass utilisation under grazing has to be optimised. However, no tools are available to support farmers to optimise grass utilisation under CG.

Materials and methods

To develop a tool to optimise grass utilisation in CG, three steps were undertaken in the project Amazing Grazing: (1) development of a first version of the tool (Version-1), (2) testing of the tool and (3) finalising the tool. These steps were carried out in close interaction with farmers, advisers and researchers. In the first step the farmers' needs were identified and version-1 of the tool was built. Version-1 was tested by five farmer-adviser combinations for six weeks during the grazing season and experiences were collected fortnightly by interviews (Step 2). Based upon the test, Version-2 was developed and finalised (Step 3).

Results and discussion

Discussions with farmers and advisers on grass utilisation showed that farmers lack information to decide when and how to adjust the supplemental feeding of the animals that are grazing. It appeared that not only the information on the amount of grass available to adjust supplemental feeding failed, but also that required to optimise grassland management under CG. To keep up grass production and quality in CG systems it is essential to maintain the target sward height (SH) under grazing. Therefore, it was decided to develop a tool to monitor SH for use in optimising both feeding and grassland management.

To monitor SH in Version-1, the 'Grass-window' (Figure 1) was set up to compare actual SH with target SH (in clicks or cm) of the offered area for grazing. Actual SH should be around target SH and between the borders of the target range. The prognosis showed SH for the next week when management and growth conditions will remain the same. The test showed that farmers considered this tool to be useful for improving grass utilisation in their CG system.

Based upon change in actual SH – comparable to the methodology used by Hutchings *et al.* (1992) – the Feed-window in Version-1 showed the farmer how to adjust supplemental feeding (kg dry matter cow^{-1}). However, from the test it appeared that farmers not only adjusted supplemental feeding to maintain SH but also hours of grazing during the day, and the size and composition of the area offered for grazing. Since the area offered for grazing comprises the fields of a farm, the size and composition of the grazing platform can be adjusted. The number of animals grazing was not adjusted at all, and supplemental feeding was only changed when the other options to maintain SH had reached their limits (Groen and Teune, 2017). So, the Feed-window in Version-1 of the tool appeared not to be applicable.

The test also showed that the lay-out of the tool to register measured SH did not support farm grassland management as CG was set up and executed differently at the farms (Groen and Teune, 2017). CG was carried out in one or more platforms, either with or without compartmented CG. Combining this information with the flexible use of the area offered for grazing lead to the setup of the 'Input calendar' in Version-2 (Figure 2).

A grazing, growing and cutting platform were defined. The cutting platform consists of the paddocks planned to be cut. The growing platform consists of the paddocks recently cut and regrowing for the next grazing. The grazing platform consists of the paddocks currently in use for grazing. During the grazing season it has to be indicated to which platform a paddock belongs, and SH has to be registered weekly.

Farmers, in composing the grazing platform of their farm, indicated in the test the need for the actual SH of the individual paddocks, of both the grazing and growing platforms, to be able to adjust the grazing platform. Therefore the 'Paddock-window' was set up (Figure 1).

A dashboard comprising a 'Grass-window' and 'Paddock-window' was developed to provide insight into actual SH for various CG systems. The dashboard is fed with actual SH and size and composition of the grazing platform from the 'Input-calendar'. Combining SH with grass growth predictions and the animal feed rations will provide the farmer information on when and how to adjust grassland and feeding management. Since measuring grass is not common practice in the Netherlands, automatic collection of SH is necessary for the dashboard to become available for farmers. The dashboard and the knowledge behind are free for use: https://edepot.wur.nl/503841.

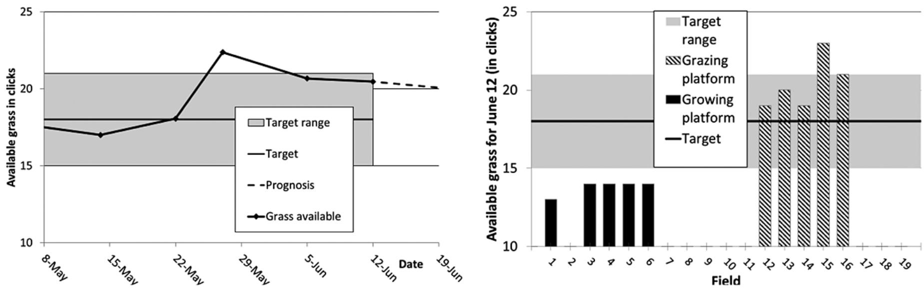

Figure 1. Grass window (left) and paddock window (right) to support continuous grazing.

Fill out in clicks		Date	Target	Date	Target	Date	Target	Date	Target	Date	Target	Date	Target
		7-May	18	14-May	18	22-May	18	27-May	18	5-Jun	18	12-Jun	18
Field	ha	Platform	Clicks	Platform	Clicks	Platform	Clicks	Platform	Clicks	Platform	Clicks	Platform	Clicks
1a	1.2	growing	15	grazing	17	grazing	14	growing	18	growing	21	growing	13
1b	1.2	growing	12	cutting		cutting		cutting		cutting		cutting	
2a	1.3	growing	15	grazing	19	grazing	17	growing	16	growing	19	growing	14
2b	1.3	growing		grazing	20	grazing	17	growing	17	growing	19	growing	14
2c	1.3	growing		grazing	16	grazing	20	growing	17	growing	20	growing	14
2d	1.3	growing		grazing	13	grazing	22	growing	20	growing	21	growing	14
4a	1.2	grazing	16	cutting	19	cutting	19	cutting	20	cutting	21	cutting	14
4b	1.2	grazing	20	cutting	21	cutting	19	cutting	20	cutting	21	cutting	14
4c	1.2	grazing	18	cutting	19	cutting	19	cutting	20	cutting	21	cutting	14
10a	1.3	grazing	17	cutting		cutting		cutting		cutting		cutting	
10b	1.3	grazing	17	cutting		cutting		cutting		cutting		cutting	
10c	1.2	cutting	42	growing	14	growing	16	grazing	22	grazing	19	grazing	19
10d	1.2	cutting	42	growing	14	growing	16	grazing	24	grazing	20	grazing	20
11	1.3	cutting	41	growing	14	growing	16	grazing	22	grazing	20	grazing	19
12	1.4	cutting	41	growing	14	growing	16	grazing	22	grazing	23	grazing	23
13	1.3	cutting	41	growing	14	growing	16	grazing	22	grazing	21	grazing	21
H1	1.2	cutting	46	growing	14	growing	16	cutting		cutting		cutting	
H2	1.2	cutting	46	growing	14	growing	16	cutting		cutting		cutting	
H3	1.2	cutting	46	growing	14	growing	16	cutting		cutting		cutting	

Figure 2. Input calendar filled out for continuous grazing in 3 composed platforms.

Conclusions

A dashboard, comprising a 'Grass-window' and 'Paddock-window', based upon input from the 'Input-calendar', was developed providing insight into actual SH on the grazing platform in various CG systems executed in one or more platforms, with or without restricted animal access. Combining the dashboard with information on grass growth predictions, animal feed rations as well as automatic collection of SH will make it feasible for common use in practice.

Acknowledgements

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References

Groen P. and Teune J. (2017) *Standweiden tool analyse rapport*. Aeres Hogeschool, Dronten, NL, 27 pp.

Holshof G., Zom R.L.G., Schils R.L.M., Philipsen A.P., Van den Pol-van Dasselaar A. and Klootwijk C.W. (2018) Amazing Grazing: substantial fresh grass intake in restricted grazing systems with high stocking rates. *Grassland Science in Europe* 23, 234-236.

Hutchings N.J., Bolton G.R. and Barthram G.T. (1992) Decision rules for controlling the sward height of continuously grazed experimental pastures. *Grass and Forage Science* 47, 41-49.

Biorefined grasses and legumes – effect of species and spring cutting date on extractable protein yield

Stødkilde L.[1], Eriksen J.[2] and Jensen S.K.[1]
[1]*Department of Animal Science, Aarhus University, 8830 Tjele, Denmark;* [2]*Department of Agroecology, Aarhus University, 8830 Tjele, Denmark*

Abstract

Grasses and forage legumes can produce a high protein yield per ha, but knowledge is needed with respect to effect of plant species and harvest time on chemical composition of the protein and pulp fractions produced by biorefining. Spring cut herbage of three legumes (red and white clover, lucerne), and two grasses (perennial ryegrass and tall fescue) harvested at four consecutive weeks were processed in a lab-scale screw press, resulting in two feed-relevant fractions: a pulp and a protein isolate. Cutting date affected nitrogen (N) content of the produced pulps, with delayed cutting date leading to decreased pulp N content. Early and late cutting dates produced protein isolates with low N contents. Interestingly, there was species variation in N recovery in both pulp and protein isolate, with high recovery in lucerne protein isolate, and in pulp from perennial ryegrass, tall fescue, and red clover. Cutting date affected recovery in protein isolates for tall fescue and for pulp in the two clovers. The study demonstrated large variations in composition of the biorefined fractions, highlighting the importance of selecting optimal input material for the specific feed productions.

Keywords: biorefining, legumes, grasses, protein, extractability

Introduction

The use of grasses and legumes as locally produced protein sources for both monogastrics and ruminants has gained increasing attention in Northern Europe, partly due to their high per-hectare dry matter (DM) and protein yields (Manevski *et al.*, 2017; Wilkins and Jones, 2000) and an amino acid composition comparable to soybean (Stødkilde *et al.*, 2019). Whereas the main use of grasses and legumes previously has been as feed for ruminants, the introduction of biorefining potentiates a more efficient nutrient utilisation by monogastrics (Colas *et al.*, 2013; Pirie, 1978), thereby being an alternative to traditional protein sources. Biorefining results in a fibrous pulp suitable for ruminants, a protein isolate for monogastrics, and a residual brown juice. The extraction efficiency, i.e. how much of the DM and protein that is distributed into the three fractions, and the quality of the fractions are expected to depend on selected input material. Time of harvest and selection of plant species are expected to affect composition, recovery and quality of the produced fractions. A prerequisite for biorefining of grasses and legumes is that the produced protein has a nutritional quality that is acceptable for the livestock sector. The aim of the present study was to investigate the nitrogen (N) and dry matter content of fractions produced by screw-press processing of three legumes and two grasses harvested on four consecutive weeks in order to determine optimal harvest time and species.

Materials and methods

The spring cut of white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), lucerne (*Medicago sativa* L.), perennial ryegrass (*Lolium perenne* L.), and tall fescue (*Festuca arundinacea* L.) was harvested in a replicated plot experiment at Foulumgaard, Aarhus University, Denmark. The cut herbage was frozen until processing in a lab-scale screw press (Angel 8500s, Angel juicers, AM Naarden, the Netherlands) resulting in a pulp and a green juice. Soluble proteins in the juice were precipitated by adding 12 M phosphoric acid until pH was lowered to 4. The acidified juice was left overnight at 4 °C and subsequently centrifuged for 10 min at 2,000×*g* and 4 °C. The precipitated protein was separated from the leftover liquid (brown juice).

The DM contents of all samples were determined on freeze-dried material by drying at 103 °C for two hours. Nitrogen was analysed by the Dumas procedure (Hansen, 1989). Composition and recovery was analysed with a linear model using Proc mixed in SAS (version 9.4. Copyright, SAS Institute Inc., Cary, NC, USA).

Results and discussion

Nitrogen content of herbage of the five plant species decreased with delayed spring cut and, in agreement with recent studies, legumes had higher N content than grasses at all spring cuts (Larsen *et al.*, 2019; Stødkilde *et al.*, 2019) (Table 1). The N content of the produced pulps was affected by plant species and spring-cut harvest date, generally with higher values in the early spring cuts and in the legume pulps (Table 2) corresponding well to the N content in the input plant material. The N content of the protein isolate varied with plant species and with harvest time, legumes having higher N contents than the grasses. For the legumes, N content was highest at the intermediate spring-cut harvest dates, whereas there was a tendency in the grasses for low N contents in late spring cuts. Nitrogen recovery, i.e. the proportion of plant N distributed to either pulp or protein isolate, was calculated (Table 3). In the pulp, species and time of harvest affected the N recovery, with higher values in pulp from late-cut plants and from grasses.

Table 1. Nitrogen content in spring cut harvested grasses and legumes.[1]

Spring cut harvest date	Nitrogen content (g kg^{-1} dry matter)				
	Lucerne	Red clover	White clover	Perennial ryegrass	Tall fescue
May 17	37.0^{a}	37.4^{a}	43.2	30.6	30.1^{a}
May 24	39.1^{a}	35.7^{ab}	42.3	24.8	26.7^{a}
May 31	33.2^{a}	29.0^{bc}	35.1	18.7	18.2^{b}
June 6	25.6^{b}	22.7^{c}	34.2	20.5	19.6^{b}
SEM	1.9	1.8	2.0	2.2	1.6
P-value (harvest)	0.02	0.01	0.06	0.07	0.02
P-value (species)	<0.0001				
P-value (harvest)	<0.0001				

[1] Different superscripts in a column indicate significant difference (P<0.05); SEM = standard error of the mean.

Table 2. Nitrogen content of pulp and protein isolate from spring cut grasses and legumes harvested at four consecutive weeks.

Spring cut harvest date	Pulp nitrogen content (g kg^{-1} DM)					Protein isolate nitrogen content (g kg^{-1} DM)				
	Lucerne	Red clover	White clover	Perennial ryegrass	Tall fescue	Lucerne	Red clover	White clover	Perennial ryegrass	Tall fescue
May 17	31.4^{a}	35.5	43.2^{a}	31.2^{a}	25.4^{a}	56.0^{c}	61.3^{a}	60.4^{bc}	50.4	58.9
May 24	27.8^{a}	26.7	33.8^{b}	23.3^{b}	20.9^{b}	68.9^{a}	63.1^{a}	66.7^{a}	55.9	59.3
May 31	22.0^{b}	26.0	32.6^{b}	19.5^{c}	17.9^{c}	66.8^{a}	57.6^{b}	60.9^{b}	48.7	50.4
June 6	23.3^{b}	25.6	29.2^{b}	19.4^{c}	16.4^{c}	61.6^{b}	52.1^{c}	56.5^{c}	49.0	38.1
SEM	1.0	2.9	1.5	1.0	0.7	0.8	0.9	1.0	1.7	3.9
P-value (harvest)	0.01	0.07	0.01	0.003	0.003	0.002	0.003	0.01	0.06	0.05
P-value (species)	<0.0001					<0.0001				
P-value (harvest)	<0.0001					<0.0001				

[1] SEM = standard error of the means; DM = dry matter; different superscripts in a column indicate significant difference (P<0.05).

Table 3. Nitrogen recovery (% of plant N) in pulp and protein isolate from spring cut grasses and legumes harvested at four consecutive weeks.[1,2]

Spring cut harvest date	Pulp					Protein isolate				
	Lucerne	Red clover	White clover	Perennial ryegrass	Tall fescue	Lucerne	Red clover	White clover	Perennial Ryegrass	Tall Fescue
May 17	47.83	51.08[b]	52.76[a]	63.44	44.22	45.88	31.74	35.07	35.81	28.27[a]
May 24	39.65	32.75[c]	30.30[c]	66.81	50.83	55.49	50.11	39.24	41.19	32.24[a]
May 31	33.03	47.56[bc]	41.89[b]	64.21	73.14	66.60	45.78	41.10	29.31	32.10[a]
June 6	58.25	73.21[a]	48.29[a]	75.12	65.36	60.14	46.50	41.85	24.00	17.01[b]
SEM	5.31	4.37	1.62	7.99	6.34	4.27	8.73	5.71	4.68	2.45
P-value (harvest)	0.10	0.01	0.002	0.73	0.09	0.10	0.53	0.84	0.19	0.03
P-value (species)	<0.0001					<0.0001				
P-value (harvest)	0.0002					0.08				

[1] Recovery was calculated from mass balances and N analysis of individual samples. As a consequence, recovery in pulp and protein isolate exceeds 100% for some samples.

[2] SEM = standard error of the means; different superscripts in a column indicate significant difference ($P<0.05$).

For the protein isolate, there was a species variation in recovery, with higher values in legumes than in grasses. Overall, there was a tendency for higher recovery for intermediate cutting dates. The results for both pulp and protein isolate were consistent with a higher proportion of protein being fibre-bound in the more developed plants (Buxton and Redfearn, 1997), thereby distributing a larger proportion of N to the pulp.

Conclusions

The study demonstrated that N content of pulp and protein isolate was affected by the spring-cut harvest date and by the plant species. Lucerne harvested at intermediate spring-cut dates had a high protein content and recovery, which combined with a high DM yield per ha makes this legume interesting from a monogastric feed perspective. For ruminant feed purposes, pulp produced from perennial ryegrass and tall fescue harvested at late spring cut showed greatest potential; however, the recovery and N content in the related protein isolate was low. From an economically and environmentally sustainable point of view, the potential of both feed-relevant fractions, the pulp and protein isolate must be maximized. The results from this study highlight the challenge of optimising both fractions, and that selection of optimal species and time of harvest will depend on whether the purpose is production of pulp for ruminants or protein isolate for monogastrics.

References

Buxton D.R. and Redfearn D.D. (1997) Plant limitations to fiber digestion and utilization. *The Journal of Nutrition* 127, 814-818.

Colas D., Doumeng C., Pontalier P.Y. and Rigal L. (2013) Twin-screw extrusion technology, an original solution for the extraction of proteins from alfalfa (*Medicago sativa*). *Food and Bioproducts Processing* 91, 175-182.

Hansen B. (1989) Determination of nitrogen as llementary N, an alternative to Kjeldahl. *Acta Agriculturae Scandinavica* 39, 113-118.

Larsen S.U., Jørgensen H., Bukh C. and Schjoerring J.K. (2019) Green biorefining: effect of nitrogen fertilization on protein yield, protein extractability and amino acid composition of tall fescue biomass. *Industrial Crops and Products* 130, 642-652.

Manevski K., Lærke P.E., Jiao X., Santhome S. and Jørgensen U. (2017) Biomass productivity and radiation utilisation of innovative cropping systems for biorefinery. *Agricultural and Forest Meteorology* 233, 250-264.

Pirie N.W. (1978) *Leaf protein and its by-products in human and animal nutrition*. Cambridge University Press, Cambridge, UK.

Stødkilde L., Damborg V.K., Jørgensen H., Lærke H.N. and Jensen S.K. (2019) Digestibility of fractionated green biomass as protein source for monogastric animals. *Animal* 13, 1817-1825.

Wilkins R.J. and Jones R. (2000) Alternative home-grown protein sources for ruminants in the United Kingdom. *Animal Feed Science and Technology* 85, 23-32.

Characteristics of organic manure from 'Freewalk' housing, compared with slurry, and their appreciation by farmers

Van Middelkoop J.C.[1], De Boer H.C.[1], Galama P.[1], Brügemann K.[2], Leso L.[3], Blanco-Penedo I.[4], Zentner A.[5] and Klopčič M.[6]

[1]Wageningen University and Research, the Netherlands; [2]University of Kassel, Germany; [3]Università degli Studi di Firenze (UniFi), Italy; [4]Swedish University of Agricultural Sciences, Uppsala, Sweden; [5]HBLFA Raumberg-Gumpenstein Institut für Tier, Technik und Umwelt, Irdning-Donnersbachtal, Austria; [6]University of Ljubljana, Slovenia

Abstract

In the EU-project 'Freewalk' a new housing system for dairy cattle is investigated in eight countries. In a freewalk housing system cows are provided with an open bedded-pack area for resting and exercise, rather than the individual stalls and concrete alleys in cubicle systems. In most of the freewalk housings a slatted floor is also present in the eating area. The two floor types result in slurry and solid manure. Slurry has a relatively high mineral and low organic matter content. Solid manure has a relatively high organic matter content. In a questionnaire, freewalk farmers and reference cubicle farmers were asked about their appreciation of the two manure types as fertiliser and/or as soil improver on grassland and arable land. Liquid manure types were more frequently appreciated as fertiliser and solid manure as soil improver.

Keywords: freewalk housing, animal manure, soil improver, fertiliser

Introduction

Since the 1970s in Europe, cubicle-housing systems have become widely used by the majority of farmers, replacing the traditional tie stalls. The animals can roam freely on slatted or solid concrete floors in the eating area and lie down to rest and ruminate in cubicles. The system has increased the labour efficiency greatly and saved bedding material compared with the traditional tie stall system. Since the type of animal manure is inextricably associated with the type of housing, the handling of manure changed with the housing, from mainly solid to mainly slurry.

Recently a new housing system is under development in Europe, the so called 'freewalk housing'. The concept of freewalk housing for dairy cattle has been initiated for improved animal welfare and manure quality. The cattle can roam freely through the whole housing on a packed bedding floor, and can lay down to ruminate wherever they choose. The assumption is that cattle can express their natural behaviour more in a freewalk system than in cubicle housing, and have fewer health problems, especially claw disorders and lameness (Leso *et al.*, 2019).

Regarding the environment and nutrient efficiency, it is essential that freewalk housing does not lead to any deterioration, but preferably to improvements compared with cubicle housing. The faeces and urine are absorbed, mixed and more or less composted in the bedded pack. This results in a solid type of manure and is removed only a few times a year. In most of these housings the floor in the eating area is slatted or solid with a manure scraper, by which slurry is also available. Due to differences between the two manure types in their dry matter contents (Table 1) the techniques for storage, transport and application differ. In addition, the composted bedding has a relatively high content of both organic nitrogen and organic matter, expressed in C content, and could be used as a soil improver to increase soil organic matter. The slurry has a low dry matter content and a high mineral N content, which makes it more suitable for use as fertiliser. It is of interest to determine how farmers appreciate the fertiliser and soil improver values of the different manure types.

Table 1. Characteristics of a freewalk composted bedding and slurry, in g kg product^{-1} (from: de Boer, 2014).

Parameter	Dry matter	Total N	Mineral N	Total P	Total C	C/N-ratio
Composted bedding	454.5	9.7	0.5	1.9	132.5	14.5
Liquid manure (slurry)	84.0	3.9	1.9	0.5	33.0	8.4

Materials and methods

In the EU-project FreeWalk (ERANET-SUSAN-ID: 117) farmers with cubicle and freewalk housing are involved. For the Freewalk farmers it was required that they had used the freewalk system since at least before April 2017. Farmers from Austria (n=6), Germany (n=14), Italy (n=7), Sweden (n=2), Slovenia (n=2) and the Netherlands (n=10) filled in a questionnaire regarding their appreciation of manure types. Responses were received from 20 freewalk farmers and 21 reference cubicle farmers. The types of manure were slurry, liquid and solid fraction, compost from woodchips and/or straw, other compost, compost from outside the farm, and straw from a deep straw barn. It was asked if the different manure types are appreciated as fertiliser, soil improver or both, on grassland and on maize and/or other arable land.

Results

On most farms slurry is applied on grassland and arable land (Table 2). Composted wood chips are, as expected, only applied on freewalk farms. It can be expected that this solid type of manure would be mainly applied to arable land. Ten farmers, however, applied it on both grassland and arable land. Composted straw and straw from deep straw barns is mainly applied on the reference cubicle farms. On the reference farms the solid manure types are mainly applied on arable land.

The total number of observations, n=41, was not enough to statistically analyse differences between the two farm types in terms of farmer appreciation of all the manure types. Therefore, no differentiation between farm types was made in appreciation of manure types, as summarized in Figure 1. On both grassland and arable land the liquid types of manure, slurry and liquid fraction, were appreciated by two-thirds of the farmers solely as fertiliser and one-third as a soil improver or both. Solid manure types were mostly appreciated as soil improver. Only 15% of the farmers appreciated the solid types solely as fertiliser, both on grassland and on arable land.

Appreciation of liquid types and solid types were compared between the two housing systems (data not shown). The freewalk farms appreciated the liquid types more frequently as solely fertiliser, and the solid types as both fertiliser and soil improver. The reference cubicle farms appreciated the liquid types more frequently as both fertiliser and soil improver and the solid types as solely soil improver.

Table 2. Number of freewalk and reference farms that applied manure types to grassland and arable land.

Manure type	Freewalk farms, n=20		Reference farms, n=21	
	Grassland	Arable land	Grassland	Arable land
Slurry	17	10	17	13
Liquid fraction	3	3	3	2
Solid fraction	0	1	1	2
Composted wood chips	10	10	0	0
Composted straw	1	1	1	6
Other compost	1	1	0	0
Deep straw barn	2	2	3	6

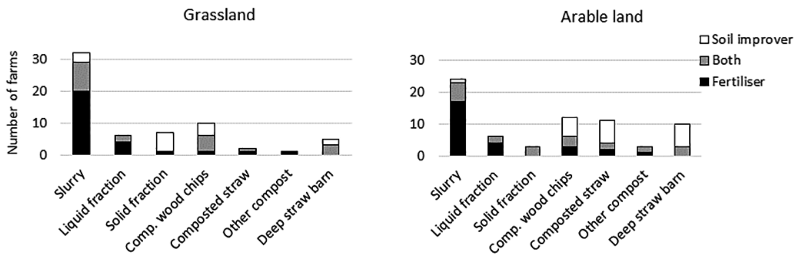

Figure 1. Number of farmers that appreciate manure types as soil improver and/or as fertiliser.

Conclusions

Freewalk farms apply solid manure types on both grassland and arable land. Reference cubicle farms apply solid manure types mainly on arable land. Two-thirds of the farmers appreciate liquid manure types solely as fertiliser and one-third as both fertiliser and soil improver. More than half of the farmers appreciate the solid types of manure solely as soil improver and one quarter as both fertiliser and soil improver. The cubicle farmers appreciate liquid manure types more as soil improver than the freewalk farmers; the freewalk farmers appreciate the solid manure types more as fertiliser than the cubicle farmers.

References

De Boer H.C. (2014) *On farm development of bedded pack dairy barns in The Netherlands: nutrient balances and manure quality of bedding material.* (eport Wageningen UR Livestock Research; No. 709. Lelystad: Wageningen UR Livestock Research.

Leso L., Barbari M., Lopes M.A., Damasceno F.A., Galama P., Taraba J.L. and Kuipers A. (2019) Compost bedded-pack barns for dairy cows. *Journal of Dairy Science* 103, 1072-1099. https://doi.org/10.3168/jds.2019-16864

The use of multispectral images to estimate yield of grassland

Vervisch B. and Demeulemeester K.
Inagro VZW, Ieperseweg 87, 8800 Roeselare, Belgium

Abstract

An optimal and intensive grassland management can reduce cost of concentrated protein feed to a minimum. For an optimal use of grassland, intensive follow up of the growth and quality is therefore crucial. Hence, to estimate time of mowing, information is needed about potential yield and feed value in the field. Multiple visual assessments are, however, both time and cost consuming. For the use of grassland as low cost fodder it is important to be able to assess the quality of the harvested biomass for ensilage. Even though studies have demonstrated the effectiveness of remote sensing in grassland monitoring, it is still a challenge to use remotely sensed data in intensively managed grasslands to predict yield potential and feeding value of the biomass. Remote sensing can deliver objective estimates of on-field parameters, as a decision support system. In our research, assessment with remote sensing was performed before every harvest date, to evaluate reflectance of the grass with aboveground biomass, pigment, water content and feeding value parameters. Ultimately this study aims to investigate correlation between remote sensing parameters and yield measurements for assessing annual grassland production.

Keywords: grassland, yield predictions, multispectral sensing

Introduction

The estimation of forage quality of grassland is an important factor and has great promise for performance of livestock, both grazed and silage fed. However, there is a lack of effective tools to evaluate and predict the quality of grassland (Hund *et al.,* 2019). The objective of this study is to explore the possibilities in assessing grassland yield through canopy reflectance. Four different grassland mixtures in Beitem, Belgium were analysed with remote sensing techniques and compared with vegetation samples collected on site. The mixtures consisted of (1) perennial grass, white and red clover, (2) perennial grass and white clover, (3) perennial grass and (4) tall fescue. These mixtures were assessed on yield and quality parameters. Plant parameters Normalized Difference Vegetation Index (NDVI) and Normalized Difference Red Edge (NDRE) were analysed with a multispectral camera to asses yield predictions. The traditional approaches usually implemented require expensive laboratory analyses, whereas remote sensing techniques could provide crucial information without these destructive and expensive analysis. The results of this study show that it is possible to make yield predictions according to the NDVI value, but the prediction of forage quality is at this point still too complex. Model-based research may deliver results in the near future.

Materials and methods

This study was conducted at the trial location of Inagro, Beitem, Belgium (50°54'12.7'N; 3°07'26.4'E). The area has a temperate maritime climate, with an annual mean precipitation of 925 mm and mean annual temperature of 9.8 °C (KMI, 2019). The trial consists of four mixtures of grassland in (n=4): (1) perennial grass (80%), white (10%) and red clover (10%), (2) perennial grass (80%) and white clover (20%), (3) perennial grass (100%) and (4) tall fescue (100%). All plots were managed according to good agricultural practices and evenly fertilised. Field data were collected during the growing season of 2019 (April – September). During the season 4 grass cuts were sampled: 15/04/19, 06/06/19, 15/07/19 and 12/09/19. Grass yield was weighed from 12 m^2 per plot on these four dates. At each yield moment biomass samples of approximately 500 g were taken for analyses of dry matter in the lab of Inagro. Forage quality was determined at BAB LAB Belgium. Shortly before each grass cut, field spectral measurements were analysed for comparison with Micasense RedEdge-M on bands blue (475 nm), green (560 nm), red (668 nm), red-edge (717 nm) and near-infrared (840 nm).

Results and discussion

At the first cut the monoculture of perennial grass showed the highest yield of 3,172.9 kg dry matter (DM) per ha. The second cut on 06/06/2019 showed no differences. A mean yield of 6,215.9 kg DM ha^{-1} was harvested at this date (Table 1). Approximately one month later the monoculture of perennial grass again showed the highest yield, 3,160.5 kg DM ha^{-1}. By the end of the season at the fourth grass cut no significant differences were detected between mixture of perennial grass and white clover, perennial grass monoculture and tall fescue. No assessments were made on the relative abundance of clover, but according to yield data clover seems not to be well established on this field.

As can be seen in Figure 1, there is a positive linear trend between DM yield and NDVI values, except at the second cut. There were R^2 of 0.7554, 0.7864 and 0.6149 for the yield determinations of respectively the first, third and fourth grass cut. At the second assessment a negative trend is noted. This can be

Table 1. Yield data (kg dry matter ha^{-1}) on the four yield assessments.[1,2]

	17/04/2019		06/06/2019		15/07/2019		12/09/2019	
PG + WC + RC	1,381.5	d	6,811.8	a	1,070.3	c	2,111.6	b
PG +WC	2,269.4	b	6,068.5	a	2,302.1	b	3,241.7	a
PG	3,172.9	a	6,114.0	a	3,160.5	a	3,398.4	a
TF	1,778.8	c	5,869.2	a	2,604.7	ab	3,285.3	a
Mean	2,150.6		6,215.9		2,284.4		3,009.2	
LSD	248.4		1,899.3		770.9		674.6	
VC	5.230		13.840		15.290		10.150	
P-value	0.000	***	0.472	NS	0.000	***	0.001	***

[1] Values followed by the same letter are not significantly different ($P \geq 0.05$). * $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS = not significant $P \geq 0.05$.

[2] PG = perennial grass; WC = white clover; RC = red clover; TF = tall fescue; LSD = least significant difference; VC = Coefficient of variation (%).

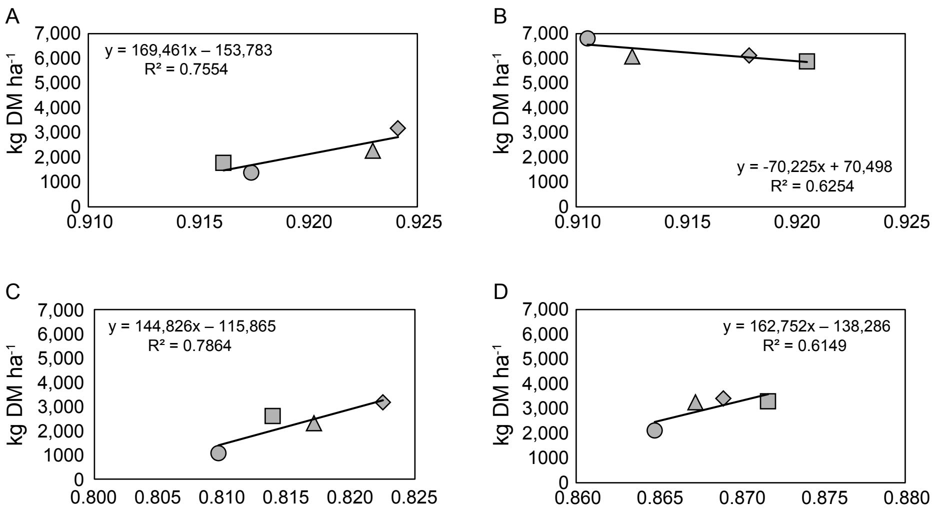

Figure 1. Correlations between dry matter (kg dry matter ha^{-1}) yield and NDVI values for the four timings of grass yield assessment (with (A): first grass cut, (B) second, (C): third and (D): fourth; ● perennial grass (PG) + white clover (WC) + red clover (RC); ▲ PG + WC; ♦ PG; ■ tall fescue (TF)).

explained by the fact that the grass sward was lodged at that time. Lodging was estimated according to correlation with blue intensity, according to the method described by Andreasen *et al.* (2019). The plots with perennial grass in combination with clover (red and white) showed a significant higher level of relative blue intensity, hence lodging, than tall fescue (Table 2). This had effect on the value of NDVI, which is lower than expected. The plot with tall fescue showed the lowest level of lodging and the lowest yield level, but as there was no lodging in this plot, the NDVI showed a false positive.

Table 2. Relative value of blue.[1,2]

	Blue intensity (relative %)	
PG + WC + RC	115.0493	a
PG +WC	108.7174	a
PG	93.79443	b
TF	82.43893	c
Mean	100	
LSD	10.64	
VC	4.82	
P-value	0	***

[1] Values followed by the same letter are not significantly different ($P \geq 0.05$). *** $P<0.001$.

[2] PG = perennial grass; WC = white clover; RC = red clover; TF = tall fescue; LSD = least significant difference; VC = Coefficient of variation (%).

Conclusions

The parameter of NDVI shows great promise in predicting the yield per cut, with R^2 of 0.7554, 0.7864 and 0.6149 for respectively first, third and fourth grass cut. Special attention is needed regarding lodging of grass swards. In lodged fields no adequate prediction can be made with the parameter NDVI. This is an important factor in upscaling these results to larger grass fields. These analyses will be made in the growing season of 2020.

Acknowledgements

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References

Andreasen C., Bitarafan Z., Westergaard, J.C. and Rasmussen J. (2019) Using drone imagery to estimate lodging in red fescue (*Festuca rubra*). *Grassland Science in Europe*, 24, 309-311.

Hund A., Feuerstein U., Roth L., Kirchgessner N., Aasen H., Studer B. and Walter A. (2019) Methodological advances, challenges and perspectives in field phenotyping and its application to forage crops. *Grassland Science in Europe*, 24, 295-305.

KMI (2019) *Climate atlas*. Collected from Royal Meteorological Institute of Belgium: Available at: https://www.meteo.be/nl/klimaat/klimaatatlas

Grass as a raw-material for biogas production – a case study with a farm-scale leaching bed digester

Winquist E.[1], Virkkunen E.[1], Koppelmäki K.[2], Vainio M.[1], Tampio E.[1] and Seppänen A.M.[1]
[1]*Natural Resources Institute Finland (Luke), Maarintie 6, 02150 Espoo, Finland;* [2]*University of Helsinki, Agroecology, P.O. Box 27, 00014, University of Helsinki, Finland*

Abstract

Grass silage from green manure leys was used for a farm-scale biogas production together with horse and chicken manure. The realised biomethane production in a batch process (117 d) was 86% of the calculated production. Altogether 76% of the total nitrogen in raw materials was left in the fresh digestate, and 63% remained in the digestate after three weeks storage.

Keywords: green manure, grass silage, farm-scale, biogas, leaching bed, nitrogen

Introduction

In organic arable farming, grass is cultivated for green manure typically every third year as part of a crop rotation. Biogas production from grass biomass could sustainably intensify the production by combining food and energy production (Koppelmäki *et al.*, 2019). In addition, biogas plant digestate is a better fertiliser than green manure. Firstly, part of the organic nitrogen is degraded into soluble ammonium nitrogen, more easily available for the plants (Möller and Müller, 2012). Secondly, digestate can be spread onto the fields according to the fertiliser requirements. In this study, green manure leys were ensiled and used for biogas production in a dry-type digester together with small amounts of horse and chicken manure. Grass silage has high organic matter content (volatile solid, VS) and high biochemical methane potential (BMP) (Prochnow *et al.*, 2009). Horse and chicken manure used as co-substrates provide additional biomethane production capacity but also valuable trace elements for the micro-organisms and alkalinity to maintain buffering capacity (Thamsiriroj *et al.*, 2012). Moreover, manure increases the nutrient content of the grass-based digestate. The aim of the study was to follow-up a new type of farm-scale bioreactor, a leach bed reactor, and evaluate its suitability and efficiency for energy production and nutrient circulation on the farm.

Materials and methods

The biogas plant on Knehtilä farm in Hyvinkää, southern Finland had two batch leach bed reactors (2×810 m^3), and a percolation tank (200 m^3). The basic principle in a leach bed reactor is that the raw materials are packed airtight in a silo (anaerobic conditions), percolation liquid is circulated through the bed and biogas is collected both from the silos and the percolation tank. Grass (mainly clover-grass) was grown as green manure during summer 2018, harvested and ensiled in a pile without additives. Chicken manure was obtained from a nearby egg producer and horse manure from several stables in the area (Table 1). The plant started operation in September 2019. The follow up of the plant started during the third batch (Reactor 1, 11.3. – 8.7.2019, 117 d). Data were obtained from the on-line measurements of formed biogas (methane content and volumetric production) and by sampling (total and soluble N, BMP) raw materials, fresh digestate, and digestate after storage in a pile (26 d). Only one sample was taken from each of the raw materials, while three samples were taken from the digestate (one sample represented a composition sample from ten sub samples). The BMP experiment was carried out with inoculum from a farm-scale biogas digester (Luke, Maaninka). Inoculum/substrate VS-ratio in bottles was 0.5 and the experiment lasted 53 d. Assay was performed according to Tampio *et al.* (2015).

Table 1. Raw materials and calculated biomethane production.[1]

	Mass	Mass	BMP	CH_4 production	CH_4 production
	Mg FM	%	Nm^3 CH_4 $(Mg\ VS)^{-1}$	m^3	%
Grass silage	407	79.8	245±5	26,490±510	88.5
Chicken manure	50	9.8	146±9	2,010±130	6.7
Horse manure	53	10.4	91±4	1,450±60	4.8
Total	510	100.0	217±5	29,940±700	100.0

[1] FM = fresh matter; BMP = biochemical methane potential; VS = volatile solids, i.e. organic matter.

Results and discussion

Grass silage provided 80% of the total mass of raw materials but 89% of the biomethane production (Table 1). Based on measured BMPs, the total calculated biomethane production was 29,940 m^3. However, the realised biomethane production was only 25,760 m^3 (86% from calculated) (Figure 1). Often the whole biomethane potential in raw materials is not achieved in production scale. The degradation of organic nitrogen to soluble nitrogen was assumed to be 60% from the degradation of organic matter (VS). The calculated amount of organic nitrogen left in the digestate is almost equivalent to the amount of organic nitrogen analysed from the fresh digestate (Figure 2). However, the amount of soluble nitrogen is considerably less than what could have been expected according to the model calculations. One reason for this is that liquid nitrogen is partly dissolved in the percolation liquid circulating in the process. Also, the sampling contributed some of the uncertainty in the results. The raw materials were not thoroughly mixed when the reactor was loaded. Thus, the samples taken from the digestate may not represent the mixture adequately. Furthermore, the total nitrogen content was decreased even during the storage in a pile. Altogether 76% of the total nitrogen in the raw materials was left in the fresh digestate, and 63% after three weeks of storage.

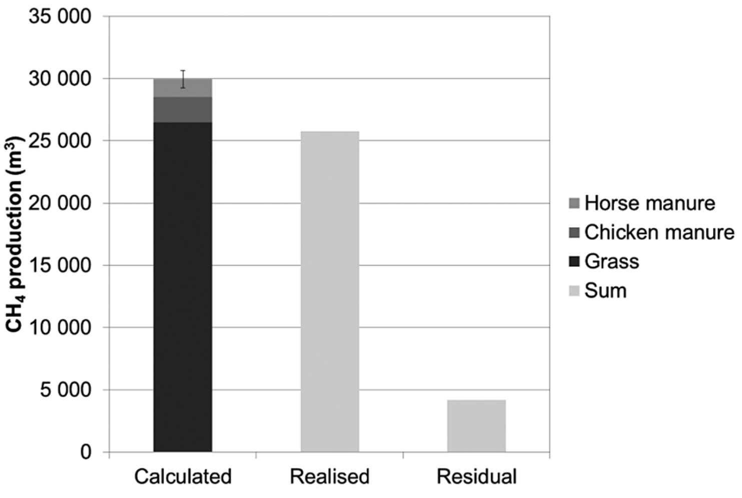

Figure 1. Methane production from one batch (residual = calculated – realised).

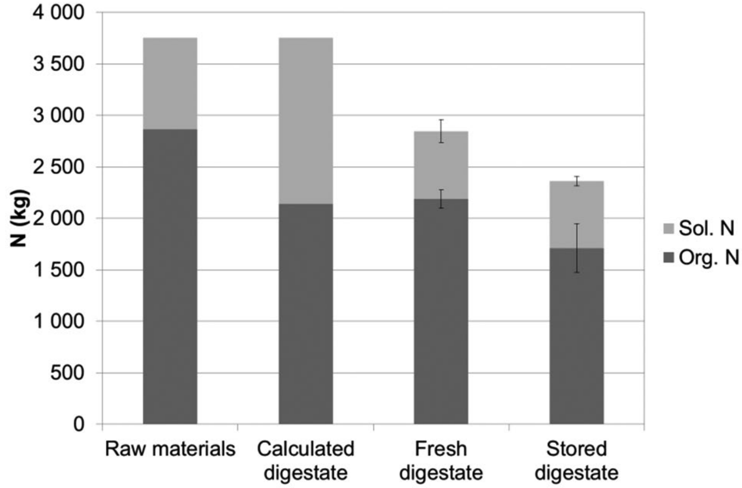

Figure 2. Soluble and organic nitrogen in the raw materials (sum), calculated digestate, digestate (analysed) and stored digestate (analysed).

Conclusions

The follow-up of the biogas plant continues. This was the first batch where samples were taken and analysed. With the next batch the residual biomethane potential of the digestate also will be analysed. In addition, more attention will be given to the nitrogen balance through the biogas process. The monitoring helps to understand and make improvements to the process and thus enable more efficient use of raw materials and nutrients in farm-scale environment.

Acknowledgements

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References

Koppelmäki K., Parviainen T., Virkkunen E., Winquist E., Schulte R.P.O. and Helenius J. (2019) Ecological intensification by integrating biogas production into nutrient cycling: modeling the case of Agroecological Symbiosis. *Agricultural Systems* 170, 39-48.

Möller K. and Müller T. (2012) Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. *Engineering in Life Sciences* 12, 242-257.

Prochnow A., Heiermann M., Plöchl M., Linke B., Idler C., Amon T. and Hobbs P.J. (2009) Bioenergy from permanent grassland – a review: 1. Biogas. *Bioresource Technology* 100, 4931-4944.

Tampio E., Ervasti S. and Rintala J. (2015) Characteristics and agronomic usability of digestates from laboratory digesters treating food waste and autoclaved food waste. *Journal of Cleaner Production* 94, 86-92.

Thamsiriroj T., Nizami A.S. and Murphy J.D. (2012) Why does mono-digestion of grass silage fail in long term operation? *Applied Energy* 95, 64-76.

Theme 5.
Knowledge exchange

Knowledge exchange approaches for better decision-making and innovation processes

Kelly T.
Teagasc, Oak Park, Carlow, Ireland

Abstract

There are three approaches which underpin knowledge exchange and proactive support for innovation in professional farm advisory services that guide the type of support advisers provide to farmers. (1) Knowledge exchange is a human contact game. It is based on the interactivity, relationships, trust and motivations of the people involved. Advisers in their professional roles use peer-to-peer influences, digital technologies, practical demonstration grounded in the needs of users, language of the user. They must show empathy with the challenges and generate the interest of actors. (2) Multiple channels of communications are needed. There is no one system that does it all for everyone. For any one individual, the knowledge exchange process benefits from exposure to a range of communication channels, creating awareness, interest and a demand for more support. (3) Multi-actor approaches are better for complex innovations; they address real issues, deliver clearer messages, more consistently, with less risk of contradiction and loss of relevance to targeted users. Farm advisers are service providers who go far beyond the role of service provider in helping farmers and their families. This developmental work extends beyond the linear knowledge transfer of technologies from science to practice. They may even go beyond the role of knowledge exchange into roles that challenge their abilities to support networks of multi-disciplined actors to resolve issues in groups or networks. For most advisers the immediate need is to deliver a valued service to a client base of farmers who demand these services. There is a clear value in having strong institutional support from advisers' organisations or associations, and the backup of applied research and industry partners working in harmony towards common farm and rural development objectives.

Keywords: farm advisers, innovation support, relationships, decisions

Introduction

This paper reflects on three basic approaches that are important in the delivery of successful innovation and entrepreneurial supports to farmers. This reflection is drawn from the experience of the author from his role as Director of Knowledge Transfer in Teagasc (Ireland) and in EUFRAS (European Forum for Farm and Rural Advisory Services). This experience was framed in the context of diverse grass-based family farm production systems.

The three important approaches are:
1. Human interactivity and direct contact is valuable.
2. Multiple channels are needed for the diversity of messages, needs, wants of people.
3. Multi-actor approaches are better for complex challenges; they support interactive innovation.

The value of interactivity and human contact

Human contact is basic to learning in an interactive way. It is fundamental to satisfying a need or want, with the possibility that knowledge and information may be exchanged and questioned, increasing the probability that it will be used to support innovation on farms.

Agriculture extension organisations provide innovation support through people who have roles as farm advisers, agricultural instructors, extension agents, agricultural consultants, innovation brokers, facilitators or intermediaries. The latter three use more interactive learning approaches. In fact, we see that this interactivity is encouraged in formal education and society in general, for example; see how classrooms are laid out in modern schools.

Within agricultural extension there is still a strong component of linear information and knowledge transfer from applied research through publications, books or best practice manuals to wider adoption on farms. The linear extension methods is predominant as it has been extremely successful in leading and assisting change in agriculture and rural development for over 100 years of formal and informal farm advisory programmes. The objective of these programmes is to help farmers and rural communities survive, develop and prosper by providing food, environmental and other services for our increasingly urban world and its growing population. 'One-to-one exchange of information and advice, whether from farmer to farmer or from professional adviser to farmer (and vice versa), will continue to be important' (Black, 2000) who also stated that 'There is a need to provide for active participation by farmers in research and development processes.' Knowledge transfer has been central to a continuous learning process ranging from soils to consumer and society issues, within the framework of an agriculture and knowledge innovation system (AKIS). This system is made up of organisations, individuals and groups who facilitate the flow of information, the generation of new knowledge and the interactions necessary to motivate and empower all the actors involved. Fundamental to all learning processes is the transfer or exchange of knowledge and support for action in applying that knowledge.

How we teach others and learn ourselves has been continuously challenged and while professional advisers may be comfortable with classroom, lecture, workshop, practical demonstration and job work placement, not all adults are. There is a realisation now that learner-centred approaches are more important. Are all people eager to learn? Do we learn in the same way? How do interactive tools improve engagement, dialogue and the interaction/participation of learners? How do we facilitate adult learning? These questions are now more important in the rush towards more online training platforms, distance learning, blended learning, etc. The recent Teagasc Education Vision (2018) and the EU SCAR AKIS (2019) report both support a move towards a more inquiry-based learning including problem-based learning (PBL). PBL is a student-centred learning approach where teachers become facilitators of group work as students work their way through a presented problem (Barrett, 2017).

We have a well-educated population of farmers and farm families. They have a huge appetite for knowledge, but increasingly want information on a just-in-time basis. Their retention span is short and they need constant reminders and routines. The role of extension is to ensure that the flow of information is constant and relevant to the time of year and issues on that farm. A good education is the platform for life-long learning. An analysis of economic returns to formal agricultural education (Heanue and O'Donoghue, 2014) confirmed a positive and significant return both in terms of the internal rate of return from a human capital perspective as well as the internal returns from agricultural education to farm level yields, intensity and income when viewed from a production function perspective. The analysis confirms patterns in the international literature on returns to education. It also supports the contribution of learning to the enabling environment for innovation and an effective and resilient AKIS.

One to one is the traditional model of advisory and consultancy. It is now delivered through different means: in the office, through social media, on the phone, on the farm etc. The adviser can be both reactive (answering questions, giving information and opinions) and proactive asking questions, showing interest, challenging and encouraging. Personal contact is important in building relationships, all actions, words, tone and visual appearance affects the person we are communicating with and the message we are giving. We must look at this from the perspective of the farmer who will form an independent opinion of the message giver very quickly. Their opinions are influenced by the interest shown in them, their farm, their family and the story of the person they meet. The interest in the farm is a key factor and needs to be genuine and deliberate; it should appreciate the farmer's emotional attachment to the land, the livestock, crops, environment and history of the farmer and farm. Adviser-client relationship and trust was recently studied by Grogan (2017) who identified the value of personal and organisational trust relations around the flow of credible and reliable information supporting changed and improved practices and new technologies. In this study, trust in the relationship was seen to have been gained quickly where there was trust in the adviser's organisation (Teagasc) because of its reputation, agency status, independence and resources. This was influenced by the standards set and the support provided in the selection and appointment of advisory staff. Apart from this, the relationship was hugely influenced by the individual characteristics and behaviour of the adviser. First impressions were important and client preferences for younger, more proactive, honest and interested advisers who had up-to-date technical knowledge were apparent in this study. Experience in the adviser role or in farming made it easier to build a relationship quickly. The study also showed the value of availability and responsiveness with higher value put on face-to-face meetings where possible.

The increased focus on interactivity is key to better innovation support and calls for more participatory programmes where they combine critical factors such as experiential learning, integrating local information, effective facilitation, group autonomy and ongoing relationships (Millar and Curtis, 1997). The change in role of extension personnel from expert to facilitator (Barman and Kumar, 2011) has opened up the potential for advisers to harness the power of peer-to-peer learning and to exploit the shared motivation, experience and energy for learning and improvement in practices, outcome and impacts. Among Irish dairy farmers, studies by Hennessy and Heanue (2012) have shown increased rates of good practice adoption and increased profitability among active participants in adviser-facilitated dairy discussion groups. How we engage and build relationships in society also extends to working with groups of farmers. These groups take many forms, including peer-to-peer informal discussions, e-messaging platforms, listening groups, benchmarking groups and facilitated discussion groups.

An analysis of agricultural knowledge spill-overs and the degree to which these drove innovation in agriculture in Ireland, showed a significant special relationship with research, education and advisory service locations and centres. Läpple *et al.* (2016) reinforce the point that the diffusion of technology and innovation is related to the level of contact, interaction and ownership of the process. It follows that farmers' knowledge and trust of the adviser and their organisation is also important; 'Local and personal contacts generally have more influence on farmers' intentions than more distant and impersonal sources. In particular, many farmers are not disposed to follow advice from institutions that they feel do not fully understand their situation' (Garforth *et al.*, 2004).

Multiple communication channels and extension methods

People are different; they have different personalities, communication preferences and habits. The knowledge transfer challenge is to use the available channels effectively and with a view to effective knowledge sharing, learning and adoption. We should not assume that all farmers are actively seeking knowledge, as many need to be engaged proactively. This leads to a potential for a miss-match or divergence of purpose and communication preferences and even cultural divide that can frustrate the adviser and the farmer. One of Vanclays' social principals of extension (Vanclay, 2004) is that 'Farmers construct their own knowledge ...; they create their own knowledge through experimentation, trial and through their own theorising'. From their own view the very act of adoption is in itself is a form of scientific enquiry and contributed to the knowledge resource of the farmer and those who communicate with that individual. This may vary from looking over the fence, to one-to-one communication or group work.

The value of using multiple communications and extension methods is acknowledged by Vanclay (2004). It creates a knowledge platform which is flexible and supports the varied needs, habits and preferences of individuals. The approach in organisations like Teagasc is to employ a menu of communications and extension methods both directly and indirectly, which exploit the full potential of the AKIS and are justified by the feedback received.

The value of old fashioned paper

Paper-based information sources vary from published science, farming press, company magazines, newsletters, posters and infographics, and are widely used and have strong readership. Despite the fact that over 95% of Irish farmers have mobile access to information, they generally value a variety of printed information, photographs and infographics. In a Teagasc digital-use survey in 2019 over 89% of a sample of 200 Irish advisers agreed or strongly agreed that digital media and print media would co-exist for the next five years (Moore, unpublished data). Teagasc, for now, continue to produce printed publications for monthly newsletters, a bimonthly farmer magazine, booklets for events and conferences as well as supporting the weekly farming press with relevant articles and advertising even though these are published online.

Digitisation

New forms of communication are being increasingly used and enabled by the array of digital tools now in use: e-mail, text, websites, YouTube, podcasts, webinars, messaging and social media networks. These all enable broad delivery and exchange of information in easy to access forms with a strong tendency towards mobile devices and cloud support. However, the real added value from digitisation is the degree to which it expands and speeds up the flow of knowledge in the AKIS and empowers advisers and farmers to work at a higher level of interaction. For example, advisers can record and edit a radio programme and upload it to the local radio station from their own office or from a farm. In terms of big data, farmers are currently gathering and processing huge amounts of information that needs to be interpreted and used more in decision-making. The motivation and the means of making this change to greater digital application needs to be led by impartial intermediaries; this is a new role for advisers.

Public events

The bringing together of people to listen, see, share, discuss, question, experience and evaluate information is well organised in conferences, demonstration/open days, seminars, workshops, meetings, training days, shows etc. These have the added value in that they are shop windows for the target audience and the knowledge gaps identified. A farmer or member of the public will assess if the topics are relevant to them. They can also be influenced by the skill, leadership and attitude of the presenter, or by the value of the message to their personal situation. The experience gained in Ireland from the BETTER Beef Programme (Kelly, 2011) and other demonstration farm programmes is that there is a need to brand and market events over a period of 4-5 years, supporting them through other channels. This includes using all farming media, advertising and promotional channels.

Communication and extension channels serve two main purposes. Firstly, they disseminate information and messages that someone has decided are important, they carry new information or reminders of seasonal routines to be followed. The second purpose is that they create a public profile, they stimulate interest and the receiver will pick-up on not just the information but also the prominence given to and importance of the source, topic, specific action or decision to be influenced. While we often hear of demand-led services, pull rather than push approaches. Even though many of these media channels rely on push rather than pull approaches, the structure within the various communication channels is important, the source, the relevance, quality and consistency of the methodology used feed the habits of the receiver and create a dependency and therefore a demand. The debate is open as to whether this dependency is good or bad. Good; it allows the user/innovator to make simple decisions based on the experience of others or the scientific expertise. Bad; in that it does not stimulate the users' own rational decision-making processes, and therefore it may be harder to make other decisions where support is not readily available and therefore to be creative or innovative.

The reality is that it allows both to exist in equilibrium; this equilibrium shifts for one decision to the next depending on the knowledge, complexity and importance of the action. The role of the adviser is to support the decision making, and this is helped if there is lots of information available to inform both the adviser and the farmer. However, the adviser must accept that human diversity exists and that opinion is formed over a lifetime and embedded in habits and experiences of the past. The shift from top-down linear knowledge transfer (KT) to more participatory knowledge exchange (KE) and co-creation has been enabled largely by the much easier access to ever increased knowledge and amounts of information. There also has been increased specialization of farming activity, isolation of farmers and a need for reassurance through other like-minded individuals. This, however, leads to the adviser's dilemma; which is that as they want to enable the client to find the best decision and implement it, like it was their own solution, but in order to be paid for a service they need to impress that they found the solution and that there was a real added value from their services. Despite all the information and knowledge, the challenge of constantly updating and reminding farmers will continue creating the awareness, even urgency, using all the channels available. However, to ensure better support decisions and actions more effort is needed to persuade farmers and support the implementation of these decision. All the channels of communication used by advisers must always support the 'why?' as well as the 'what?' and 'when?'

Targeting extended family, wider rural community or customer base in order to influence farmers' decisions also exploits the wider informal networks and their relationship/influence on farmer decisions. This approach is used extensively in targeting issues like farmer health and safety, environmental and market awareness and diversification opportunities for farm business. This strategy is good at generating awareness, expensive if using advertising, and has been aided considerably by the advent of social media messaging platforms.

Multi-actor approaches

In addition to multiple channels the influence of multiple actors who interact with farmers in their day-to-day life are valuable resources in innovation support and better decision making. Their influence is directly proportional to their status in the farmer's mind and to the degree to which their opinion and advise is localized, contextual and timed. The co-creation of innovative solutions occurs through multi-actor interactions in formal and informal networks, including groups, partnerships and related actor groupings (Faure *et al.,* 2019). One actor supporting innovative decision-making cannot be as effective as a range of different actors who are consistent in their advice, opinion or encouragement. It is even better if these actors have something to gain, or have implemented these decisions on their own and gone through a similar decision-making process.

Multi-actor approaches allow farmers and other actors in the AKIS to cooperate or work in partnership with each other to achieve common objectives. A simple example from Ireland is where Teagasc has worked with industry partners in multi annual joint development programmes. These have advanced the level of support to farmers and fostered innovation in improved demonstration farm and discussion group facilitation activities by advisers leading to synergies and teamwork which has endured for almost 30 years (Kelly *et al.,* 2004) The value of industry collaboration is evident in the continued financial support by industry for interdisciplinary applied research and knowledge transfer programmes. These programmes are grounded by the common objectives and the desire to collaborate and solve problems or issues through common language and coordinated engagement and messaging of the target audience.

The degree to which these multi-actors are a co-ordinated group or network who have different skills, backgrounds and services but who work together to solve a problem is important and brings knowledge exchange to an even higher level. In these groups there is a cross fertilisation of ideas, inspiration and planned action, which requires a friendly or warm relationship. The social capital created in these networks is valuable not just in terms of interaction but in generating trust. Fisher (2013) showed that simply providing access to information is insufficient; instead, trust between the informant and recipient is essential as without it any information transfer activity will be futile.

The innovation spiral which was used in the H2020 Agrispin project to analyse a large number of diverse innovation case studies proved useful in looking at the stages of the innovation from idea generation to embedding in practice and actors involved and their interaction. The role of intermediaries at different stages and value of the range of multidisciplinary support actors was apparent but unpredictable due to the different AKIS contexts and diverse nature of each case study (Faure *et al.,* 2019). The project findings supported a broader innovation broker role for farm advisers and roles in innovation network support also. The European innovation partnership for agricultural productivity and sustainability (EIP-Agri) supports the goals of rural development by encouraging innovation in agriculture and rural communities. The EIP-Agri was created to bridge the gap between the innovative solutions created, and the use of new technologies by those living and working in rural areas. By creating partnerships between those who will eventually use new technology and those that create them, EIP-Agri aims to accelerate the uptake of change. In measure 16 of the EU CAP 2014-2020 Rural Development Programme, the option of financial support for bottom-up, farmer-led groups was provided and adopted widely with over 1000 EIP Operational Groups established, funded and working around Europe by 2019. In a previous study of the needs for innovation support, Klerkx and Leeuwis (2008) concluded that the innovation support roles of intermediaries in farmer networks and groups was important in the context of reduced public funding of research and advisory services and the absence of measures which articulate demand for these services in the supply chain. Wielinga (2014) identifies three useful components for creative interactive innovation processes within networks. These are vital space, free actors and responsive capacity as these underpin the co-creation process and allow individuals to pool resources and share their ambitions effectively.

There is a clear commitment from the EU through the European Innovation Partnership (EIP) to speed up the innovation process in Agriculture and Forestry (EU SCAR AKIS, 2019). Currently a number of EU H2020 projects are evaluating interactive innovation in Agriculture and Forestry, the LIAISON project is looking at a range of projects and cases to establish good practice and measure impact, and two other projects, EURAKNOS and EUREKA, are looking at how EU H2020 Thematic Networks and EIP Operational Groups are working and to share the learning from these. Specifically, in innovation support, the AGRILINK, FAIRshare and i2connect projects aim to equip farm advisers in their roles to support interactive innovation in decision support, digital technologies and skills development. H2020 Thematic Networks and EIP Operational groups provide financial support for specific actions in areas of need identified by the beneficiaries. They empower people to help themselves through bottom-up innovation processes rather than by top-down. These offer huge hope for faster adoption of new technologies and better innovation. They also will ensure that the lessons learned from this huge investment in speeding up innovation and the adoption of new technologies will have positive and lasting impacts for farmers and society.

References

Barman U. and Kumar B. (2011) Requirement of facilitation skills of extension Personnel under ATMA – review. *Agricultural Reviews (ARCC)* 32, 127-133.

Barrett T. (2017) *A new model of Problem Based Learning*. AISHE-All Ireland Society for Higher Education.

Black A.W.(2000) Extension theory and practice: a review. *Animal Production Science* 404, 495-502.

EU SCAR AKIS (2019) *Preparing for future AKIS in Europe*. Brussels, European Commission.

Faure G., Knierim A., Koutsouris A., Ndah H., Audouin S., Zarokosta E. and Heanue K. (2019) How to strengthen innovation support services in agriculture with regard to multi-stakeholder approaches. *Journal of Innovation Economics and Management* 28, 145-169.

Fisher R. (2013) 'A gentleman's handshake': the role of social capital and trust. *Rural Studies* 3,13-22.

Grogan P. (2017) *An approach to building effective agricultural advisor – client relations*. M.Agr.Sc. Thesis University College Dublin.

Heanue K. and O'Donoghue C. (2014) *The economic returns to formal agricultural education.* Teagasc, Ireland, ISBN: 978-1-84170-6139.

Hennessy T. and Heanue K. (2012) Quantifying the effect of discussion group membership on technology adoption and farm profit on dairy farms. *Journal of Agricultural Education and Extension* 18, 41-54.

Kelly T., Kirley T. and O'Dwyer T. (2004) *The importance of industry/extension joint development programme in a relevant and effective extension service*. AIAEE 20th International Conference Dublin.

Kelly T.G. (2011) *BETTER farms: their role in more effective technology transfer.* BSAS Knowledge Transfer Workshop, 3 March 2011.

Klerkx L. and Leeuwis C. (2008) Matching demand and supply in the agricultural knowledge infrastructure: experiences with innovation intermediaries. *Food Policy* 33, 260-276.

Läpple D., Renwick A., Cullinan J. and Thorne F. (2016) A spatial analysis of knowledge spillovers. *Land Use Policy* 56, 238-250.

Millar J. and Curtis A. (1997) Moving farmer knowledge beyond the farm gate: an Australian study of farmer knowledge in group learning. *European Journal of Agricultural Education and Extension*, 4, 133-142.

Teagasc (2018) *Teagasc education vision-meeting future needs*. Teagasc Education Programme, Oak Park, Carlow, Ireland.

Vanclay F. (2004) Social principles for agricultural extension to assist in the promotion of natural resource management. *Australian Journal of Experimental Agriculture* 44, 213-222.

Wielinga E. (2014) Concepts for co-creating innovations in the EIP. In: Aenis T., Knierim A., Riecher M.C., Ridder R., Schobert H. and Fischer H. (eds.) *11th European IFSA symposium, farming systems facing global challenges: capacities and strategies, proceedings*. Berlin, Germany, 1-4 April 2014, pp. 824-834.

Grasslands uses and animal health management: perceptions of dairy farmers in western France

Petit T., Gotti V., Manoli C. and Couvreur S.
Unité de Recherche sur les Systèmes d'Élevage, ESA, 49007 Angers, France

Abstract

Grasslands are considered as a pillar of the agroecological transition by researchers, advisers, politicians and dairy farmers due to several services at farm and territory scale (zootechnical, agronomic, environmental). The effects of grasslands on animal health and their perceptions by farmers are not known. We led a farm-scale survey to understand the place and role of grasslands on farms in terms of animal health management. A qualitative analysis was done of 20 dairy farmers' discourses from Maine-et-Loire (France). We have identified common knowledge: most of the farmers do not link grassland and animal health. They consider that being outdoors, more than grazing, is beneficial for animal health, but they cannot give the positive effects objectively. We then classified farmers according to: (1) their perception about forage management and (2) health management. We highlighted five types of farmers' perceptions about forage and animal health management. Finally, we performed a cross-analysis to understand the perceptions of the links they made between grass use or, on a higher-level of animal feeding, and animal health. Our study underlines that a continuum of several perceptions about health management exists, crossing alternative medicine vs allopathic on one hand, and holistic vs reductionist approaches management on the other hand. It suggests taking into account a need to develop relevant advice at farm level for a better integration of grassland as a lever for health management.

Keywords: grasslands, forage management, animal health, farmers perception

Introduction

In the context of agroecological transition, livestock farming systems have to face various stakes at animal, herd, farm and territory scale. For this purpose, Dumont *et al.* (2013) have proposed five principles to design sustainable animal production systems: (1) adopting management practices to improve animal health, (2) decreasing the inputs needed for production, (3) decreasing pollution by optimising the metabolic functioning of farming systems, (4) enhancing diversity within animal production systems to strengthen their resilience, and (5) preserving biological diversity in agroecosystems by adapting management practices. Grasslands are more and more popular for research, agricultural development and dairy farmers due to several services at farm and territory scale (zootechnical, agronomic, environmental) to answer these agroecological issues. Mainly focused on the grazing use, studies underline that grasslands can improve animal health by reducing lameness and mastitis occurrences (Arnott *et al.* 2017). This can reduce the use of pharmaceuticals (within antibiotics) and the cost of animal health management (Sulpice *et al.*, 2019). However, grazing may present threats to animal health, as in cases of parasitism, vector-borne diseases, ingestion of toxic plants, and in cases of mismanagement of feed transition. In order to advise farmers about practices for combining integrated animal health management and grassland uses, there is a lack of knowledge about how farmers design their forage system towards these aims. The hypothesis we made is that farmers consider health risks/benefits of grassland use to drive their use of grassland in the forage system.

Materials and methods

In 2019, we selected 20 dairy farms to be the most diversified possible, regarding the farming systems in the Maine et Loire area (north west France) in terms of Utilized Agricultural Area (UAA), herd size, hectares of grasslands, hectares of grazed grasslands, work force. Data were collected via one-to-one qualitative survey with farmers. The interview dealt with the: (1) general organization of the farm (UAA, work force, production facilities, size and performance of the dairy herd, crop rotations), (2) forage system (types of grasslands, types of forages, grass forage quality and quantity, grazing management), (3) animal health management, (4) relations between animal feeding and animal health identified by the farmer, (5) advice concerning feeding, forage and health managements. The interview was fully transcribed, focussing on the reasons given for farming practices. At first, we developed an analytical framework based on farmers' perceptions about: (1) forage management, and (2) health management. Then we classified farmers according to contrasting perceptions to highlight types of ways of thinking. Finally, we have characterised the types on the links they made between grass use or, on a higher-level animal feeding, and animal health.

Results and discussion

At first, the discourses analysis allowed us to identify common knowledge and perceptions of farmers about animal health. A majority of farmers (n=18) have rough indicators to assess health at herd level: by observing animals and checking the milk quality criteria (somatic cell count, protein and fat contents, milk yield). The relation between grasslands and animal health management is largely unknown by farmers. Most of them (n=16) consider that going outdoors, more than grazing, is beneficial for animal health, but they cannot identify objectively the positive effects except for the milk quality criteria cited above.

Based on our analytical framework, we highlighted five ways of thinking about the forage and the animal health management (Table 1): (1) systemic farming management with grass used as a natural forage for self-sufficiency and health benefits (n=7) (2) grassland for grazing and a good health through an adapted feeding (n=3), (3) high use of alternative medicine but disconnected from the forage management (n=2), (4) animal health managed separately from livestock management based on allopathic treatment and with little grass in the diet (n=4), (5) grasslands only as economic lever, and health management based on high level of intervention (both allopathic and alternative) on animals with both allopathic (n=4).

Farmers who have the best knowledge about the significance of grass in animal diets with regard to its health are those who are developing an integrated management of animal health at farm level (Type 1). The others mainly manage their farming systems through a compartmentalisation of animal feeding on one hand, and animal health management on the other hand (Types 3, 4 and 5). Farmers in Type 2 have no common perception of animal health management even if the cow's diet mainly comes from grasslands.

Table 1. Ways of thinking about the forage and the animal health management.

Forage management	Health management	Types
Grassland used for food self-sufficiency by covering animals' requirements	Animal health passes through a global approach on livestock conditions	1. Systemic farming management with grass used as a natural forage for self-sufficiency and health benefits (7/20)
Grassland used as part or forage system in spite of unpredictability of production	No particularity	2. Grassland for grazing and a good health through an adapted feeding (3/20)
	Alternatives treatments and techniques used on priority	3. High use of alternative medicine but disconnected from the forage management (2/20)
Grassland used sometimes to improve dairy production	Allopathic treatments used as priority	4. Animal health managed separately from livestock management based on allopathic treatments and with few grass in the diet (4/20)
Grassland used to optimise economy and work efficiency	Fast intervention whatever the health problem diagnosed	5. Grasslands only as economic lever and health management based on high level of intervention on animals (4/20)

The results have shown various animal health perceptions, no matter the level of production. Some farmers talk about holistic health management in which grasslands contribute, with other elements, to the good animal health status (Type 1) as in Hellec and Manoli (2018). At the opposite, other farmers consider their farming system based on reductionist approaches, integrating, or not, grasslands as a lever of health management (Types 2, 3, 4 and 5).

A majority of farmers (n=14, present in the 5 types) do not link grassland use and animal health. However, most of them (n=15, present in the 5 types) link feeding (grass excluded) and animal health especially through mineral complementation during critical physiological phases (peri-partum, feeding transitions).

Based on our sample, we have found no relation between farm structure (herd size, part of grassland in the UAA), advice and the way of thinking about grasslands and animal health management. In contrast, we have seen that farmers still separate a lot forage management and animal health management. This seems to be largely due to the fact that advisers also separate it. It could illustrate the learning process while farmers assimilate, appropriate and implement new health practices mobilizing technical, cognitive and organizational learnings as shown by Fortané *et al.* (2015).

Conclusions

Our study underlines that a large part of farmers do not connect animal health and grassland managements. Moreover, there is a continuum of several perceptions about health management crossing alternative medicine vs allopathic treatment, and holistic vs reductionist approaches of farm management. Based on these observations, our work questions the compartmentalization of advice: animal feeding on the one hand and health management on the other. It suggests the need to develop knowledge leading to objectify the health benefits of grass in animal diets under farm conditions. Furthermore, new ways of advising, which involve several farm advisers such as agronomist, nutritionist and veterinarians could be relevant. A lever to the significant agroecological transition expected within a few years could be an improvement of the farmers' formations offered, which link grass use in animal diets and their health, so that integrated health management may be improved.

References

Arnott G., Ferris C.P., and O'Connell N.E. (2017) Review: welfare of dairy cows in continuously housed and pasture-based production systems. *Animal*, 11 (02), 261-273.

Dumont B., Fortun-Lamothe L., Jouven M., Thomas M. and Tichit M. (2013) Prospects from agroecology and industrial ecology for animal production in the 21st century. *Animal* 7(6), 1028-1043.

Fortané N., Bonnet-Beaugrand F., Hémonic A., Samedi C., Savy A. and Belloc C. (2015) Learning processes and trajectories for the reduction of antibiotic use in pig farming: a qualitative approach. *Antibiotics*, 4 (4), 435-454. doi:10.3390/antibiotics4040435

Hellec F. and Manoli C. (2018) Soigner autrement ses animaux: la construction par les éleveurs de nouvelles approches thérapeutiques, *Économie Rurale*, 363, 7-22.

Sulpice P., Manteaux J.P., Michaud A., Fauriat A., Ollivier A., Otz P. and Longfellow H. (2019) Quels effets bénéfiques du pâturage sur la santé animale ? Première approche à partir de suivis d'élevages bovins laitiers par des vétérinaires conventionnés. *Fourrages*, 238, 133-138.

Generating and transferring grassroots innovations in a multi-actor participatory process

Herzon I.[1,2], Puig De Morales M.[3], Gaki D.[4], Kazakova Y.[5], Moran J.[6], Pinto Correia T.[7], Jitea I.M.[8], Vlahos G.[9], Faraslis I.[4] and Ljung M.[10]

[1]Department of Agricultural Sciences, P.O. Box 27, 00014 University of Helsinki, Finland; [2]HELSUS, P.O. 20 Box 65, 00014 University of Helsinki, Finland; [3]CIHEAM-IAM, Montpellier, France; [4]Laboratory of Rural Space, Department of Planning Engineering, Planning & Regional Development, University of Thessaly, Greece; [5]Society for Territorial and Environmental Prosperity, Bulgaria; [6]Department of Natural Sciences, Galway-Mayo Institute of Technology, Ireland; [7]ICAAM, Institute for Mediterranean Agrarian and Environmental Sciences, Portugal; [8]Economic Sciences Department, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania; [9]Department of Agricultural Economics and Rural Development, Agricultural University of Athens, Greece; [10]Swedish University of Agricultural Sciences SLU, Sweden

Abstract

A thematic network called HNV-Link (High Nature Value Farming: Learning, Innovation and Knowledge) was launched under Horizon 2020 in 2016. It brought together 13 research, advisory, regional administration organizations, farmer, LEADER and other civic groups from 10 countries in an innovation brokering process. These agricultural areas are renowned for their outstanding natural and cultural values. Through a structured participatory process, multi-actor groups (MAG) engaged in establishing the baseline conditions of their activities; identifying innovative solutions and innovation needs; and matched these with those of other groups. The MAG conducted cross-visits and learning trips to exchange innovations and expertise, and developed innovation transfer plans for their own regions. The network created over 100 outcomes illustrating its participatory brokering process. This includes innovations linked to identified needs in each area, and educational resources with hands-on teaching activities. Over three years, HNV-Link reached an estimated minimum of 400,000 people through about 500 activities and outputs. The marginality of High Nature Value farming areas in mainstream research and development means that their innovation needs and solutions are rarely explored in academic fora. This transnational network filled the gap by strengthening the grassroots innovations, with farmers at the core of the process.

Keywords: High Nature Value farmland, peer-learning, rural livelihoods, thematic network

Introduction

High Nature Value (HNV) farmland refers to those areas in Europe where agricultural activities support and are associated with exceptionally high biodiversity (EIP-AGRI, 2016). These areas are also important components of cultural heritage, quality agricultural products, and rural employment. Low intensity livestock farming based on extensive grazing is the most common HNV production type (*ibid.*). Abandonment, intensification of production, and socio-economic decline are long-standing threats. Despite numerous public benefits, these areas receive only limited attention in agricultural policy, research and extension.

In order to fill in the gap, the HNV-Link thematic network (High Nature Value Farming: Learning, Innovation and Knowledge, 2016-2019) was launched under the Horizon 2020 research and innovation programme. The aim was to build a multi-actor network which identifies and spreads innovations while strengthening HNV farming actors in a participatory approach. Thematic networks are a new format of work in the EU Horizon 2020 programme, which aims at encouraging coordination and support actions by bringing together actors from science and practice. This serves to create practical outputs on a specific theme within agricultural research and innovation. Here we demonstrate how the innovation brokering for multi-actor groups (MAG) was implemented and reflect on key success factors.

Project approach

HNV-Link network developed a novel approach to the innovation brokering process based on a structured participatory process at local and European level. The process was initiated by mobilising existing local MAG or developing these further if the initial group was weak and under-represented by critical stakeholders. The main stakeholders were research, advisory, local and regional administration organisations, farmers and farming organisations, LEADER and other civic groups. Each group had a coordinator to liaise with the network. The project engaged 10 such MAG called Learning Areas (LA) in Bulgaria, Croatia, France, Greece, Ireland, Portugal, Romania, Spain, Sweden, and the UK.

The entry point for each LA group was to assess the context of their territory from its development perspective (e.g. environmental and socioeconomic character, governance, challenges and opportunities) and to co-develop a shared vision of a desirable future for each LA in a participatory grassroots process. Simultaneously, the LA groups identified the existing innovations of relevance for their HNV territories and innovation needs, i.e. persistent challenges that could be overcome with an innovative solution. The network deployed four innovation themes (EIP-AGRI, 2016): (1) Social and Institutional, (2) Regulatory Framework and Policy, (3) Products and Markets, and (4) Farm Techniques and Management.

Each LA group developed an innovation action plan which is an iterative and reflexive tool, aimed at coherent and complementary local strategies that account for the LA vision and its environmental and socio-economic context. Next the groups implemented their action plans reinforcing the local governance of the HNV farming systems. The LA groups matched their innovation needs with the innovative solutions available within the network at an HNV farming innovation fair. The network organised innovation brokering activities through 20 cross-visits (over 250 participants, mostly farmers). The role of the LA coordinators operating as 'HNV Innovation Brokers' was particularly critical at this stage in engaging local stakeholders and ensuring the effectiveness of the learning processes at the grassroots level.

Cross-visits proved beneficial for enhancing motivation and strengthening collaboration among the groups. Intense discussions revealed to both the visiting and hosting groups that, despite different locations and administrative settings, the challenges facing HNV farmers across Europe are very similar. Being exposed to innovations and the catalysers behind them inspired and motivated the cross-visits participants to 'try-it-at-home'. The multi-actor profile of the groups ensured that the necessary expertise and skills were available to formulate transferable 'at-home-solutions' and develop new ideas on adding values to HNV areas and goods. This strengthened collaboration between the LAs has outlasted the project.

At the later regional meetings, the local stakeholders analysed how the most relevant innovations could be transferred and adapted to their territorial context. Over 750 stakeholders (30% farmers) participated in 17 regional meetings. In many cases, the meetings were coordinated with complementary events to enable higher impact.

Effective, continuous and diverse communication across and outside the network was vital for keeping up the network team spirit, raising awareness on the importance of HNV in sustainable agriculture and creating new initiatives. In three years, the HNV-Link project reached an estimated minimum of 400,000 people through its 500 activities and outputs. It produced over 100 outcomes illustrating its participatory innovation brokering process, including educational resources with hands-on teaching activities. Each output was carefully tailored to a specific user group or groups. The whole network was involved in creating these, thus ensuring their relevance across territorial cultures and contexts. The outputs remain available, post-project, online. The most valuable outputs – innovation transfer and uptake – will take time to emerge. By the end of the project new initiatives had already started: business, networking, and novel policy incentives of a results-based payment scheme.

Reflection on the process and conclusions

The network's major achievement was strengthening the grassroots innovation process with farmers at the core of the process. Supporting HNV farmers' empowerment and cooperation with other stakeholders in a forward vision-oriented innovative process is key to improving their working and living conditions. This needs to occur while maintaining the exceptional environmental values of HNV farming regions.

A central finding was that for practically all the innovation needs that the local groups identified, there were existing examples of relevant solutions in other locations. A challenge is to spread and apply existing innovations more widely. This requires favourable social, institutional and regulatory conditions. Farmers meeting farmers across the network was the most rewarding experience, as many video-testimonies on the project website demonstrate.

The engagement of MAG at local level was crucial. In several cases, it was an outsider to the farming community who performed the role of 'catalyser', engaging people in the visioning and peer-learning innovation process. Motivated and locally trusted catalysers proved to be critically important for sustaining an effective innovation process. Through a network such as HNV-Link these leaders can get support and encouragement.

In the words of the external evaluators, the network has 'delivered exceptional results' and 'supported local empowerment, effective commitment of local stakeholders to HNV innovations and ownership of the project results'.

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References

EIP-AGRI 2016. How to make HNV farming more profitable without losing the HNV characteristics? Report of the EIP Focus Group on HNV farming profitability. Available from https://ec.europa.eu/eip/agriculture/en/focus-groups/high-nature-value-hnv-farming-profitability

Getting our message across

Butler G.[1], Malisch C.[2], Nadeau E.[3], Woodhouse A.[4], Flø B.E.[5], Sakowski T.[6], Gottardo F.[7], Ruzzi G.[7], Davis H.[1] and Steinshamn H.[5]

[1]Newcastle University, Newcastle NE1 7RU, United Kingdom; [2]Christian-Albrechts University, Kiel 24118, Germany; [3]Swedish University of Agricultural Sciences, P.O. Box 234, 532 23 Skara, Sweden; [4]Research Institutes of Sweden, Frans Perssons vag 6, 41276 Göteborg, Sweden; [5]Norwegian Institute of Bioeconomy Research, P.O. Box 115, 1431 Ås, Norway; [6]Institute of Genetics and Animal Breeding, Polish Academy of Sciences, 05-552 Magdalenka, Poland; [7]University of Padova, 35122 Padova, Italy

Abstract

The SusCatt project investigates alternative systems to improve sustainability in European cattle production, taking different approaches in Norway, Sweden, Germany, Poland, UK and Italy – all making greater use of pasture and forage, reducing damaging or external inputs. Rather than us deciding on how we tell everybody about findings, one project task is to ask potential audiences about their sources of information – how they gain knowledge? Ideally, this will offer guidance on an effective dissemination strategy. Project messages are relevant to multiple sectors: farmers, extension workers, consumers and policy makers. Attempts were made to survey these multiple stakeholders. We collected 236 opinions and found considerable variation, not only between groups but also between the same sectors in different countries. The most popular and highest-ranking sources overall were traditional press formats of newspapers and magazines. On the other hand, accessing information from social media was very polarised; almost non-existent for German and Polish stakeholders but widely used by UK farmers (possibly skewed by the dominance of face-to-face rather than on-line data collection). Findings suggest that each message from research projects needs a customized approach in dissemination, depending on the target audience and their regular habits of sourcing information.

Keywords: dissemination, stakeholder's opinions, survey

Introduction

If research is worth conducting, an effective dissemination strategy is essential, providing suitable messages in appropriate formats for optimum, rapid uptake – making a real difference. Publicly funded agricultural research ought to create a positive impact on the farming community and, possibly (via policy makers), ultimately to society at large. In the past this appeared relatively straightforward since most European countries funded national extension and development services. For instance, the UK's Agricultural Development and Advisory Service supported the pathway of research to the wider industry, with an army of advisers *translating* ongoing findings into practical guidelines. Over the last 20 years, however, challenges have changed. On one hand, national extension services have been abandoned or commercialised, although, we now have a plethora of internet and social media options, many with global reach. In theory, this ought to make the job easier but how do we know the most effective media to use?

The SusCatt ERA-NET project investigates management to improve sustainability in European milk and beef production, with interventions in Norway, Sweden, Germany, Poland, UK and Italy. Broadly we aim to increase grazing and forage-use, minimizing concentrate feeds, especially those with the potential for use as human food, like cereals or soya. If, as hoped, we improve efficiency this will only make a difference across Europe if widely adopted, ideally supported by educated consumers sourcing sustainable products. Each partner's activities are described on the website: https://www.nibio.no/en/projects/suscatt.

Our findings are relevant for multiple sectors of society and may need a different approach to reach these different audiences. The farming industry could reduce input costs or possibly improve the nutritional quality of their milk or meat, introducing the scope for premium pricing. If a premium is to be realised, we need to convey information on potential changes in nutrition and environmental impact to consumers, generating a demand for more sustainable milk and meat. These messages are also highly relevant for policy makers with the power to change our food systems and/or reward farmers for *public good*. Rather than predetermine our outreach activities, one project task is to ask potential audiences about information sources – how they gain knowledge.

Materials and methods

Each partner took a slightly different approach to collecting relevant information although all stakeholders were targeted with questionnaires listing between 14 and 19 potential sources (presented in alphabetical order), including many online media. In most countries, partners collected information from farmers, consumers, industry representatives and fellow scientists in person, at various gatherings through 2018 and 2019, although Italy also used an online approach. A total of 236 questionnaires were completed: 44 in Norway, 19 in Sweden, 70 in Germany, 19 in Poland, 35 in UK and 49 in Italy. Since each partner asked about slightly different sources, some country specific, all data was allocated into common categories to allow uniform analysis. These are shown in Table 1. Information collected was assessed in 2 ways: (a) the numbers or proportion of participants using each category and (b) the relative importance of sources used, on a scale of 1-6 with 6 being the most relevant and 1 the least. Unfortunately, results from Italy were not fully compatible with others and are excluded from the comparison under analysis (a).

Results and discussion

Figure 1 shows the proportion of participants in each country relying on the media categories. The most variable between countries was *associations* which only appeared to be used by 39% of participants in Norway, but in UK, where it was one of the most popular sources, was used in 83% of replies. There was no record of this type of organisation playing a role in Sweden, Germany or Poland, possibly because of the definitions used in these countries. *Social media* was also very variable in its use, ranging from only 3% in Germany and 11% in Poland (both 2 records) to 63% of UK participants. The most popular media across all countries was clearly use of the *press* – 77% of all participants overall accessed information in magazines and newspapers, although this ranged from 89% of German participants down to only 51% of UK farmers. Accessing information from *personal communication*, *events* and the *internet* were very similar with 62, 63 and 66% of all replies respectively – although again seeing different priorities between the countries.

The relative importance of these media categories to participants is presented in Figure 2 and, as with their use, the relative ranking varies between countries. The most relevant and consistent category across the countries was the traditional *press* with a ranking of 3.9 overall, ranging from 4.4 in Italy to 3.5 for

Table 1. Common categories with information sources covered.

Category	Sources covered
Literature	academic publication, books
Associations	levy boards, trade unions, trade organisations
Internet	blogs, Google, websites, YouTube
Social media	Facebook, Instagram, email forums, Twitter
Communications	consultants, friends, neighbours, family
Events	discussion groups, farm open days or visits
Press	newspapers, magazines, newsletters

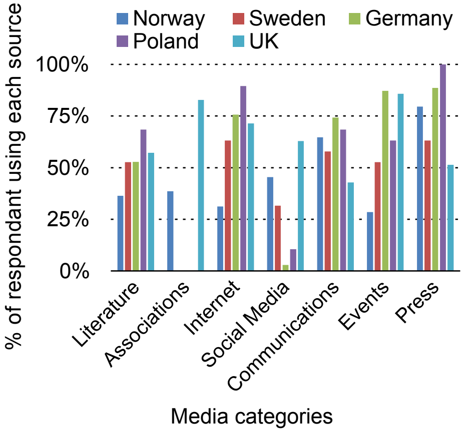

Figure 1. Use of media sources.

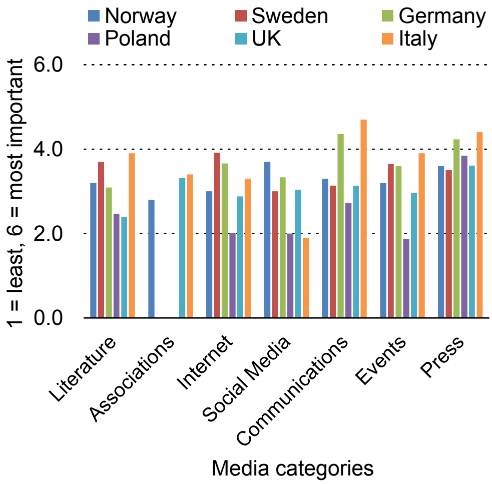

Figure 2. Ranking of information source.

Swedish participants. Again, the use of *social media* saw the greatest variability between countries. In Norway these were ranked as the most important source of information at 3.7, yet only reached 1.9, the least important source, for Italian participants – despite the fact some of these data were actually collected on-line.

As academics we often communicate with other scientists in peer reviewed journals or conferences. However, these often lack the opportunity for in-depth discussions and rarely include *other* stakeholders, especially consumers. The main objective here is to help broaden communication of our SusCatt findings, beyond academia to reach farmers, others involved in the livestock industry, policy makers, diet related health professionals and consumers.

However, we may need to consider our survey results with caution, since all data shown in Figure 1 and the vast majority in Figure 2 were derived face-to-face contact, rather than on-line access to the questions. Hence, this poll may not represent views of individuals regularly using social media and internet platforms, which would be collected more effectively with an on-line survey. That said, the extent of variability between countries and the different groups come as a surprise, reinforcing the point that, to be effective, dissemination needs to be customized depending on the target audience and their practices.

Conclusions

Each SusCatt partner will use their data to decide on an effective way for each message to reach the appropriate sector.

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Identifying barriers to improving grass utilisation on dairy farms

McConnell D.A.[1], Huson K.M.[1], Gordon A.[2] and Lively F.O.[1]
[1]Agri-food and Biosciences Institute, Large Park, Hillsborough, Co. Down, Northern Ireland; [2]Agri-food and Biosciences Institute, 23a Newforge Lane, Belfast, Northern Ireland

Abstract

The positive relationship between grass utilisation and net margin on dairy farms has been well established across a range of dairy production systems. Within Northern Ireland grazed and ensiled grass remains the predominant forage source; however, grass utilisation rates remain suboptimal. A face-to-face survey was conducted during 2018 with the aims of: (1) detailing current grassland management practices on commercial farms, (2) understanding the factors affecting farmers' attitudes to grassland production and utilisation and (3) identifying the perceived challenges farmers face when seeking to improve grass utilisation. Survey respondents had a median herd size of 150 cows, milk output per cow of 8,000 kg cow^{-1} yr^{-1}) and grassland area of 96.4 hectares. The perceived importance, and use, of grazed grass reduced with increasing herd size and milk yield. Grassland management was also considered to be less important than most other areas of farm management. The primary barriers identified to increasing dairy cow performance from grazed grass were weather, soil trafficability and sward productivity. Similarly, the primary barriers identified to increasing dairy cow performance from grass silage were weather, cutting stage and sward productivity.

Keywords: dairy, grassland, grazing, utilisation, survey, attitudes

Introduction

Well-managed forages remain among the most cost-effective feedstuffs for UK dairy cows and the positive relationship between forage utilisation and net margin on dairy farms has been well established across a range of dairy production systems (AHDB Dairy, 2019). With increased global demand for, and fluctuations in both the availability and cost of imported feedstuffs, efficient forage utilisation is and will continue to be a key driver of profitability on UK dairy farms. In Northern Ireland (NI) grazed or ensiled grass remains the dominant forage source. Despite this, grass utilisation remains sub-optimal with an estimated annual utilised grass yield of 7.5 Mg DM ha^{-1} on commercial dairy farms, 50% of the potential yield observed in plot trials (DAERA, 2016). In addition, little is known about current grassland management practices on commercial farms or of the value attributed to grassland by livestock farmers. To address this gap, a face-to-face survey was conducted during 2018 with the aims of (1) detailing current grassland management practices on commercial farms, (2) understanding the factors affecting farmers attitudes to grassland production and utilisation and (3) identifying the perceived challenges farmers face when seeking to improve grass utilisation.

Materials and methods

A subset of 500 farms stratified by herd size were randomly generated from the 2016 NI DAERA Agricultural Census database and asked to participate in the survey. One-to-one interviews were conducted on-farm during November 2017 – July 2018 by a single interviewer. The 137-question survey contained closed and open questions, split into eight sections: (1) farm background, (2) attitudes towards grassland management, (3) grazing, (4) zero-grazing, (5) silage, (6) sward renewal, (7) soil, and (8) grassland advice. The 112 responses were categorised by milk yield per cow (MY1-4) and herd size (HS1-4) using the following limits: MY1 = <6,501 kg cow^{-1} yr^{-1}, MY2 = 6,501-7,500 kg cow^{-1} yr^{-1}, MY3 = 7,501-8,500 kg cow^{-1} yr^{-1}, MY4 = >8,500 kg cow^{-1} yr^{-1}; HS1 = <101 head, HS2 = 101-150 head, H3 = 151-225 head, HS4= >225 head. Average cow numbers for categories HS 1-4 were 70, 131, 182 and 337, respectively (s.e.d. = 13.1, P<0.001). Statistical analysis was carried out using Genstat (VSN International Ltd 19.1, 2018) with the effect of MY and HS on continuous variables determined by ANOVA. Ordinal data was assessed using Wilcoxon Matched-Pairs Test, grouped data was assess using Mann-Whitney U test. No significant interaction between MY and HS was observed.

Results and discussion

Survey responses reflected a median area farmed of 96.4 ha (range: 28.3-384.6 ha) of which 96.4% (range: 50-100%) was classified as grassland. An estimated 48.1% (range: 5-86%) and 51.9% (range: 14-95%) of grassland was considered by participants as predominantly managed for grazing and silage, respectively. Median herd size and milk yield of respondents were 150 head and 8,000 kg cow^{-1} yr^{-1}, respectively. Performance from forage and use of grazed grass and grass silage differed significantly between MY categories; however, there was no significant impact of HS (Table 1).

Recording of grass information remained limited with records of annual grass performance, reseeding and fertiliser events kept on 9.8, 16.1 and 33.0 of farms, respectively. 29% of farms were measuring grass on a regular basis throughout the growing season (measurement comprised 13.6% eyeball, 4.5% cut and weigh and 9.1% platemeter methods). The average sward renewal interval was 10.2 and 9.5 years for grazing and silage swards, respectively. Only 2% of respondents failed to reseed swards on owned land; however, 15% of respondents did not reseed swards on rented land.

The relative importance of grassland management was not influenced by MY or HS. Areas of breeding (+0.176), business (+0.234), animal health (+0.696), animal nutrition (+0.436) and youngstock (+0.228) management were all considered to be more important than grassland management (0). Only environmental management (-0.402) and labour (-0.329) were ranked as less important. In addition, grass silage ranked significantly more important than grazed grass (means scores (out of 10): silage 9.174 vs grazing 7.326; r=0.553; P<0.001) with the importance of grazed grass declining with increasing HS and MY (Figure 1). In line with this, the proportion of herds grazing reduced significantly with increasing HS (P=0.002) and MY category (P=0.004; Table 2). With a trend for increasing herd sizes and milk output per cow on N.I. dairy farms, it is likely that the role of grazed grass will continue to reduce.

Primary barriers to improving dairy cow performance from grazed grass were weather (67.4% of respondents), soil trafficability (30.4%), sward productivity (28.3%), grazing management (25.0%) and land availability (18.5%). Similarly, when considering barriers to improving dairy cow performance from grass silage the following challenges were highlighted: weather (53.3%), cutting stage (48.9%), silage quality (39.1%), soil and sward productivity (37%), ensiling processes (8.7%), access to land (7.6%) and contractor (7.6%).

Table 1. Concentrate feeding, use of grass and performance from forage of survey participants as classified by milk yield category.

	Milk yield category[1]				s.e.d.	P-value
	MY1	MY2	MY3	MY4		
Milk yield (kg cow^{-1} yr^{-1})	5,993^{a}	7,115^{b}	8,186^{c}	9,270^{d}	140.9	<0.001
Concentrate fed (kg cow^{-1} yr^{-1})	1.41^{a}	1.86^{b}	2.31^{c}	2.87^{d}	0.17	<0.001
Milk from forage (kg cow^{-1} yr^{-1})	2,861	2,996	3,152	2,689	370.7	NS
Proportion of forage as grazed grass	0.53^{b}	0.43^{a}	0.36^{a}	0.33^{a}	0.061	0.002
Proportion of forage as grass silage	0.41^{a}	0.57^{b}	0.62^{b}	0.63^{b}	0.063	0.007

[1] Milk yield categories: MY1 = <6,501 kg cow^{-1} yr^{-1}; MY2 = 6,501-7,500 kg cow^{-1} yr^{-1}; MY3 = 7,501-8,500 kg cow^{-1} yr^{-1}; MY4 = >8,500 kg cow^{-1} yr^{-1}.

[2] Values with different superscript letters in a row are significantly different (P<0.05).

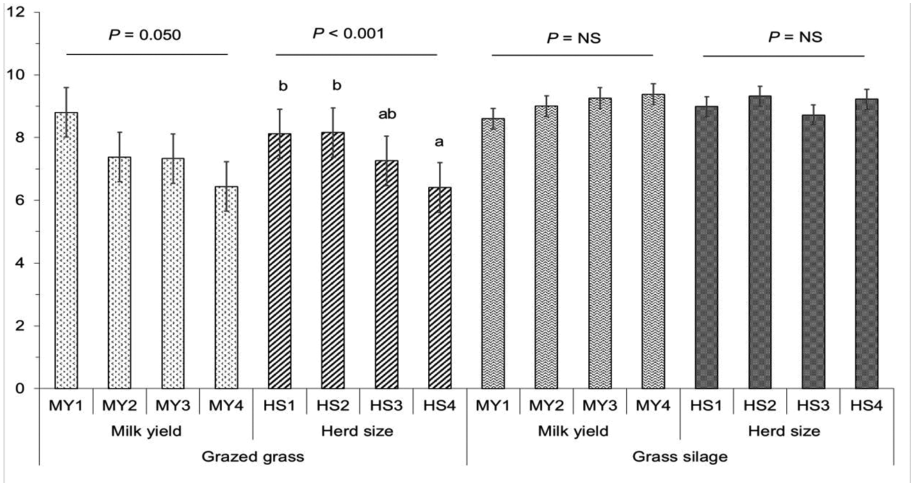

Figure 1. Perceived importance (0 = not important, 10 = very important) of grazed grass and grass silage across a range of milk yield (MY 1-4) and herd size (HS 1-4) categories. Bars containing different letters are significantly different (P<0.05).

Table 2. Proportion of respondents employing different grazing strategies by milk yield and herd size category.

		All herd: full time grazing	All herd: part-time grazing	Part herd grazing	No grazing	sig.
Milk yield[1]	MY1	0.842	0.105	0.000	0.053	0.004
	MY2	0.600	0.160	0.120	0.120	
	MY3	0.571	0.107	0.143	0.179	
	MY4	0.225	0.300	0.200	0.275	
Herd size[2]	HS1	0.765	0.176	0.059	0.000	0.002
	HS2	0.567	0.133	0.133	0.167	
	HS3	0.360	0.200	0.160	0.280	
	HS4	0.174	0.261	0.217	0.348	

[1] Milk yield categories: MY1 = <6,501 kg cow^{-1} yr^{-1}; MY2 = 6,501-7,500 kg cow^{-1} yr^{-1}; MY3 = 7,501-8,500 kg cow^{-1} yr^{-1}; MY4 = >8,500 kg cow^{-1} yr^{-1}.

[2] Herd size categories (lactating cows only): HS1 = <101 head; HS2 = 101-150 head; H3 = 151-225 head; HS4 = >225 head.

Conclusions

Despite grazed or ensiled grass remaining the predominant forage available to N.I. farms, the perceived importance of grassland management practices to farm business remains low. The role of grazed grass is expected to reduce as herd sizes and milk yields continue to increase.

Acknowledgements

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References

AHDB (2019) *Dairy performance results 2017/2018*. AHDB, Kenilworth, UK, 32 pp.

DAERA (2018) *Sustainable agricultural land management strategy for NI*. DAERA, Belfast, UK, 129 pp.

GrassCheck: monitoring grass growth and maximizing grass utilisation on UK farms

Huson K.M.[1], Lively F.O.[1], Aubry A.[1], Takahashi T.[2], Gordon A.[3] and McConnell D.A.[1]
[1]Agri-Food and Biosciences Institute, Hillsborough, Co. Down NI, United Kingdom; [2]Rothamstead Research, North Wyke, Okehampton, Devon, United Kingdom; [3]Agri-Food and Biosciences Institute, Newforge Lane, Belfast, Co. Down NI, United Kingdom

Abstract

GrassCheck originated in Northern Ireland (NI) in 1999 as a grass growth and quality monitoring project, and recently expanded to include commercial farms alongside research plots, with 45 pilot farms in NI (since 2018), and 50 in Great Britain (GB) (since 2019). GrassCheck collates grass growth and weather data, and disseminates regional summary data weekly through the growing season (March-October). Bulletins published via social media and the farming press include timely grass management notes, current regional grass growth rates and soil data (temperature and moisture). The NI bulletins also include 7- and 14-day grass growth predictions. GrassCheck engages with farmers across the UK through social media and bulletin publications to encourage a 'measure to manage' culture for grazing systems, and provides information to support farmers in improving grass utilisation and grassland management. In 2019 across both GB and NI, GrassCheck produced 63 bulletins, and recorded average yields of 10.3 and 12.2 Mg DM ha^{-1} across GB and NI project farms. In 2019 average grass utilisation on these farms was calculated at 79 and 84% respectively.

Keywords: grass utilisation, grass growth, knowledge transfer

Introduction

In the UK 71% of the utilised agricultural land area is grassland (DEFRA, 2018). With grass the cheapest feedstuff available, and the primary dietary component for UK ruminant livestock (DAERA, 2016), well managed grassland can support efficient and profitable production systems. Regular grass growth monitoring is essential to enable accurate herbage allocation for grazing stock, and has been highlighted as a key factor in enabling farmers to maximise both animal performance from grass and the utilisation of grass grown on their platform (Murphy *et al.*, 2018). However, relatively few (<15%; McConnell *et al.*, 2020) grassland farmers around the UK currently undertake any regular measurements. Furthermore, long-term data from research plots, managed to replicate grazing, has demonstrated potential annual yields from improved pastures in the UK of >12 Mg DM ha^{-1} (GrassCheckNI long-term data), but average utilised grass yields on UK livestock farms are estimated to be significantly lower, at just 5-8 Mg DM ha^{-1} (AHDB, 2018; DAERA, 2016). The GrassCheck projects, which focus on engagement through social and online media and the farming press, encourage grassland farmers to take regular measurements of herbage growth on pastures throughout the grazing season. By making regular assessments of pasture covers and grass growth rates farmers can tailor their grassland management to optimise grass production and maximise grass utilisation. Through the publication of weekly bulletins, GrassCheck communicates current trends in regional grass growth and soil conditions, and in NI, 7- and 14-day grass growth predictions are published each week, providing relevant information for on-farm decision support. GrassCheck captures a significant volume of data, which illustrates the breadth of variability in grass growth and utilisation across grass-focused UK farms, and contributes to ongoing research into the relationships between climate factors, management decisions, grass growth and animal performance metrics on commercial farms.

Materials and methods

On-farm grass growth was recorded each week on 95 commercial farms (NI: 20 dairy, 25 beef/sheep, GB: 23 dairy, 27 beef/sheep). Farms all had a focus on grassland management and operated rotational paddock grazing systems. Paddocks were measured weekly, with a rising plate meter (Jenquip EC10/20), and grass covers inputted through AgriNet (Irish Farm Computers Ltd). Weekly growth rates for each individual farm were calculated, and the data used to provide regional summary data. Grass utilisation was calculated as the percentage offtake of grass available >1,500 kg DM ha^{-1}, based on paddock pre- and post-grazing covers. On-farm weather data were recorded by a Davis Pro2 automatic weather station, with current weather data uploaded to cloud-based storage every 30 min, and summarised weekly. For NI, 7- and 14-day grass growth predictions were produced weekly via the GrazeGro model (Barrett *et al.*, 2005), using up-to-date weather forecasts for the same period. Each week individual farm data were uploaded to the GrassCheckGB (grasscheckgb.co.uk) and NI (agrisearch.org/grasscheck) websites, along with weekly bulletins, which were also published in relevant farming press and on twitter (@GrassCheckGB/@GrassCheck for GB/NI respectively). Statistical analysis of meteorological conditions (weekly total rainfall, average solar energy and radiation, min, max and average air temperatures, average soil temperature and soil moisture readings at 100 mm, and total evapotranspiration) alongside weekly grass growth rates was performed in Genstat® (V20.1, VSN Int. Ltd) by multivariate linier mixed model analysis (REML), with farm and week set as random effects. Regression analyses with soil moisture and grass growth rate data were performed in Excel.

Results and discussion

GrassCheck farms recorded average yields of 10.3 and 12.2 Mg DM ha^{-1} in GB and NI from March-October 2019. Individual farm yields recorded in this period were extremely variable, ranging from 5.1-15.6 and 8.4-15.9 Mg DM ha^{-1} in GB and NI respectively, likely reflective of differences in both the topography and management of each individual farm. Differences between UK regional average monthly grass yields were also evident (Figure 1). Overall, average grass utilisation on NI and GB farms was on-par with the expected values for well-managed paddock grazing systems (80%; AHDB, 2018). However, grass utilisation calculated for individual farms was also variable, ranging from 47-97% in GB and 66-94% in NI, averaging 79 and 84% respectively. Whilst location and management factors play a significant role in grassland performance, meteorological conditions also have a major impact on grass growth variability between seasons (Hurtado-Uria *et al.*, 2013). Mild winter and early spring weather seen in 2019 (Met Office, 2019) appeared to boost grass growth, particularly in the south of England (SE) where March yields were +89% above the long-term average. In NI, on-farm March grass growth was highest in the north-west (Derry), yielding +180% above the NI long-term average for this month. Conversely, hot, and for isolated areas dry, conditions recorded in SE during June and July 2019 coincided with dry average soil moisture readings (>60 centibar) and reduced grass growth rates in this region compared to the rest of GB (Figure 1). Regression analysis (2nd order) of GB grass growth data and soil moisture readings showed that in 2019 GB soil moisture readings >60 centibar were associated with a decreasing trend in grass growth rate. Linear mixed model (REML) analysis was performed for the combined GB and NI 2019 dataset. Univariate analysis showed solar radiation, soil moisture and temperature, average and max/min air temperature and solar energy were significantly ($P \leq 0.002$) associated with grass growth rates, but in the final multivariate model only solar radiation and minimum air temperature remained as significant factors ($P \leq 0.003$). Rainfall and evapotranspiration were not found to be significant. Further data collected in subsequent seasons will continue to strengthen data analyses, with data contributing to ongoing research into the relationships between meteorological conditions and grass performance. Beginning in March 2019, 32 bulletins were published weekly in NI in the farming press, with a circulation of >23K. In GB, 31 bulletins were produced covering April-October 2019 (press circulation ~29.5K). The GrassCheckNI and GB twitter pages also posted each weekly bulletin, achieving between

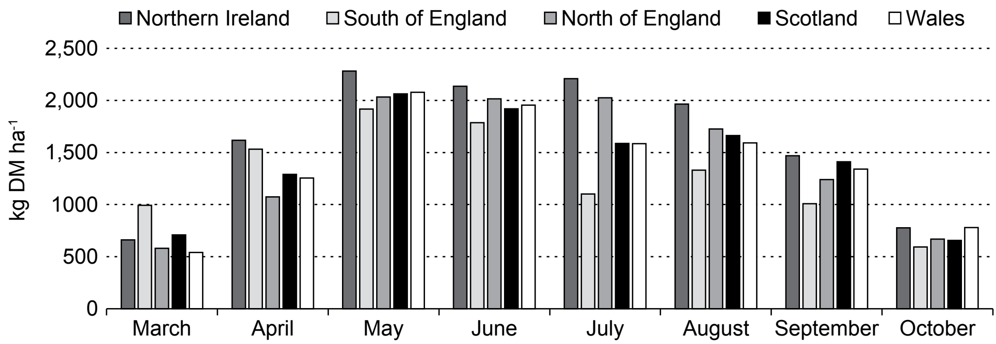

Figure 1. Average monthly yield of grass grown per hectare on GrassCheck farms in NI (45) and in each region of GB (South of England=14, North of England=8, Scotland=14, Wales=14) from March-October 2019.

10k and 20k impressions each per month. Bulletins covered regional (GB + NI) and plot (NI) grass growth rates and relevant weather information (weekly rainfall and soil temperature in the shoulders of the season, and soil moisture readings in the mid-season period) captured from a network of commercial farms, along with 7- and 14-day growth predictions (NI). By providing relevant, timely information for grassland farmers GrassCheck facilitated clear knowledge transfer to support and inform grassland management decisions, assisting farmers in increasing both grass production and utilisation in grazing systems.

Conclusions

Engaging with farmers through social media and the farming press allowed the GrassCheck projects to reach a wide-ranging farmer audience during 2019. Published management notes were available to support farmer decision making throughout the growing season, with a focus on maximizing the utilisation of grass grown on UK farms. The grass growth predictions currently provided through GrassCheck in NI, (with the intention to validate regional forecasts across GB), provided further decision support for short-term planning.

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References

AHDB (2018) *Improving pasture for better returns*. AHDB Beef & Lamb, Kenilworth, 1-36.

Barrett P.D., Laidlaw A.S. and Mayne C.S. (2005) GrazeGro: A European herbage growth model to predict pasture production in perennial ryegrass swards for decision support. European *Journal of Agronomy*, 23, 37-56.

DAERA (2016) *Delivering our future, valuing our soils: a sustainable agricultural land management strategy for Northern Ireland*. DAERA, Belfast. 16.

DEFRA (2018) *Agriculture in the United Kingdom*. DEFRA, London. 4.

Hurtado-Uria C., Hennessy D., Shalloo L., O'Connor D. and Delaby L. (2013) Relationships between meteorological data and grass growth over time in the south of Ireland. *Irish Geography*, 46, 175-201.

McConnell D.A., Huson K.M., Gordon A. and Lively F. (2020) Identifying barriers to improving grass utilisation on dairy farms. *Grassland Science in Europe* 25, ###-###.

Met Office (2019) Met Office – UK weather summary 2019, Met Office, Exeter. 1-4.

Murphy D.J., O' Brien B. and Murphy M.D. (2018) Development of a labour utilisation decision support tool to efficiently measure grass herbage mass using a rising plate meter. *2018 ASABE Annual International Meeting*, ASABE Paper No. 1800806.

Assessing variability in grass growth and quality on commercial farms using grassland management software

Aubry A.[1], Rankin J.[2] and Meeke T.[1,3]
[1]*Agri-Food and Biosciences Institute, Hillsborough, Co. Down, Northern Ireland, United Kingdom;* [2]*AgriSearch, Large Park, Hillsborough, Co Down, Northern Ireland, United Kingdom;* [3]*School of Biological Sciences, Queen's University Belfast, Belfast, Northern Ireland, United Kingdom*

Abstract

Increasing the uptake of grass growth monitoring by producers is key to making informed grass management decisions and for improving grass utilisation. One of the aims of this study was to evaluate the ease of use and quality of the data generated at farm level. Another objective was to provide more accurate values of grass growth and overall production from sheep farms. Over two successive grazing seasons, five sheep producers throughout Northern Ireland measured grass heights from their grazing fields every week using rising plate meters and took grass samples once every two weeks for NIRS analyses to estimate nutritional quality. Participating farmers entered grass and animal data using an online grassland management software. The data thus generated contributed to a larger farm network as part of the GrassCheck programme to produce weekly advisory bulletins. Data obtained during the 2018 and 2019 seasons indicate that it is possible for sheep farms to produce more than 10,000 kg DM ha^{-1} yr^{-1} of grass. The data from this on-farm project was also very valuable to inform research projects, in particular in relation to the study of different rotation systems.

Keywords: sheep, grassland production, grass quality, grass growth, perennial ryegrass

Introduction

Grass is the cheapest feed source available to the ruminant sectors in the United Kingdom (UK) and Ireland. Grass yields and utilisation levels in Northern Ireland (NI) are currently estimated to be below optimal levels, especially on beef and sheep farms where grass utilisation is estimated to be only 4,100 kg DM ha^{-1} yr^{-1} (DAERA, 2016). Improving herbage utilisation while maintaining optimal animal performance remains a challenge for many producers (Earle *et al.*, 2017). However, most sheep farms do not know their actual levels of grass production. Therefore, increasing the uptake of grass growth monitoring by producers is key to making more informed grass management decisions and for improving grass utilisation. One of the aims of this study was to evaluate the ease of use and quality of the data generated at farm level. Another objective was to provide more accurate values of grass growth on sheep farms, in order to define more ambitious, yet realistic, targets for grass production.

Materials and methods

The present study was conducted from April to November 2018 and 2019 at five commercial sheep farms across NI, UK. The areas of permanent grassland used for the study consisted predominantly of perennial ryegrass (*Lolium perenne* L.) swards. At each farm, two grazing groups were rotated using 4 and 8 paddock rotational systems. The average paddock sizes were 1.1 ha (4 paddock system) and 0.6 ha (8 paddock system), with the same stocking rate of 14 ewes ha^{-1}. Sheep on these farms were of a similar breed type, and grazing groups were balanced at turn out for ewe live weight, body condition score and lamb sire breed. The target pre-grazing cover was 2,600 kg DM ha^{-1} for the duration of the study period and the target post-grazing cover was 1,600 kg DM ha^{-1}. Ewes and lambs were weighed, and body condition scored at specific time points by AFBI and farm staff in a consistent manner across all study farms. Lambs were weaned on average at 14 weeks of age.

Pre- and post-grazing compressed sward heights were determined on each paddock (n=12) each week, as well as before and after each grazing movement, by taking 30 measurements across the diagonal of the paddock with a rising plate meter (Jenquip, New Zealand). Grass covers, grazing events and animal numbers were then recorded on grass management software (AgriNet). These data were used to estimate weekly and total grass production for each farm. At each farm, fresh herbage samples (one from each grazing group) were also analysed once every two weeks using near infrared reflectance spectroscopy for compositional quality. All statistical analyses were completed using Genstat (16^{th} edition). Grass quality parameters included dry matter (DM), metabolizable energy (ME), crude protein (CP), acid detergent fibre (ADF) and water soluble carbohydrates (WSC) contents. These were analysed using linear mixed models, with month as a fixed factor and farm as a random factor.

Results and discussion

The participating farmers found that the grass monitoring exercise, despite being time consuming, was particularly useful. In particular, it helped them to ensure that the targets for pre- and post-grazing covers were followed as best as possible, in order to optimise the grass production for their grazing platform. They also indicated that the grassland management software was easy to use; however, they felt that it was not best suited to sheep farms. In particular, greater flexibility is needed to be able to monitor multiple rotation grazing groups, and to differentiate between ewes and lambs. This would enable a more accurate estimation of the demand curves for each grazing group and associated predicted number of grazing days ahead.

Grass quality parameters varied greatly among farms and throughout the season (Figure 1). In 2018, grass DM content was significantly higher in July (P<0.001) due to a period of drought (Figure 1A). Energy content (Figure 1B) varied between 9.5 and 13 MJ kg^{-1} DM, with significant differences between months (P<0.001). These results indicate that these farmers managed to produce very good quality grass, especially at the start of the grazing season. Total grass production at each of the five farms in 2018 ranged from 9,700 to 10,900 kg DM ha^{-1} yr^{-1}, with a median value of 9,800 kg DM ha^{-1} yr^{-1} which is well above the current estimated utilisation rate of 4,100 kg DM ha^{-1} yr^{-1} for beef and sheep farms in Northern Ireland (DAERA, 2016). Despite challenging weather conditions in 2018, particularly wet spring and dry summer, as reflected by the two troughs in May and July (Figure 2) these results demonstrate that high grass production levels, similar to levels found with dairy systems, are also achievable on well managed sheep farms.

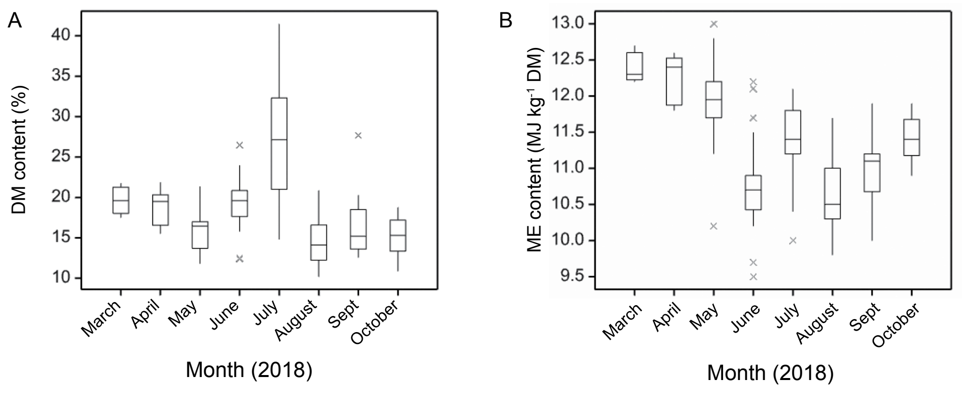

Figure 1. Dry matter content (A) and metabolizable energy (B) at each study farm for the period 14 March to 31 October 2018.

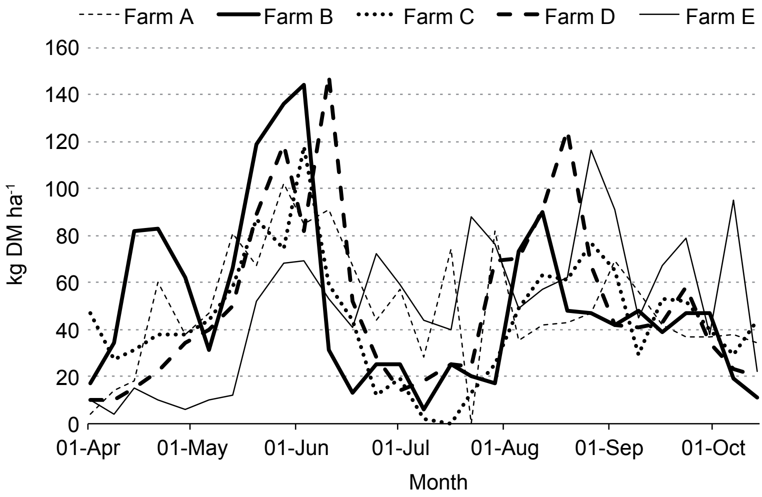

Figure 2. Grass growth curves for the five sheep farms in 2018.

Conclusions and implications

Levels of grass production achieved at the study farms indicate that there is a real potential for sheep farms to produce more grass. Grass monitoring and management tools are available and easy to use; however, they need to be modified and improved to better reflect multiple grazing groups in rotational grazing systems. This on-farm study also provides valuable information on both spatial and seasonal variations of grass quality, which can be used to review the methods currently used to predict grass growth throughout the grazing season.

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References

DAERA (2016) *Delivering our future, valuing our soils: a sustainable agricultural land management strategy for Northern Ireland.* Report produced by an independent Expert Working Group on Sustainable Land Management for the Department of Agriculture, Environment and Rural Affairs (DAERA), 130 pp.

Earle E., Boland T.M., McHugh N. and Creighton P. (2017) Measures of lamb production efficiency in a temperate grass-based system differing in ewe prolificacy potential and stocking rate. *Journal of Animal Science* 95, 3504-3512.

Analysis of the innovation system for dairy on grassland in the Wesermarsch-Region

Becker T.[1], Feindt P.H.[2] and Janker J.[2]
[1] *Grünlandzentrum Niedersachsen-Bremen, Germany;* [2] *Humboldt-Universität zu Berlin, Germany*

Abstract

The diffusion of innovations depends on a variety of factors beyond their technical characteristics. While the perceived costs and advantages of innovations have long been established as key factors, more recent research emphasizes the relational embeddedness of innovations in so-called innovation systems, i.e. networks of organizational, economic, informational, legal and other interactions related to the innovations and their potential use. Research on innovation systems related to the dairy sector and grassland utilisation has been limited so far. This paper presents first findings from a case study analysis of regional innovation systems in the German dairy sector. Farmers in the Wesermarsch region in Lower Saxony struggle with low milk prices, high production costs and increasing regulatory requirements. Adaptation of production methods seems inevitable, either by adopting production methods and marketing channels developed elsewhere or by developing and implementing novel techniques and methods. Our analysis of the regional innovation system found that all the important actors, institutions and infrastructures needed for a functioning innovation system are present. We discuss the implications of this finding for the innovativeness of the dairy sector in the case study region.

Keywords: agricultural innovation system, grassland, stakeholder

Introduction

Grassland-based agriculture is considered a major contributor to climate change alleviation, agrobiodiversity and ecosystem service functions (Schröder and Wider, 2013). Innovations could support these functions of grassland-based agriculture. As innovation literature has indicated, innovation transformation processes are particularly successful if farmers and other stakeholders are involved and if conflicting interests and institutional barriers are detected (Schut *et al.*, 2015). In the German project Green Grass the exchange among the central stakeholders is intensified for the implementation of remote sensing technologies for farming systems based on grazing. The precondition for this type of transdisciplinary research is the system identification process, which was conducted in the grassland-based Wesermarsch-region in the Northern part of Lower Saxony.

Theory

Recent approaches to agricultural innovation have put emphasis on the development and diffusion of technologies, integrating elements of both economic (Schumpeter, 1939) and social (Rogers, 1983) innovation theory. Our study follows this mixed approach, assuming that the implementation of any new product or service in agriculture is only successful if it answers to real-world agricultural transitions (Meuwissen *et al.*, 2019). Grasping the complex processes behind and supporting agricultural transformations through reciprocal knowledge exchange with involved stakeholders is necessary to identify barriers to innovations. We utilise the Farming Systems Approach by Meuwissen *et al.* (2019) and determine all stakeholders with vested interests in grassland agriculture, as well as the institutional frame conditions for grassland in the region Wesermarsch. Secondly, we examine the farming system of Wesermarsch through the Agricultural Innovation System (AIS) approach of Schut *et al.* (2015), in order to detect barriers for innovations: We show how stakeholders interact within the innovation system (e.g. public and private functions), how they are institutionally framed (e.g. laws and regulations) and how they may conflict, therefore unveiling the implications for technologies under development.

Materials and methods

For the analysis of the innovation system in the Wesermarsch region, a combination of literature analysis and semi-structured interviews was used. For approaching the stakeholders for grassland agriculture in and beyond the region of Wesermarsch, snowball sampling was carried out. We started with an established contact in the applied research Centre for Grassland in Lower Saxony. According to this advice, we interviewed farmers, a milk processor and scientists as experts in (local) grassland agriculture about past, present and future developments (transition processes). We used the type of factual interviews by, e.g. Kvale and Brinkmann (2009), thereby considering interviewing as social production of different forms of knowledge, according to the interview partners. From the literature review and the interviews, we identified the typical farming system in the region, and summarized and categorized according to the AIS framework of Schut *et al.* (2016).

Results and discussion

The dairy sector is characterized by more than 400 family farms, with an average of 110 cows and a milk yield of 7,750 litres per cow, and the main source of income being milk and subsidies (Landesamt für Statistik, 2011; 2017). Six important dairies collect the milk and sell the products (Landvolk Wesermarsch, 2017). The proximity to the North Sea enables the dairies to export milk and milk products to European and non-European countries. Through the chamber of agriculture, private consultants and professional journals, farmers have access to advisory services on varying topics, such as animal nutrition, machinery and management.

At the institutional level, one of the most important influences on the farming system is the political aim to increase the competitiveness of European food markets and to compete with international markets, while simultaneously protecting the existing farms. The milk quota (1983-2015) was one instrument to reduce the previous overproduction. In 1992, farmers got direct payment for land, to compensate for the stronger adaptation to the world market (European Commission, 2019). Many farmers struggled under these conditions or did not find a successor and had to give up their farms. Their land was then used by neighbouring farms; these in turn became bigger, and the number of cows increased (Eurostat 2009; Eurostat, 2018). The political developments resulted in an intensification of the dairy sector and a reduction of grassland-based agriculture. Meanwhile, the consolidation of fragmented land parcels supported the introduction of larger machines, owned by contractors, which in turn reduced the cooperation among neighbouring farms. The innovations for specialisation and production increases were supported by consultants, banks and tax accountants, professional journals and by farming schools. They have the important role of knowledge enablers in the innovation system.

Parallel to the increasing production, new critical actors evolved. Nature protection groups, the green party, the AbL (peasant farmers' association) criticised the intensification and tried to establish more environmentally friendly methods. While organic farmers had been discriminated by the other farmers in the past, this changed during the last decade. The demand for organic products increased and more and more farmers changed to organic farming. Farmers, consultants, scientists and members and milk processors expect that the next decades in agriculture will be characterised by transformation. Many of them consider stricter regulations (for the protection of grassland, peatland and water) accompanied by voluntary agri-environmental and climate protection programmes important measures to respond to environmental concerns. Other farmers are afraid that stricter regulations could ruin their farms because they already struggle with low milk prices. Remote sensing, later cutting dates and the use of insect-friendly and fuel-saving bar mowers instead of rotary mowers are discussed as promising techniques to react to climate change, and to sustainably intensify production and reduce the impact on the climate and declining insect populations.

Conclusions

The drastic changes over the last decades and the adoption of several innovations to increase production efficiencies indicate that the dairy sector in the Wesermarsch region is open for innovations. From the interviews, we can see already that awareness for the need for a transformation process is increasing, and there is a demand for corresponding innovations. With political frameworks influencing agricultural reality in the region strongly, and a recent political demand for more biodiversity enhancing measures, a potential arises particularly for those grassland innovations that enable enhanced economic efficiency and agrobiodiversity. In collaboration with the stakeholders, our innovation platforms will explore how the political, public and farmer interests may come together.

Acknowledgements

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References

European Commission (2019) The common agricultural policy at a glance. https://ec.europa.eu/info/food-farming-fisheries/key-policies/common-agricultural-policy/cap-glance_en.

Eurostat (2009) Viehhaltung: Anzahl der Betriebe und Tiere nach landwirtschaftlicher Fläche und Gebietsstatus. https://appsso.eurostat.ec.europa.eu/nui/show.do.

Eurostat (2018) Indikatoren landwirtschaftlicher Betriebe nach landwirtschaftlicher Fläche, betriebswirtschaftlicher Ausrichtung, Standardproduktion, Rechtsform und NUTS-2-Regionen. https://appsso.eurostat.ec.europa.eu/nui/submitViewTableAction.do.

Kvale S. and Brinkmann S. (eds.) (2009) *InterViews: Learning the Craft of Qualitative Research Interviewing. Sage Publication*. Thousand Oaks, London, UK, 357 pp.

Landesamt für Statistik (2017) Landwirtschaftliche Betriebe mit Viehhaltung und Viehbestand am 1. März 2016. Hannover, Germany.

Landesamt für Statistik (2011). Landwirtschaftszählung 2010 – Hauptnutzungs- und Kulturarten. Hannover, Germany.

Landvolk Wesermarsch (2017) Landwirtschaft in der Wesermarsch. www.klv-wesermarsch.de/.

Meuwissen M., Feindt P., Spiegel A., *et al.* (2019) A framework to assess the resilience of farming systems. *Agricultural Systems* 176.

Rogers E.M. (1983) *Diffusion of innovations*. 3rd ed., Macmillan, London, UK, 453 pp.

Schröder S. and Wider J. (eds.) (2013). Agrobiodiversität im Grünland – nutzen und schützen. Tagungsband eines Symposiums am 12. und 13. November 2013 in Berlin. Berlin, Germany.

Schumpeter J.A. (1939) *Business cycles. a theoretical, historical and statistical analysis of the capitalist process.* McGraw-Hill Book Company, New York, NY, USA, 461 pp.

Schut M., Klerkx L., Rodenburg J., *et al.* (2015). RAAIS: Rapid Appraisal of Agricultural Innovation Systems (Part I). A diagnostic tool for integrated analysis of complex problems and innovation capacity. *Agricultural Systems* 132, 1-11.

Preferences for grassland-management innovations in dehesa farms from Andalusia (Spain)

Fernández-Habas J.[1], Fernández-Rebollo P.[1], Vanwalleghem T.[2], Luis-González A.[2], Sánchez-Zamora P.[3] and Gallardo-Cobos R.[3]

[1]Department of Forestry, School of Agricultural and Forestry Engineering, University of Cordoba, Spain; [2]Department of Agronomy Hydraulic Engineering Area, School of Agricultural and Forestry Engineering, University of Cordoba, Spain; [3]Department of Agricultural Economics, School of Agricultural and Forestry Engineering, University of Córdoba, Spain

Abstract

Permanent grasslands (PG) are of key importance for the provision of ecosystem services (ES). Suitable management is essential to guarantee their persistence and functionality. Currently, there is a growing interest in innovations and new technologies aimed to facilitate and to improve the management of PG while increasing their provision of ES. It is important to know which innovations are preferred by PG managers and farmers so they can be prioritized by researchers and policymakers. In this study, we produced a list of the main innovations and technologies that can be applied to the management of PG of dehesa farms concerning in Andalusia. Through surveys, we gathered information on the importance that farmers give to these innovations according to their preferences and needs. The willingness to implement innovations on dehesa farms correlated positively with the education level and negatively with high age levels of the farmers.

Keywords: innovations, farmers, permanent grasslands, pastures, dehesa

Introduction

The dehesa is a savanna-like ecosystem composed of scattered oak trees and pastures. This ecosystem covers 3 million hectares in the Iberian Peninsula, and it is considered as one to be a highly biodiverse and multifunctional system (Bugalho *et al.*, 2011).

The development of new technologies and species over the last decades has made available different tools and innovations that could be applied to grassland management on dehesa farms. These innovations could be essential to guarantee the provision of ecosystem services while making their management more efficient and effective (Berckmans, 2017; Maroto-Molina *et al.*, 2019). Remote sensing and GPS-collars technology have been proposed as some of these innovations for grassland and grazing management (Gómez-Giráldez *et al.*, 2019; Moreno *et al.*, 2015). Other tools that focus on grass production and quality control are also been applied. However, little is known about the preferences and needs of grassland managers about these innovations. This is essential as it could help to focus research on the real needs of grassland managers in order to answer the real demand for innovations. A better understanding of these preferences might also explain which profile of managers are willing to apply certain innovations.

The objectives of this study were: (1) to compile a list of the main innovations and technologies that can be applied to the management of PG of dehesa farms, (2) to evaluate the preferred innovations by farmers and grassland managers, and (3) to investigate the correlation between some attributes of farmers and the preferred innovations.

Materials and methods

A list of innovations was gathered through a literature review. This list was evaluated and completed by a panel of four experts on grassland management and ecosystem services. An innovation was considered to be any tool, change, improvement or technology that could be applied to permanent grasslands of dehesa farms.

Once the innovation list was completed, a survey was carried out in two focus groups with farmers. Each innovation was rated according the following range of relevance: 1 = very low, 2 = low, 3 = medium, 4 = high, 5 = very high. A total of 36 surveys were answered. The survey also gathered information about the farmers (e.g. education level; from 1 = primary education to 5 = university studies) and farms attributes (e.g. farm size, farming system). Correlations between innovations and farmers/farms attributes were explored by Chi-Square test and Pearson correlation coefficients.

Results and discussion

The average farm size was 247 ha with a large range of sizes from 2 ha to 1,400 ha. The age distribution of the farmers in classes of <25, 26-35, 36-45, 46-55, 56-65 was 14, 26, 14, 26 and 20% respectively. The main enterprise of the farms was sheep breeding for meat (55%) followed by mixed systems of sheep with pigs or dairy/beef cattle (24%), the rest (21%) were farms specialised in either dairy or beef cattle.

Those innovations aimed to increase pasture performance and drought tolerance were rated with high scores (I1, I2, I3, I4 and I12, Table 1). These results denote the importance that farmers give to grasslands as the main source of feed for livestock. Farmers might consider grassland improvement as crucial to maintain their livelihood in the future. That need aligns with the predictions that climate change threatens quality and ecosystems services of Mediterranean pastures (Bernués *et al.*, 2011). New technologies such as virtual fences or GPS collars that are useful for grazing management (I6 and I7) were poorly rated. This might be caused either by a lack of knowledge about these technologies or because the farmers do not find them useful. Other factors could be the cost and the need for technical assistance to use them. Research efforts should be directed to make these technologies useful and applicable to special systems such as extensive farming.

Correlation coefficients showed that innovations I2 (0.305), I3 (0.316), I4 (0.389), I5 (0.313), I7 (0.310) and I10 (0.322), had a positive significant correlation ($P<0.05$) with higher education level of farmers.

Table 1. List of innovations and scores assigned by the farmers (n=36).[1]

	Innovations	Average score	Standard Dev.
I1	Sowing with new mixes of seeds	4.36	0.83
I2	Searching for drought-tolerant grassland species	4.58	0.84
I3	Increasing the knowledge about the quality of grassland species in the Dehesa and their evolution during the year	4.25	0.91
I4	Grassland fertilisation: products and fertilisation guidelines	4.06	1.12
I5	Monitoring and guidance for soil health assessment	3.94	1.14
I6	GPS collars	3.18	1.51
I7	Virtual fences	3.06	1.54
I8	Remote sensing of grasslands as monitoring tool	3.37	1.24
I9	Analysis of manure and slurry	3.82	1.17
I10	Software for short-term estimation of grass growth based on current pasture status and weather conditions	3.66	1.14
I11	Software for GHG emissions estimations on the farm, with existing farm management, and recommendations on how to reduce them	3.15	1.35
I12	Extension and dissemination of research results on permanent pasture and its management through specialized websites, workshops and courses	4.34	0.87

[1] Range of relevance: 1 = very low, 2 = low, 3 = medium, 4 = high, 5 = very high.

A new generation of farmers could be willing to improve the management of grassland, demanding the implementation of some of the proposed innovations. Dissemination of research results and specialized courses could be essential to spread the use of some innovations, since higher education leads to a greater willingness to implement them. This aligns with I12 being highly rated, as it might indicate a demand for closer research-farmer trade-offs on permanent-grassland management.

Conclusions

The farmers rated positively the pasture performance-related innovations, highlighting the need for productive and drought-tolerant pastures. Low relevance was given to new technologies, and this might be related to the complexity of adapting these technologies to dehesa farms. Younger farmers and higher education level correlated with a positive willingness to implement innovations on dehesa farms.

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References

Berckmans D. (2017) General introduction to precision livestock farming. *Animal Frontiers* 7, 6-11. https://doi.org/10.2527/af.2017.0102.

Bernués A., Ruiz R., Olaizola A., Villalba D. and Casasús I. (2011) Sustainability of pasture-based livestock farming systems in the European Mediterranean context: synergies and trade-offs. *Livestock Science* 139, 44-57. https://doi.org/10.1016/j.livsci.2011.03.018.

Bugalho M.N., Caldeira M.C., Pereira J.S., Aronson J., and Pausas J.G. (2011) Mediterranean cork oak savannas require human use to sustain biodiversity and ecosystem services. *Frontiers in Ecology and the Environment*, 9, 278-286.

Gómez-Giráldez P.J., Aguilar C., Caño A.B., García-Moreno A., and González-Dugo M.P. (2019) Remote sensing estimation of net primary production as monitoring indicator of holm oak savanna management. *Ecological Indicators* 106, 105526. https://doi.org/10.1016/j.ecolind.2019.105526.

Maroto-Molina F., Navarro-García J., Príncipe-Aguirre K., Gómez-Maqueda I., Guerrero-Ginel J.E., Garrido-Varo A. and Pérez-Marín D.C. (2019) A low-cost IOT-based system to monitor the location of a whole herd. *Sensors* 19, 10. https://doi.org/10.3390/s19102298.

Moreno G., Berg S., Burgess P.J., Camilli F., Crous-Duran J., Franca A., Hao H., *et al.* (2015) *Innovations to be examined for high nature and cultural value agroforestry*. Agforward Milestone 2.2 (MS3) Report 2.

A survey analysis of farmer practices and perceptions of zero-grazing on Irish dairy farms

Holohan C.[1], Russell T.[2], Mulligan F.J.[3], Pierce K.M.[1] and Lynch M.B.[1]
[1]School of Agriculture and Food Science, University College Dublin, Lyons Farm, Lyons Estate, Celbridge, Naas, Co. Kildare, Ireland; [2]School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Co. Dublin, Ireland; [3]School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Co. Dublin, Ireland

Abstract

Understanding the knowledge requirements of farmers is essential for the uptake of technologies like zero-grazing (ZG) (the mechanical harvesting and feeding of fresh grass). Zero-grazing is increasingly used in grass-based milk production systems alongside conventional grazing to supply freshly cut grass from outside land blocks to cows during periods of grass shortages on the main grazing block and where farm fragmentation limits grazing opportunities. This study aimed to develop an understanding of current ZG practices on Irish dairy farms and to highlight the knowledge requirements of farmers. An online survey was distributed and completed by 130 dairy farmers who use or have used ZG. Zero-grazing was used alongside conventional grazing by 92% of respondents. These farms were particularly fragmented, with between 1 and 14 separate land blocks. Respondents felt ZG helped them overcome fragmentation, increase grass utilisation, and extend grazing season length. Extra cost and time input associated with ZG were recognised as key challenges. The majority of respondents rated current technical information available on ZG as 'poor' or 'very poor', and several knowledge deficits were identified in the areas of cow performance, grass/nutrient management, and the cost benefit analysis of ZG.

Keywords: zero-grazing, dairy, survey

Introduction

Zero-grazing (also known as 'cut and carry' or 'green chop') is an alternative feeding system where fresh grass is cut and fed directly to housed cows. The fresh grass is typically cut standing by one machine which also transports the grass from the field to the housed cows. This is commonplace in some parts of mainland Europe (Meul *et al.*, 2012), however conventional paddock grazing predominates in the temperate grass-based dairy production systems typically operated in Ireland. Although well-managed grazed grass is the most economical feed available for dairy cows in Ireland (Hanrahan *et al.*, 2018) there is growing interest in the role of ZG and its potential to compliment grazing systems (Agrisearch, 2018). Farm fragmentation and land availability are major barriers to expansion on Irish dairy farms and are considered the main driver in the uptake of ZG to date, as it allows farmers to utilise outer land blocks as part of their grazing rotation. The objective of this survey study was to establish an understanding on the use of ZG by Irish dairy farmers and to capture their perceptions and knowledge requirements on the implementation of this system at farm-level.

Materials and methods

A questionnaire survey was developed using Google Forms and distributed to dairy farmers across the Republic of Ireland who used zero-grazing (ZG) on their farms. Following expert consultation it was estimated that the number of farmers who use ZG in the Republic Ireland is 2,000 (approximately 12% of dairy farmers). The survey was circulated using the Twitter and WhatsApp social media platforms between January and July 2019 and was completed by a total of 130 respondents. Survey responses were captured on Google Forms and analysis was conducted using the IBM SPSS Statistics 24 software package.

Results and discussion

Responses were geographically spread across 21 of the 26 counties in the Republic of Ireland, with the highest number of respondents from the larger dairy-producing counties of Tipperary, Cork and Kilkenny. Average respondent age was 40, and 65% were members of farmer discussion groups. The main dairy system operated by respondents was spring-calving milk production, with 65% calving in spring, 30% operating split-calving herds between spring and autumn, and 5% calving all year round. Automated milking systems (robotic milking) were used on 11% of farms. The average number of cows on farms surveyed was 146. Average reported annual milk yield was 6,702 litres, while average reported concentrates fed per cow per year was 1,195 kg. Average farm size was 90 hectares, with 43% of this leased land. On average, these farms consisted of 5 separate land blocks, (ranging from 1 to 14 land blocks per farm), indicating a significant degree of fragmentation.

The most common reason for farmers to use ZG was to overcome farm fragmentation, which was followed by the use of ZG as a means of filling feed gaps and to offer flexibility with conventional grazing systems. Survey respondents considered grass an important part of their production systems. On a scale of 1 to 10, respondents indicated that the importance of grass to their farm business was 9. Furthermore, they rated their skill level in grassland management as 8/10. Grass measuring was routinely carried out by 74% of farmers. Survey results indicate that target pre-cutting herbage mass on ZG farms differs from target pre-grazing herbage mass for conventional grazing which is 1,500 kg DM ha^{-1} (>4 cm). Average pre-cutting herbage mass for ZG was 1,785 kg DM ha^{-1} (>4 cm), with 68% stating that they zero-graze higher covers than what they would graze The main rationale for cutting these higher covers was to reduce time taken to cut grass and to minimise field traffic.

Based on survey results, the use of ZG on farms can be categorised into three main systems: (1) full-time ZG (cows housed year-round) which was used by 8% of respondents, (2) part-time ZG (cows routinely fed zero-grazed grass in addition to grazing at pasture) which 44% of respondents used, and (3) seasonal ZG (grazing at pasture complimented with ZG in spring, summer, and/or autumn) which 48% of respondents used. Summer users predominantly used ZG in periods of drought where grass from outside land blocks is used. Spring and autumn users utilise ZG to introduce grass into the diet earlier in spring and/or extend grass feeding further into autumn. According to respondents the most effective season for ZG is spring, followed by autumn and lastly summer. 55% of farmers carry out ZG themselves, while 43% hire an agricultural contractor to do this work. The main variable in contractor cost was the distance required to travel between farmyard and field. The average round-trip distance travelled to zero-graze grass on farms surveyed was 5 km. This ranged from 0.5 to 38 km.

A number of benefits of ZG were identified by survey respondents. The most common of these was 'extended grazing', whereby ZG is used to increase the number of days that grass is the main component of the diet, with 85% of respondents stating that ZG has allowed them to extend the grazing season on their farms. On average, it allowed them to achieve an additional 33 days with fresh grass in the diet. Survey participants also felt that ZG offers a level of flexibility with grazing systems, where ZG is used as a means of supplementing the diet of grazing cows during periods of grass deficits on the grazing platform. A number of respondents cited herd expansion as a benefit of ZG, while others noted that it allows them to make better use of outside land blocks, improve cow performance, and reduce concentrate feeding. Another reported benefit was that ZG allowed respondents to deliver fresh grass to cows at times where wet field conditions do not allow for conventional grazing.

In terms of its impact on cow performance, 85% indicated that ZG had an effect. The most common impacts reported were increased milk yield, higher dry matter intake, and higher milk solid yield. Farmers also reported improved body condition score. Acidosis/loose dung was reported as the negative effect on cow performance. The most significant challenge associated with ZG was the additional time/labour input required, most notably the time spent 'cutting' and 'pushing in' grass to the feed face. 'Cost' and 'reliance on machinery' were second and third respectively. Other challenges included the storage and spreading of extra slurry, housing requirements (particularly additional feed space), acidosis, variability in grass DM and quality, and contractor reliability. While there were several challenges outlined, 92% of respondents indicated that they would continue to use ZG in the future.

Survey respondents were asked a number of questions relating to the level of information currently available to them on ZG, and also about how they could be better supported by research and extension services in the future. When asked about the quality of information available on ZG, 1.5% rated it as 'excellent', 8% rated it as 'good' 31.5% as 'Ok', 39% as 'poor' and 20% as 'very poor', which would indicate that current information on this technology is not at a sufficient level for the majority of those surveyed. There were a number of areas identified by respondents in which they need more information on ZG. The most prominent of these was 'cost', while 'cow performance' was the second-most important area that respondents require more knowledge on. There were also several knowledge gaps identified in relation to grass quality / growth / utilisation, optimum grass varieties, and sward management practices for ZG.

Conclusions

Survey results demonstrate that zero-grazing (ZG) is generally seen by farmers as a way of increasing fresh grass in the dairy cow diet; overcoming farm fragmentation; and facilitating herd expansion. Unlike much of the full-time indoor ZG systems utilised in mainland Europe, ZG in the Republic of Ireland is predominantly used as a tool to compliment pasture-based grazing systems during seasonal shortages and periods where climatic conditions limit grazing opportunities. The study quantifies current ZG practices and underlines that while there are several benefits, there remains a number of challenges that need to be addressed through targeted research and improved knowledge transfer efforts. The bottom-up approach used in this study should assist in the delivery of more relevant information to farmers on ZG in the future.

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References

Agrisearch (2018) *Zero-grazing – A best practice guide*. Agrisearch, Hillsborough, UK.

CSO (2019) Crops and Livestock Survey June Final Results. Retrieved November 2019, from https://www.cso.ie/en/releasesandpublications/er/clsjf/cropsandlivestocksurveyjunefinal2018/

Hanrahan L., McHugh N., Hennessy T., Moran B., Kearney R., Wallace M. and Shalloo L. (2018) Factors associated with profitability in pasture-based systems of milk production. *Journal of Dairy Science*, 101(6), 5474-5485.

Meul M., Van Passel S., Fremaut D. and Haesaert G. (2012) Higher sustainability performance of intensive grazing versus zero-grazing dairy systems. *Agronomy for Sustainable Development*, 32(3), 629-638.

Exploring methods to quantify on-farm fresh grass intake

Klootwijk C.W.[1], De Haan M.H.A.[1], Philipsen A.P.[1] and Van den Pol-van Dasselaar A.[1,2]
[1] *Wageningen Livestock Research, De Elst 1, 6700 AH Wageningen, the Netherlands;* [2] *Aeres University of Applied Sciences, De Drieslag 4, 8251 JZ Dronten, the Netherlands*

Abstract

The Dutch dairy sector focusses on improving nutrient use efficiency while supporting grazing. All dairy farms are obliged to use the Annual Nutrient Cycling Assessment (ANCA) tool to quantify their N, P and C efficiency. The fresh grass intake, however, seems to be overestimated on farms with high feed supplementation levels. In this study we explored how on-farm fresh grass intake can be quantified, aiming to improve the ANCA tool, using literature sources and a network of stakeholders, including (inter)national experts, consultants and farmers. The novel value of this study is in finding methods that combine practicability and reliability, which is essential as each Dutch dairy farm has to use the ANCA tool. Interestingly, similar conclusions could be drawn from literature sources and inputs from the different stakeholders. The on-farm quantification of fresh grass intake can also be improved, not only by accounting for access time, but also for actual grazing time and supplementation level during the grazing season. In the long term, a promising route is to further develop and link national databases with direct grassland measurements (e.g. sensors) like grazing hours, grass growth and grass height and include the results in the ANCA tool.

Keywords: intake estimation, on-farm assessment, grazing behaviour

Introduction

In the Netherlands, all dairy farms are obliged to use the Annual Nutrient Cycling Assessment (ANCA) tool to quantify their N, P and C efficiency. The fresh grass intake, however, seems to be overestimated at farms with high feed supplementation levels. In this study we explored how on-farm fresh grass intake can be quantified, aiming to improve the ANCA tool. Currently the fresh grass intake in the ANCA tool is calculated by equation 1 for daily grazing periods between 2 and 20 h. The aim of this study is to explore if this calculation method can be improved based on novel insights from literature, expert knowledge and field experiments, while considering both practicability and reliability.

$$2 + 0.75 \times (\text{hours of grazing day}^{-1} - 2) \qquad (1)$$

Materials and methods

We performed a literature search to get a state-of-the-art overview of methodologies to calculate fresh grass intake. In addition, we organized a workshop and sent questionnaires to a network of (inter)national experts, consultants and farmers. In this multi-stakeholder process, we were interested in suggestions to quantify the on-farm grass quantity for the short term and the long term, and also in selection criteria to create a shortlist of suggestions. The different suggestions that were identified during the process were scored, based on these selection criteria, by using a matrix calculation in which they were independently scored on a 0-5 scale with equal weight by the four authors of this paper.

Results and discussion

Interestingly, similar conclusions could be drawn from the literature and the input from the different stakeholders. All suggestions could be categorised in the four directions for improvement (A-D, Table 1) that were identified during the workshop. In addition, seven criteria were identified to score the suggestions as a result of the multi-stakeholder process: reliable, minimal input, verifiable, explainable, future-proof, valuable and affordable. Results of the matrix calculation are shown in Table 1.

Improving Equation 1 without extra data input is the preferred route according to the stakeholders. Therefore, we first focussed on suggestion A. We used the literature review to explain the mechanisms that define fresh grass intake and used them as a basis for advice on how the current equation (Equation 1) could be improved to estimate on-farm fresh grass intake without extra data input. Fresh grass intake is determined by grazing time (min day^{-1}) × bite rate (bites min^{-1}) × bite mass (g DM min^{-1}) (Abrahamse, 2009). The grazing time is influenced by access time to pasture, which is evaluated by Chilibroste *et al.* (2015) based on 12 grazing studies. We used the equation (Equation 2) developed by Chilibroste *et al.* (2015) to relate access time to the actual grazing time.

Actual grazing time (hours) = $0.9269e^{-0.037 * \text{access time}}$, with access time in hours day^{-1} (2)

Combining Equation 1 and Equation 2 was not sufficient since the parameters in Equation 1 are based on access time. Therefore, we also defined bite rate and bite mass based on literature. The relation between grazing management and bite rate and bite mass are well described for grazing systems with low supplementation levels, or only concentrate supplementation. Few studies, however, show the effect of high supplementation levels including roughages on grazing behaviour. In Dutch grazing systems with unrestricted grazing and low supplementation levels the bite rate is around 60 bites min^{-1} and the bite mass around 0.5 g DM $bite^{-1}$, which results in an intake rate of 30 g min^{-1} (Abrahamse, 2009). Perez-Prieto *et al.* (2011) showed the effect of feeding roughages as supplement in combination with a low herbage allowance on these grazing behaviour parameters. Based on this study we found that the intake rate can become 17 g min^{-1} under these circumstances.

Figure 1 shows the fresh grass intake calculated based on the actual grazing time in combination with the intake rates for a low (30 g min^{-1}) and a high supplementation level (17 g min^{-1}). Since the level of supplementation varies in the Netherlands, we showed the surface between these two lines as a range. In addition, Equation 1 was added and the results of two recent grazing experiments in the Netherlands with high supplementation levels as part of the project 'Amazing Grazing' (Schils *et al.*, 2019).

Table 1. Suggestions for the short and long term to quantify on-farm fresh grass intake.

Short term: improvement of Equation 1		Long term: additional measurements	
A. No extra data input	**B. Extra data input**	**C. At farm level**	**D. At regional level**
Create multiple equations for different supplementation levels	Ask time of grazing during the season, size of grazing platform	Use new techniques (sensors, satellites), calibration needed	Set up a measuring network at regional level, analysis needed

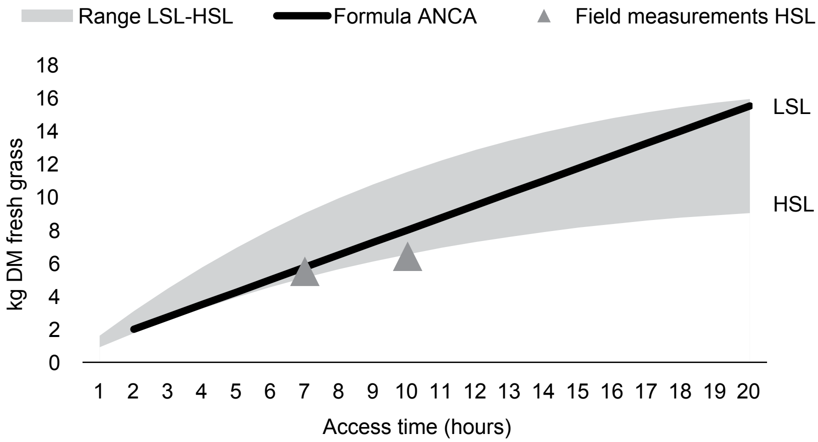

Figure 1. Effect of access time to pasture and supplementation level (LSL = low supplementation level and HSL = high supplementation level) on calculated fresh grass intake and a comparison with the calculated fresh grass intake with the current equation in the Annual Nutrient Cycling Assessment (ANCA) tool and two data points from recent grazing experiments.

Conclusions

The multi-stakeholder process proved to be valuable for exploring methods to quantify on-farm fresh grass intake. In the short term, this might be valuable to account for the relation between access time and actual grazing time to calculate fresh grass intake in ANCA. This, however, also requires an estimation of the effect of different supplementation levels on the intake rate. Since few have studies focussed on this, the next step is to do this based on expert judgement. Time of grazing during the season and the size of the grazing platform would be valuable as extra inputs. For the longer term, it would be of interest to make use of extra input factors and novel techniques such as sensors and satellites. However, this requires further calibration to be used at a large scale in practice. In addition, a promising route is to further develop and link national databases with direct grassland measurements (e.g. sensors) like grazing hours, grass growth and grass height and include the results in the ANCA tool.

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References

Abrahamse P.A. (2009) *Feeding and grazing management for dairy cattle: opportunities for improved production*. PhD thesis. Wageningen University, Wageningen.

Chilibroste P., Gibb M., Soca P. and Mattiauda D.A. (2015) Behavioural adaptation of grazing dairy cows to changes in feeding management: Do they follow a predictable pattern? *Animal Production Science* 55, 328-338.

Perez-Prieto L.A., Peyraud J.L. and Delagarde R. (2011) Substitution rate and milk yield response to corn silage supplementation of late-lactation dairy cows grazing low-mass pastures at 2 daily allowances in autumn. *Journal of Dairy Science* 94, 3592-3604.

Schils R., Philipsen B., Hoekstra N., Holshof G., Zom R., Hoving I., van Reenen K., Stienezen M., Klootwijk C., van der Werf J., Sebek L., van Eekeren N., van Dixhoorn I. and van den Pol-van Dasselaar A. (2019) Amazing grazing: a public and private partnership to stimulate grazing practices in intensive dairy systems. *Sustainability* 11, 5868.

Organic dairy cow grazing – demonstration study at Mustiala Farm

Kuoppala K.[1], Perttala R.[2], Kukkula L.[3] and Heikkonen J.[4]
[1]Natural Resources Institute Finland, Tietotie 2 C, 31600 Jokioinen, Finland; [2]University of Helsinki, P.O. Box 27, 00014 University of Helsinki, Finland; [3]ProAgria – Rural Advisory Services, Vanajantie 10 B, 13110 Hämeenlinna, Finland; [4]Häme University of Applied Sciences, Mustialantie 105, 31310 Mustiala, Finland

Abstract

Grazing is an essential part of organic milk production. It is natural for ruminants to graze and according to the rules of organic production all cattle must be grazing. Mustiala educational and research farm of Häme University of Applied Sciences (HAMK) started conversion to organic production in 2018. The dairy herd began the conversion period in January 2020. The objective of this paper is to describe pilot work for developing organic grazing of the dairy herd. Rotational grazing and establishing the pasture with rye (*Secale cereal*) were conducted. The cows were rotationally grazed and received a new strip of pasture every morning. Grass samples were taken from two pasture strips to evaluate the feed values. The grass growth was very dynamic and much of the herbage was wasted. Simultaneous indoor feeding allowed cows to maintain high milk production during grazing, and without showing reduced milk yield in response to changes in feed values of the herbage samples.

Keywords: organic milk production, grazing, network of stakeholders, farmers' discussion group

Introduction

The 'Developing Organic Production in Häme region' project started in 2019 in the Mustiala educational and research farm of HAMK, Tammela, Finland. The aim of this three-year project is to establish a network of experts from education, research, advisory services, professional kitchens and companies to work together, share ideas, build linkages between farmers and other rural stakeholders, and implement the results of multidisciplinary trials in order to develop organic production in the whole Häme region. This paper describes the pilot work with dairy cows grazing at Mustiala. Grazing is an essential part of organic milk production. It is natural for ruminants to graze and according to the rules of organic production all cattle must be grazing. Rotational grazing is one type of grazing management, where pasture is divided into smaller strips or paddocks and dairy cows graze only one area at a time while the remainder of the pasture is resting (Undersander *et al.*, 2002).

Materials and methods

The dairy herd of Mustiala educational and research farm has 80 dairy cows and replacement heifers and calves. The barn contains an automated feeding system of partial mixed ration and an automated milking system (AMS). The size of pasture for milking cows was 5 ha divided into 5 strips of 1 ha each, of which one was established in 2019 and four were pastures in their second production year. The distance from the barn to the farthest corner of the strips was 500 m. One strip (S1) was ploughed in the previous autumn, cultivated in spring 2019 and fertilised with slurry at 20 m^3 ha^{-1}. The pasture in S1 was sown on 6 May with rye (*Secale cereal*) (60 kg ha^{-1}) and a pasture seed mix (28 kg ha^{-1}) (containing timothy (*Phleum pratense*) 0.33, meadow fescue (*Festuca pratensis*) 0.15, perennial ryegrass (*Lolium perenne*) 0.15, smooth meadow-grass (*Poa pratensis*) 0.10, tall fescue (*Festuca arundinacea*) 0.10, red fescue (*Festuca rubra)* 0.05, white clover (*Trifolium repens*) 0.05, alsike clover (*Trifolium hybridum*) 0.05 and red clover (*Trifolium pratense*) 0.02 kg kg^{-1}) and herbs (2 kg ha^{-1}). The other four strips (S2, S3, S4 and S5) had been established in 2017 with pasture seed mix containing timothy, meadow fescue, perennial ryegrass and white clover with oats (*Avena sativa*) and pea (*Pisum sativum*) as cover crops. Only S4 was sampled and it was considered to represent also strips S2, S3 and S5 because during the time of establishing in 2017 they had been one paddock and they were sown with the same seed mixture.

Cows had free access to the pasture strips during the period 7:30-10:00 am and ending at 19:00-20:00 pm in the evening. Every morning the cows received a new 1 ha strip so that the resting period of each strip was 4 days. Indoor feeding (mixed silage and concentrates) was not available from 7:00 to 15:00 hours. Daily milk yield was recorded by AMS. Grass samples were taken with 0.5×0.5 m frame four times from S1 and six times from S4 (representing strips S2-S5) at morning before grazing. Four subsamples per strip were cut at 5 cm stubble length, mixed, and a representative sample of 500 g was taken to the feed analyses. The sample was analysed in the laboratory of Valio (Artturi® feed analysis using NIRS) for dry matter (DM, g kg^{-1}), D-value (the amount of digestible organic matter, g kg^{-1} DM) and the concentrations of ash, crude protein (CP), neutral detergent fibre (NDF), indigestible NDF and water soluble carbohydrates (WSC). The height of the herbage was measured from the four sides of each frame area. The strips were mowed to 20 cm height once in grazing season on 14 June for S4, S2, S3 and S5 and on 24 June for S1.

Results and discussion

The animal production team in this project consisted of an adviser (organic production, dairy cow feeding), a dairy barn manager, a teacher (animal production) and a researcher (ruminant feeds). This multidisciplinary team shared knowledge and discussed how to organize the housing and grazing in the Mustiala herd. The dairy barn was not originally built for organic production and some renovations had to be conducted inside the barn. For example, the area of solid floor per animal had to be increased with rubber mats. Within the project, a field-day event for farmers and other stakeholders was arranged in August where 150 people visited the trials of dairy cow grazing and other demonstration trials. A farmers' discussion group for organic milk producers was started in autumn and the issues discussed were conversion to organic production and first experiences of the project.

Rotational grazing began on 27 May from S2 and lasted until 21 August. Every morning, cows got a new strip of pasture. First the cows grazed only S2-S5 but after 20 June also S1 was brought into the rotations. The D-value of S4 was high in the first sampling on 29 May, but decreased rapidly during the first three weeks after first sampling, with daily decrease of 4.5 g kg^{-1} DM. Simultaneously with the decrease in D-value, the height of the herbage and the DM yield increased (Table 1). The average daily decrease of D-value has been reported to be 4.9 g kg^{-1} DM in primary growth of grass (Rinne, 2000) and 3.7 g kg^{-1} in primary growth of red clover (Kuoppala, 2010). In S1 where rye comprised the majority of the herbage and was fresh, dense and abundant, the average daily decrease of D-value was 1.78 g kg^{-1} DM over the whole sampling period. Unfortunately, it turned out that the variety of rye was not suitable for grazing. It started to develop stems and inflorescence as early as in the middle of June. S1 had to be included to the rotation earlier than was planned.

The daily milk production of the cows was on average 33.2 kg per cow during the pasture-sampling period with no reduction in milk yield after early summer. According to the feed budgeting calculations (CowCompass, ProAgria) the cows were able to maintain milk production based mainly on indoor feeding.

Table 1. Concentrations of dry matter (DM), ash, crude protein (CP), neutral detergent fibre (NDF), indigestible NDF (iNDF), water soluble carbohydrates (WSC), D-value and the DM yield of the pasture samples of the strips S1 (established in 2019) and S4 (representing strips S2-S5 which were older pasture).

		$g\ kg^{-1}$ DM							DM yield
		DM	Ash	CP	NDF	iNDF	WSC	D-value	$kg\ ha^{-1}$
Strip S1	20 June	176	94	249	436	31	78	722	1,308
	6 July	178	81	144	473	66	167	689	1,593
	17 July	233	76	113	512	92	212	676	2,318
	21 August	248	66	137	619	155	89	603	1,453
Strip S4	29 May	208	79	169	457	31	152	748	1,490
	11 June	239	72	115	549	72	126	692	2,648
	19 June	246	72	103	549	111	145	648	2,996
	6 July	283	76	120	535	108	142	643	2,788
	17 July	297	72	84	574	149	169	611	2,774
	14 August	208	76	127	595	147	109	602	2,100

Conclusions

Rotational grazing in this case did not result in effective grazing of the cows. The growth of the grass in early summer was fast and much of the herbage was wasted. Cows that were based on indoor feeding were able to maintain high milk production during grazing without showing a reduction such as that seen in feed value of the herbage samples. Rye has potential for establishing in a pasture as a fast-growing plant enabling early grazing after seeding, but a variety with delayed flowering should be tested next year.

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References

Kuoppala K. (2010) Influence of harvesting strategy on nutrient supply and production of dairy cows consuming diets based on grass and red clover silage. *MTT Science* 11: 50 p.

Rinne M. (2000) *Influence of the timing of the harvest of primary grass growth on herbage quality and subsequent digestion and performance in the ruminant animal.* University of Helsinki, Department of Animal Science publications 54. 42 p. Doctoral Dissertation University of Helsinki, 2000.

Undersander D., Albert B., Cosgrove D., Johnson D. and Peterson P. (2002) *Pastures for profit: A guide to rotational grazing.* Wisconsin, USA: Cooperative Extension Publishing.

A European survey of grassland innovations captured by practice and science meetings

Mairhofer F.[1], Van den Pol-van Dasselaar A.[2,3], Höllrigl P.[1], Baste-Sauvaire F.[4], Bastiaansen-Aantjes L.M.[2], Bogue F.[5], Carlsson A.[6], Delaby L.[7], Delaite B.[8], De Kort H.[9], Fradin J.[10], Jacquet D.[11], Kaemena F.[12], Krause A.[13], Melis R.[14], Nilsdotter-Linde N.[15], Pascarella L.[16], Paszkowski A.[17], Peeters A.[18], Przepiora A.[19], Schiebenhöfer N.[20] and Peratoner G.[1]

[1]Laimburg Research Center, Vadena/Pfatten, 39040 Ora, Italy; [2]Aeres University of Applied Sciences, the Netherlands; [3]Wageningen Livestock Research, the Netherlands; [4]APCA, France; [5]TEAGASC, Ireland; [6]Svenska Vallföreningen, Sweden; [7]INRA, France; [8]Tr@me Scrl, Belgium; [9]LTO, the Netherlands; [10]IDELE, France; [11]AWÉ, Belgium; [12]Landwirtschaftskammer Niedersachsen, Germany; [13]Grünlandzentrum Niedersachsen/Bremen, Germany; [14]CNR, Italy; [15]SLU, Sweden; [16]AIA, Italy; [17]Poznań University of Life Sciences, Poland; [18]RHEA Research Centre, Belgium; [19]Wielkopolska Izba Rolnicza, Poland; [20]University of Göttingen, Germany

Abstract

Within the Horizon 2020 thematic network Inno4Grass (www.inno4grass.eu) grassland innovations were analysed in practice and science meetings that are participatory, multi-stakeholder discussion groups. Their outcome was summarised in 115 practice abstracts (PAs) from eight countries. PAs are short summaries for practitioners containing the most important results of the activity and the main practical recommendations. In order to investigate which are the most relevant common traits characterising the innovation process in the European agricultural practice, twenty-seven descriptors of the topics addressed in the PAs were scored for presence/absence. The results were analysed by means of a Principal Component Analysis, resulting in a fairly low proportion (31.5%) of the total variation explained by the first two components. Aspects related to regional marketing are quite closely related to Component 1. The factors 'organic farming' and 'animal welfare', as well as 'conventional farming' and 'machinery and tools', were strongly related to each other. There was no obvious pattern of the data due to the agroclimatic areas but decreases in management intensity seemed to be related to the adoption of regional marketing strategies. A certain bias might be present due to partners intentionally focusing only on a specific topic.

Keywords: grassland-related innovation, innovation analysis, discussion groups, practice abstracts

Introduction

The Horizon 2020 thematic network Inno4Grass (Shared Innovation Space for Sustainable Productivity of Grasslands in Europe, www.inno4grass.eu), consisting of twenty partners from eight countries, aims at capturing grassland innovations from practice, analysing and establishing them through a better knowledge exchange between practice and science. To this aim, grassland-related innovations were analysed in 'Practice & Science Meetings' (P&SMs). P&SMs are participatory, multi-stakeholder discussion groups fostering peer-to-peer exchange. They combine the expertise of actors from science and practice to produce a comprehensive analysis of the innovations. The structure and methodologies adopted for these meetings are described in Mairhofer *et al.* (2019) and Peratoner *et al.* (2019). Innovations were defined as techniques and management methods related to grassland which (a) are well established and successful at farm level, and (b) are new and uncommon in this region. The outcome of P&SMs was summarized in the practice abstracts (PAs), which are a common format for outcome in EIP-AGRI projects (http://bit.ly/guidelines-EIP). They are a short summary for practitioners containing the main results of the activity and the main practical recommendations for practitioners who intend to implement the innovation on their farm. These PAs represent therefore a relatively broad sample of the grassland-related innovations currently being implemented across Europe. This paper aims to investigate which are the most relevant common traits characterizing the innovation process in the European agricultural practice.

Materials and methods

From 2017 to 2019, P&SMs were held in eight European countries (Belgium, France, Germany, Ireland, Italy, the Netherlands, Poland, Sweden). The twenty participating partners freely chose the innovation topics to be analysed. In total, 115 PAs were written. All PAs were assigned to one European agroclimatic area (Iglesias *et al.*, 2012): Atlantic North, Atlantic Central, Continental North, Mediterranean (merging Mediterranean North and Mediterranean South) and Alpine. Also, the targeted change of management intensity in comparison with the situation prior to the innovation implementation (increased/unchanged/decreased) was recorded. The innovations addressed in the PAs were further described by means of 29 binomial descriptors (presence/absence). The following groups of descriptors were scored (in bracket the number of descriptors of each group): farm system (2), animal type (2), animal product (3), regional marketing of products (5), a selection of grassland-relevant topics (6) according to Schiebenhöfer *et al.* (2019), and the domains of innovation according to www.encyclopediapratensis.eu (11). The domains of innovation 'marketing' and 'product processing' were excluded from the analysis, because they are described in more detail within the group 'regional marketing'. Explorative data analysis was performed by Principal Component Analysis (PCA) using 27 variables. A variance-covariance matrix with 1000 bootstrap replicates was used.

Results and discussion

The first two PCA components explained a relatively low proportion (31.5%) of the total variation (Figure 1). Component 1 is fairly strongly related to the aspects of regional marketing, which in turn seem to be more relevant for innovations targeting a decrease of management intensity, as the change of management intensity is progressing from increased/unchanged to decreased along this component. Regional marketing therefore seems to be a key strategy to achieve profitability in such cases by ensuring

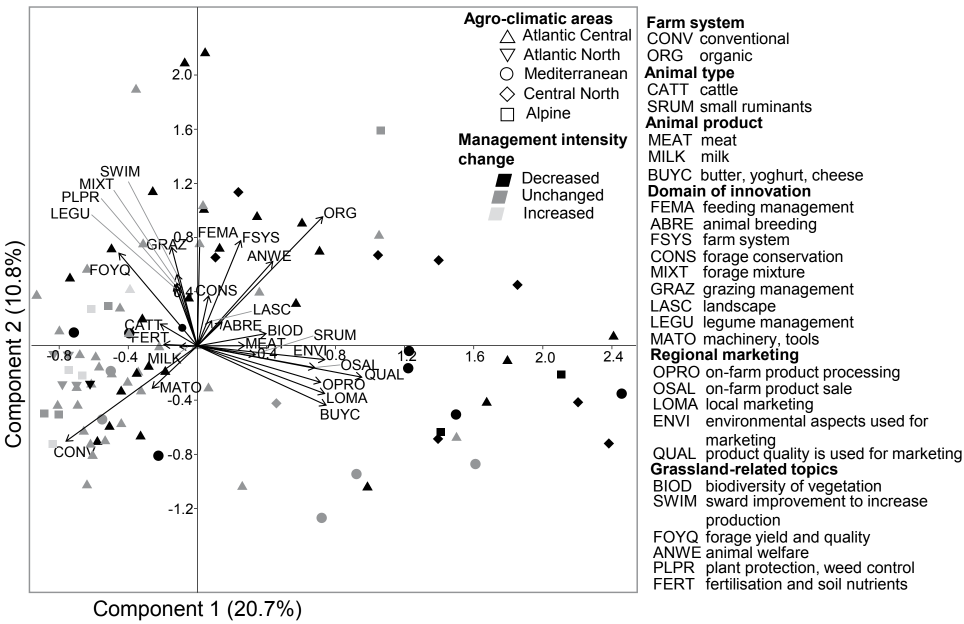

Figure 1. Biplot-ordination diagram showing the results of PCA on the topics addressed by 115 practice abstracts across Europe. Observations are shown illustrating their agroclimatic area and the targeted change of management intensity.

adequate prices for the products. The relatively close relationship between the factors 'organic farming' and 'animal welfare' on one hand, and that of 'conventional farming' and 'machinery and tools' on the other hand, suggest that animal welfare and machineries and tools are regarded to be mainly relevant for organic farming and conventional farming, respectively. However, machinery and tools would for sure also play an important role in decreasing production costs at lower management intensity.

Also 'grazing', 'forage mixture', 'legume management', and 'sward improvement to increase production' are relatively closely related to each other, but not to a specific farming type (conventional/organic), suggesting that they are equally relevant to both. 'Milk', 'meat', 'animal breeding', 'cattle', 'fertilisation and soil nutrients', and 'landscape' have a low explanatory power. There was no apparent spatial clustering of the observations according to the agroclimatic areas.

Conclusions

The results provide a picture of the relationship between farming type and other factors characterizing the grassland-related innovation in practice in Europe. No clear pattern could be detected depending on the agroclimatic areas, suggesting that innovation is not mainly driven by this factor, whilst decrease in management intensity seemed to be related to the adoption of regional marketing strategies. However, it should be mentioned that a certain bias might be present due to the selection criteria of the topics, with some partners intentionally focusing only on a specific topic.

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References

Iglesias A., Garrote L., Quiroga S. and Moneo M. (2012) A regional comparison of the effects of climate change on agricultural crops in Europe. *Climatic Change* 112, 29-46.

Mairhofer F., Florian C., Weiss A., Pfeifer A., Plitzner C., Prünster T., Van den Pol-van Dasselaar A. and Peratoner G. (2019) A method to lead discussion groups for the analysis of grassland innovations. *Grassland Science in Europe* 24, 500-502.

Peratoner G., Florian C., Mairhofer F., Baste-Sauvaire F., Bogue F., Carlsson A., …, and Van den Pol-van Dasselaar A. (2019) Effect of training and methodology development on the effectiveness of discussion groups on grassland innovation. *Grassland Science in Europe* 24, 509-511.

Schiebenhöfer N., Komainda M. and Isselstein J. (2019) Kongruenz der Themenvielfalt im Grünland: Ein Vergleich von Praxis- und Forschungsarbeiten. *Mitteilungen der Arbeitsgemeinschaft für Grünland und Futterbau* 20, 177-180.

Farmers' perceptions of grassland use are shaped through local social interactions: a case study in north-western France

Petit T.[1], Sigwalt A.[2], Martel G.[3] and Couvreur S.[1]
[1]URSE, ESA, 49007 Angers, France; [2]LARESS, ESA, 49007 Angers, France; [3]UMR Bagap, INRA, Agrocampus Ouest, ESA, 49045 Angers, France

Abstract

By cross analysing research projects on grasslands in livestock production that we took part in over the past 10 years, we seek to understand how local contexts make possible the emergence of livestock farmers' forage rationales favouring grassland maintenance. Based on the combined perspectives of sociologists and animal scientists, our analysis reveals that the production context is a key element for understanding grassland management practices on farms. Many groups of factors influence how farmers develop their perceptions of forage production (i.e. 'forage rationales'): (1) soil and climate conditions, (2) professional atmosphere and (3) existence of networks bringing together farmers and other stakeholders who discuss grassland issues. From the diversity of production contexts, we reveal perceptions that livestock farmers have about the services provided by grasslands: animal production, economic, agronomic, ecological and environmental. The structuring of these perceptions outlines an array of forage rationales in which grasslands have a relatively central place in livestock production. Finally, we show that this rationale evolves over time due to debates with peers and non-agricultural stakeholders. This leads us to discuss how evolution of livestock farmers' grassland rationales and practices can be supported, and finally to underline the importance of discussion between stakeholders with various rationales about grasslands.

Keywords: grasslands, farmers, forage perceptions, cattle

Introduction

In France, since the 1970s, the grassland area has decreased. This has depended on the interest of livestock farmers in adopting the forage-intensification techniques offered by agricultural extension services, in particular in the plains – on which this article focuses. From the 1980s to 2000s, even though the productivist agricultural model was being questioned, and environmental and landscape functions of grasslands increasingly considered (Huyghe, 2009), the grassland areas continued to decrease. Thus, from 2000 to 2015, the area of grasslands decreased in north-western France (i.e. Brittany, Normandy and Pays de la Loire) by 11.7%, while decreasing by 5.8% in the whole of France (Agreste). Nonetheless, statistical observations at a local scale identified dynamics that favour maintaining the grassland area or even increasing it (Coureur *et al.*, 2016). One may thus wonder which technical, social and economic elements contribute to this maintenance and to what degree it is possible to strengthen social dynamics that favour grasslands. By transversally analysing the research projects on grasslands in livestock production in which we have been involved over the past 10 years, we seek to understand how local contexts make it possible for livestock farmers to develop forage rationales favouring grassland maintenance. We define a forage rationale as the way farmers design their forage production.

Materials and methods

This article is based on the results of five research projects conducted from 2010 to 2018 (Table 1), each containing field surveys of cattle farmers performed by the authors. The studies were based on surveys analysed with the combined perspectives of sociologists and animal scientists. We attempt to reveal opportunities for reflection based on data that were collected in response to a variety of research questions. We used an analysis framework structured to highlight factors favouring grassland maintenance: (1) soil and climate conditions, (2) professional atmosphere, (3) existence of networks gathering farmers and

Table 1. Main research projects containing surveys used for this article.

Project	Project research objective (survey date)	No. of farmers surveyed
1	Study technical and sociological drivers of farmers' adherence to a contracting approach to agro-environmental measures for low-input forage systems (2010)	43 (dairy)
2	Identify relations between practices and representations of marsh grasslands on farms of the Marais Poitevin region (2011)	66 (dairy, beef, other)
3	Study relations between the evolution of the place of grasslands on farms and the forage rationale of livestock farmers (2014)	15 (dairy)
4	Study the potential for introducing more permanent grasslands, and its drivers, in the framework of green and blue corridors (2013)	20 (dairy, beef)
5	Identify relations between livestock-production practices and perceptions of the maraîchine breed of cattle (2018)	25 (beef)

other stakeholders dealing with grassland issues, (4) farmers' perceptions about forage and their key factors. We selected results related to the roles of grasslands, even if the analyses extended beyond this particular theme. We begin by describing production contexts that favour, or not, grasslands. Then, we focus on livestock farmers' perceptions about grasslands, which are perspectives that individuals have on grassland roles, depending upon their experiences and on how they use factors of their production contexts. These perceptions are central to the way farmers perceive forage production, what we call 'forage rationales'. Finally, we focus on how the forage rationales of livestock farmers are in motion and can contribute to evolution of grassland areas over the time.

Results and discussion

Contextual elements of farms related to grasslands

In this context, we underline a large number of factors, some of them extending beyond the local scale (e.g. public policies) and acting over the long term (e.g. existence of production chains). These factors combine in a specific way in each region: (1) the soil and climate conditions, (2) the professional atmosphere (i.e. existence of high added value chains, advisory groups, agricultural cooperatives, research centres) and (3) the existence of networks gathering farmers and other stakeholders (e.g. communes, environmental organizations, consumers, neighbours) who discuss grassland issues. The greater the diversity of these groups of factors, the greater is their impact on the expression of viewpoints and the emergence of forage perceptions and rationales.

Locally, a variety of forage rationales exist, differing in the perceptions that livestock farmers have about the services that grasslands provide

The farmers' forage perceptions depend mainly on: (1) farmers' experiences and (2) perceptions of services that grasslands provide (e.g. animal production, agronomic, economic, work, ecological, environmental). These various perceptions enable us to highlight forage rationales related to experiences and contextual factors. We show that farmers' perceptions can be impacted by the economic crisis they have experienced; depend of the moment in their career, and are related to changes occurring in their professional workgroup. In contrast, trends of increasing farm size and field dispersion reinforce the image of the complexity of managing a system based on grass production. The farmers' perceptions can be mainly analysed regarding: (1) the role given to animals in production rationales, (2) the income objectives and ideas about work, and (3) the ecological and environmental roles related to the type of grassland on the farm (Petit *et al.*, 2019). The agronomic role of grasslands is perceived positively but is seldom mentioned by farmers. All farmers' perceptions, associated with experiences and the ways farmers consider the factors of their production context, contribute to draw forage rationales that are specific to the local situation and context (Figure 1, from project 3).

Forage rationales that interact and are in motion in a region

In a specific local context, several forage rationales exist, interact and are not rigid; the concepts on which they are based evolve in line with regional socio-professional and socio-economic changes (Figure 1). Once placed in a favourable context (e.g. coexistence of rationales, interactions among various stakeholders, a local issue favouring exchange and pooling of experiences), forage rationales are debated and modified by farmers local groups. For forage rationales, this movement goes with the evolution of the design of grassland functions. Figure 1 well illustrates the movement that drives forage rationales in the agricultural profession. Through the multiple social networks in which they evolve, either professional (e.g. conventional or alternative farming groups, formal or informal work groups, facilitators or advisers specialised in grassland management) or non-professional links (family, neighbours) livestock farmers may be challenged to observe, discuss and apply forage practices developed on other farms. Depending on the production models they develop, livestock farmers are either emboldened in their production practices, in line with new expectations and issues of agriculture, or challenged about the gap between their practices and societal demands. This can lead to changes favouring grasslands through hybridization of practices between farmers (i.e. between Fulfilled graziers and Flexible optimiser farmers) (Lamine, 2011).

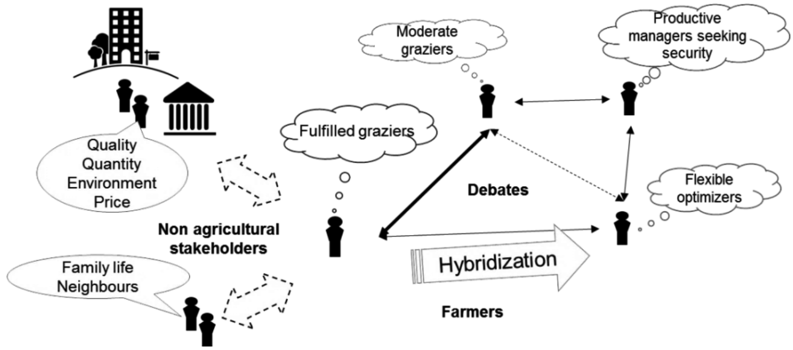

Figure 1. Farmers' forage rationales in motion (from Petit *et al.,* 2019).

Conclusions

Forage rationales are made of varied combinations of services perceptions that grasslands provide to farms and regions. Those that combine positive perceptions of the provided services are often shared by agricultural and non-agricultural stakeholders in multiple networks. At the local scale, the key to maintain grasslands on farms seems to be associated at present to the fact that they respond to the local stakeholders' expectations. This precondition may allow the emergence and coexistence of a variety of rationales as long as networks, professional or non-professional, exist. We therefore highlight the importance of 'arenas' in which farmers, in partnership with professional facilitators, technical advisers and researchers, can debate their production models and the roles that grasslands play in them, question their practices and co-design ways to maintain grasslands adapted to the diversity of systems.

References

Couvreur S., Defois J., Petit T. and Ben Arfa N. (2016). Local spatio-temporal dynamics of grassland maintenance between 2000 and 2010 in French cattle areas. *Grassland Science in Europe*, 21, 122-124.

Huyghe C. (2009). La mulifonctionnalité des prairies en France. Conciliation des fonction de production et de préservation de l'environnement. *Cahiers Agriculture*, vol. 18, no. 1.

Lamine C. (2011). Transition pathways towards a robust ecologization of agriculture and the need for system redesign. Cases from organic farming and IPM. *Journal of Rural Studies*, 27(2), 209-219.

Petit T., Martel G., Vertès F. and Couvreur S. (2019) Long-term maintenance of grasslands on dairy farms is associated with redesign and hybridisation of practices, motivated by farmers' perceptions. *Agricultural Systems*, 173, 435-448.

Petit T., Sigwalt A., Le Guen R., Martel G. and Couvreur S. (2019). Place des prairies dans les logiques fourragères des éleveurs laitiers du Grand Ouest de la France. *Fourrages*, 239, 235-245.

Grass measurement practices and attitudes amongst Irish dairy farmers

Regan A.[1], Douglas J.[2], Maher J.[3] and O'Dwyer T.[3]
[1]Rural Economy & Development Programme, Teagasc, Athenry, Galway, Ireland; [2]Grass10, Teagasc, Grange, Dunsany, Meath, Ireland; [3]Teagasc, Moorepark, Fermoy, Cork, Ireland

Abstract

Grass10 is a national strategy being led by Teagasc which aims to help family farms across Ireland realize the benefits of grass-based farming. Grass measurement is one area of focus in the Grass10 Campaign. The aim of the current study was to explore the factors acting as barriers or facilitators of grass measurement on Irish dairy farms. The current study employed a qualitative research design. One-to-one semi-structured interviews were carried out with 21 dairy farmers. A theory-based model of human behaviour called the COM-B Model was used to structure and present the analysis. Factors identified as currently facilitating or inhibiting farmers' capability, motivation, and opportunity to engage in grass measurement include knowledge, physical capacity, emotion, task effort and behavioural regulation, belief in consequences, belief in capabilities, support tools and technologies, social influences, support networks, and social identity. The findings reinforce the need for strategies to specifically target current behavioural blockages preventing farmers from engaging in grass measurement in Irish dairy farming.

Keywords: attitudes, behaviours, dairy, farmers, grass measurement

Introduction

FoodWise 2025, Ireland's national agri-food strategy, set a target to increase grass utilisation to 10 Mg ha^{-1} – a target which requires Irish farmers to be further supported in implementing and maintaining efficient grassland management practices. Grass measurement amongst Irish farmers is low (Creighton *et al.*, 2011; Hyland *et al.*, 2018). To explore the factors motivating and preventing Irish farmers to engage in grass measurement, the current study uses a behaviour change model, emerging from the health psychology literature, but applied broadly. The COM-B model is a comprehensive model used to explain human behaviour and intended to capture the wide range of factors considered to collectively bring about behaviour change (Michie *et al.*, 2011). The model identifies factors, both internal and external to the individual, which influence behaviour. In the model, 'capability', 'opportunity', and 'motivation' interact to generate behaviour. Capability is the individual's psychological and physical capacity to engage in the activity. Motivation reflects all those brain processes that energize and direct behaviour. Opportunity reflects factors that lie outside the individual, in their physical or social environment, that make the behaviour possible or deter or prompt it. Using the COM-B model as an explanatory framework, the current study explored the range of factors influencing Irish farmers' grass measurement practices.

Materials and methods

The current study employed a qualitative research design. One-to-one semi-structured interviews were carried out with 21 Irish dairy farmers. Purposive sampling was employed to ensure a reasonable spread of participants based on geographical spread, gender, farm size, and grass measurement engagement. Interviews were carried out via telephone. A semi-structured interview guide was used to explore farmers' perceptions of a visual image depicting '6 grass measurement steps' (Figure 1): this image outlined good practice in grass measurement from the perspective of a team of scientists and farm extension/advisory staff specialised in grassland management. The interview explored (1) attitudes towards grass measurement (2) personal experiences of grass measurement and (3) perceived barriers and facilitators to grass measurement. Interviews were carried out between November 2018 and January 2019. All

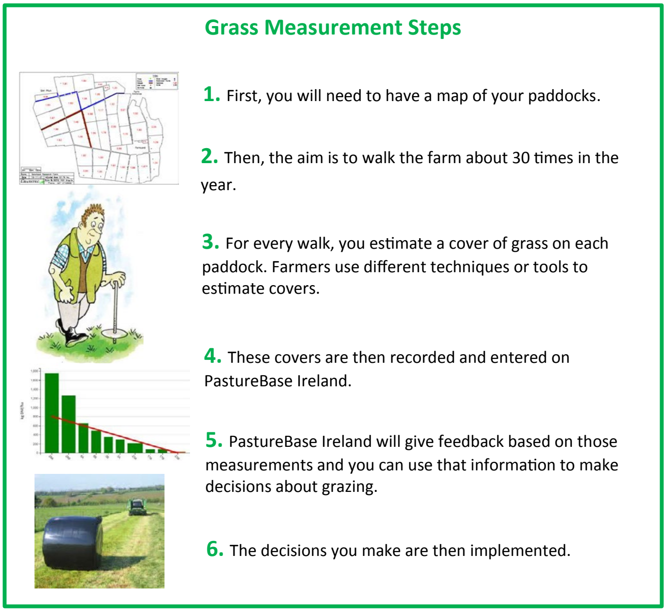

Figure 1. Grass measurement practice from the perspective of a team of scientists and farm extension/advisory staff specialised in grassland management.

interviews were audio-recorded and transcribed. A hybrid thematic analysis which combined deductive and inductive coding was carried out. The data initially underwent inductive coding, where codes were assigned freely and grounded in the data. The COM-B Model was used to deductively structure and present the codes and themes.

Results and discussion

Grass management practices, and the extent to which grass measurement was a part of a farmer's grass management, differed across the sample. For a number of farmers in the sample ('high engagers'), there was strong consistency between their approach to grass measurement and the 6 steps deemed as 'good practice' by grassland management specialists. Farmers held different meanings and interpretations of the terms 'grass measurement', 'grass management' and 'walking the farm'. For some farmers in the sample, practices taken for managing grass varied but included actively going out and walking through the farm and eye-balling grass growth (but not using any measurement technologies or recording any figures) and using a rotation system to manage grass. While all farmers valued the role of grass on their farm, they did not all prioritize grass measuring nor did they all carry out grass measurement in the same manner. Factors identified as currently facilitating or inhibiting farmers' capability, motivation, and opportunity to engage in grass measurement include knowledge, physical capacity, emotion, task effort and behavioural regulation, belief in consequences, belief in capabilities, support tools and technologies, social influences, support networks, and social identity. Table 1 summarises the barriers and facilitators in the COM-B model for engaging in grass measurement.

Conclusions

The farmers interviewed in this study were all registered to an online grass management tool, and so had baseline awareness and knowledge regarding the practice of grass measurement. Whilst knowledge and awareness levels were apparent across the sample, uptake of and engagement with the actual practice of grass measurement varied considerably. This allowed us to explore what factors beyond awareness and knowledge may contribute to shaping farmers attitudes and behaviours towards grass measurement. By exploring the opinions of farmers who actively engage in grass measurement, we identified the facilitators

Table 1. COM-B Model for Grass Measurement. Barriers (–) and facilitators (+) that influence Irish dairy farmers' uptake and engagement with grass measurement.

Capability Capacity to engage in behaviour	**Motivation** Brain processes that energise and direct behaviour	**Opportunity** Influencing factors that lie outside of the individual
Psychological Capacity to engage in necessary thought processes Knowledge • High levels of declarative ('what') knowledge (+) • Training and practice required to build procedural ('how to') knowledge (+, –) Self-regulation • High-effort task (–) • Importance of intentions, goal setting, planning, self-comparison, monitoring (+)	**Automatic** Emotions and impulses Emotion • Allows farmer to feel in control (+) • Alleviates panic/anxiety/stress (+) • Instils satisfaction and pride (+)	**Physical** Physical influences Grass measurement technologies • Availability, accessibility, & ease of use (+, –)
Physical Capacity to engage in necessary physical processes Physical stamina • 'Walking the farm' physically demanding for older farmers (–)	**Reflective** Evaluation and plans Beliefs about consequences • Belief in economic benefits (+) • Anticipated regret (+) • Beliefs that grass measurement is vital for higher-stocked dairy farms (+) • Failing to see personal value (–) Beliefs about capabilities • Lacking confidence in digital literacy and numeracy (–) Motives and values ('wants and needs') • Farm/life events as a driver of change (+) • Being a 'good dairy farmer' (+)	**Social** Social and cultural influences Social Influences • Seeing the relevance for their own farm from respected others (peers, advisors) (+) • Benchmarking: social comparisons to other farmers (+) Support network • Guidance for initial implementation (+, –) • Social support to maintain practice (+, –)

which supported and prompted them to begin, and persist with, the practice of grass measurement. By exploring farmers less active in grass measurement, we also developed an insight into potential barriers which prevent farmers from engaging in grass measurement. Future strategies should seek to specifically target current behavioural blockages so to support farmers to engage in grass measurement and increase their grass utilisation.

Acknowledgements

This study was carried out with the support of the Teagasc-led Grass10 Campaign, a multi-year campaign (2017-2020) to increase grass utilisation on Irish livestock farms.

References

Creighton P., Kennedy E., Shalloo L., Boland T.M. and O'Donovan M. (2011) A survey analysis of grassland dairy farming in Ireland, investigating grassland management, technology adoption and sward renewal. *Grass and Forage Science,* 66, 251-264.

Hyland J.J., Heanue K., McKillop J. and Micha E. (2018) Factors underlying farmers' intentions to adopt best practices: The case of paddock based grazing systems. *Agricultural Systems,* 162, 97-106.

Michie S., Van Stralen M.M. and West R. (2011) The behaviour change wheel: a new method for characterising and designing behaviour change interventions. *Implementation Science,* 6, 42.

GrasslandCam enables the monitoring of grass growth online

Tahvola E.[1]
[1] *A-Farmes Ltd, Itikanmäenkatu 3, 60100 Seinäjoki, Finland*

Abstract

GrasslandCam (GC) is a new innovative method to monitor, record and later deduce how selected farming practices and weather conditions have affected the growth rhythm and yield of a grass sward. A-Farmers Ltd's project 'Productive Grassland for Cattlefarms' launched this idea in spring 2019 on an actual farm located in Ruukki, Finland (64°40'N; 25°06'E). One of the average silage grass fields of the farm was selected to be monitored throughout the growing season. A weatherproof camera was permanently installed in the selected field and it was timed to take a photo of the growing grass three times a day. A metric measurement stick was also installed into the monitored sward in a way that it could be seen in the background of all photos while the grass height was clearly shown. Dry matter (DM) yields were measured at the time of harvest in the 3-cut regime. The collected data allowed us to create information on when, how rapidly, and how much the grass was growing throughout the growing season. A video of grass growth was also compiled from the photo material.

Keywords: grass growth rate, timothy, *Phleum pratense*

Introduction

The growth rhythm of grass swards is rather variable during the growing season in Finnish conditions. This is due to the nature of grass species and the fluctuation in their growth rate in response to the environmental conditions (Virkajärvi, 2003; Virkajärvi *et al.*, 2012). In Finland, the amount and quality of solar radiation is unequally distributed during the growing season because of the northern conditions. In many grass species, the timing of the onset of flowering is regulated by daylength and by the changes of the circadian cycle through the growing season. These species grow vigorously in the long-day conditions and, in turn, decelerate their growth in shortening days in order to prepare themselves for over-wintering. One of these long-day grass species is timothy (*Phleum pratense*), which is the most common grass species in Finnish arable grasslands. Herbage accumulation in timothy-dominated grass swards may be between 50 and 240 kg DM ha^{-1} day^{-1} depending on the time of the growing season. In previous studies it has been addressed that grass DM yield can be deduced by the height of the sward (Pakarinen *et al.* 2012). In our project, we invented a new way to monitor the variation of growth in a grass sward in order to produce an easy way for farmers to obtain real-time information and observations on the growth rate of grass swards and for the evaluation of its yield accumulation. We wanted to determine how to express and validate the effects and significance of weather conditions on the growth rate of the grass sward. Based on these ideas, we created the concept of Grassland Cam (GC).

Materials and methods

The experiment was conducted in Ruukki, Finland (64°40'N; 25°06'E) under the project 'Productive Grassland for Cattlefarms' on a private farmer's silage grass field during the growing season of 2019. The selected plot was estimated as being average for yield and growth potential and it was fertilised two times during the season with 100 and 80 kg N ha^{-1}. A Burrel Edge HD + 3G weatherproof camera was installed on the plot by attaching it to a solid wooden pole inserted into the soil. The camera was set to an appropriate height and the sward immediately in front of the camera was covered with a plastic sheet so that the growing grass would not occlude the lens. Stones were placed on the plastic sheet to keep it in place. A metric measurement was incorporated into the photographs by inserting a metric measurement stick into the sward. The camera was programmed to take photographs three times per day and to send photographs to an email address. Photographs were compiled into weekly videos that were published via the Facebook page of the project. When the sward was occluding the camera lens, it was removed by hand. The DM yield of the plot was determined for the first and second harvest by calculating the number and weight of round bales of harvested silage and analysing the silage for its DM content. In the third cut, the yield was determined with a sampling frame of 0.25 m^2 and analysing the collected fresh matter for weight and DM content. The weather data for the growing season was recorded at the near-by research station of Natural Resources Institute Finland, Ruukki.

The 2019 growing season started in Ruukki, Finland on 23 April and ended on 6 October. The monitoring with the GC was initiated on 9 May when the accumulated growing degree day sum (GDD) was 30 °C. Monitoring ended on 27 September when the grass had turned into over-wintering phase. The total GDD of the growing season was 1,123 °C and the total sum of precipitation 269 mm. In conclusion, the growing season of 2019 was dry and warm.

Results

The progression of the growth and development of silage grass sward was monitored throughout the growing season of 2019 by analysing photographs taken with the GC. In the Facebook postings, the growth rate was analysed on a weekly basis. As a result, we managed to record the sward height accumulation and the fluctuation of the grass growth rate during the growing season of 2019. The summarised results of the GC monitoring are presented in Figure 1.

In the first cut the grass yield was 3,500 kg DM ha^{-1}. The growth rate of the sward was rather constant apart from a cool period that lasted approximately for two weeks. During this time the growth rate decelerated after a one-week delay and also accelerated after the cool period with a similar delay (Figure 1). The most rapid phase of the growth occurred during the growth of the first cut with a weekly average growth rate of 190 kg DM ha^{-1} d^{-1} (Figure 1). In the regrowth, the growth rate alternated between different monitoring weeks and resulted in a yield of 3300 kg DM ha^{-1} in the second cut. The beginning of regrowth was rapid but regressed about two weeks after the first cut, with a new acceleration in the growth rate after this lag phase (Figure 1). Approximately one week before the second harvest the growth rate decreased for a short period but recovered right before the cut (Figure 1). The most rapid period regrowth occurred right before the second cut with a growth rate of 140 kg DM ha^{-1} d^{-1} (Figure 1). The

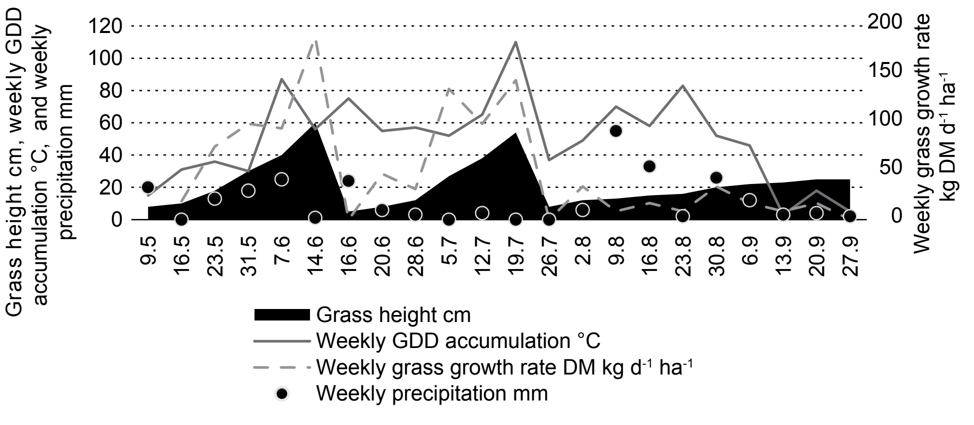

Figure 1. The summarization of the results from GC during the growing season of 2019. The cumulative height of the grass sward in a 3-cut regime (cm), a weekly accumulated GDD (°C), a weekly sum of precipitation (mm) and the average of weekly growth rate of the grass sward (kg DM d^{-1} ha^{-1}, based on the calculations of the dry matter yield of the plot) are shown.

yield in the third cut was only 1000 kg DM ha^{-1}. According to the visually collected data for changes in the sward height and the recorded data of the yield, it was estimated that the accumulation of dry matter was approximately 66-60 kg DM ha^{-1} cm^{-1}.

Discussion

After the installation the camera operated smoothly. During the most rapid phase of growth there were some problems with the quality of the photographs since the sward grew to occlude the lens of the camera. It was concluded that in the future it would be necessary to cover a slightly larger area with plastic film, both from the front and rear of the camera. Otherwise the experiences about the execution of monitoring were encouraging.

The height of the sward was estimated only visually, meaning that there is a possibility of an error in the height measurement. Fortunately, the estimation of the height of the sward was quite easy as the visual data from GC included a wide overview photograph of the whole sward. The ratio of dry matter accumulation per one centimetre of vertical growth was at the highest level during the first-cut growth and lowest during the third-cut growth. This is undoubtedly connected to the high proportion of stem-bearing shoots in the first-cut growth. This observation may be utilised in the future when developing measures to predict the accumulation of dry matter yield by the height growth rate of the sward.

The lag phase in the first cut was most likely due to the cool period and it was interesting to find out that the delayed response to the growth rate was about a week. In the second cut the first lag phase was probably due to the stresses of harvest and drought, since during this time there was almost no precipitation and the weather was warm. It is likely that this second lag phase was due to drought also. The weather was mainly warm during the second regrowth, with the exception of a cool and dry period over two weeks right after the second cut, which led to a poor initiation of the third-cut growth. At this time of the late summer, the day length was already significantly shorter and the growth of the timothy-dominated sward was not vigorous even though there was an increase in precipitation (Figure 1).

Conclusions

In conclusion, the collected data showed that a grass sward on an average field developed mainly in an expected manner. In the future, monitoring applications like GC may prove to be a quite simple and concrete method to collect scientifically valid datasets on the growth of grass swards and their reactions to environmental conditions. This method can also be used on farms to monitor the growth rhythm of grass on remote fields. In addition, this method may help the dissemination of research-based information to farmers and advisers.

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References

Pakarinen K., Hyrkäs M. and Juutinen E. (2012) Development and validation of practical methods for the determination of dry matter yield in grass silage swards. In: Stoddard F. and Mäkelä P. (eds.) *Abstracts of ESA12*. The 12th Congress of the European Society for Agronomy, University of Helsinki, Helsinki, Finland, pp. 542-543.

Virkajärvi P. (2003) Effects of defoliation height on regrowth of timothy and meadow fescue in the generative and vegetative phases of growth. *Agricultural and Food Science in Finland* 12, 3-4, 177-193.

Virkajärvi P., Pakarinen K., Hyrkäs M., Lemettinen J.-P., Rinne M., Manninen O. and Seppänen M. (2012) Crop physiology of grasses as a tool for higher quality of silage. *MTT Science* 56. 123 p.

Grazing management tool for Finnish cattle farms based on grass growth model

Tahvola E.[1], Ryhänen J.[1] and Mustonen A.[2]

[1] *A-Farmers Ltd, Itikanmäenkatu 3, 60100 Seinäjoki, Finland;* [2] *Natural Resources Institute Finland, Maaninka, Halolantie 31 A, 71750 Maaninka, Finland*

Abstract

It is estimated that over 80% of suckler-cow farms in Finland overgraze their pastures. This problem could be decreased by improved grazing management, including appropriate allocation of pasture herbage during the growing season. A-Farmers Ltd and Natural Resources Institute Finland created a grazing management (GM) tool in the project 'Productive Grassland for Cattlefarms'. The main aim of this tool is to encourage farmers into grazing by optimising the grazing rotations and the use of pasture resources. The GM tool describes the pasture rotation using variable parameters: herd size, grass growth rate, average herbage intake, total grazing area and the number and size of grazing plots. The tool gives an estimate of grazing time and how to divide the available grazing area into suitable number and size of grazing plots per each grazing rotation and for certain sized herds in terms of good grazing practices. The tool also predicts how much buffer feeding is needed. This tool has already been taken into practical use and has been developed even further.

Keywords: grazing, rotational grazing, grazing management

Introduction

Grazing is a challenging task under Finnish conditions as the growing season is short (ca. 120 d) and the growth rhythm of the pasture is uneven (e.g. Virkajärvi *et al.*, 2003). Due to these issues farmers tend to start the grazing season too late and with too large grazing plots. This can easily lead to overgrazing, which is a notable problem in suckler cow farms in Finland (Junnonaho, 2017). Failure in the initiation of the grazing season, the favouring of constant grazing and the use of oversized grazing plots lead to the risk of running out of productive pastures in the middle of the grazing season, even though the total grazing area seems to be sufficient. In overgrazing, the pasture is utilised constantly and buffer feed is brought to the pasture to feed the cattle. Constant grazing after the deceleration phase of the grass growth results in poor over-wintering, higher proportion of weeds in the grassland, and lower herbage yields during subsequent years. The compaction of the top soil and the buffer feed may add to the risk of nutrient leaching and to decreases in the carbon stock in the soil.

Overgrazing is problematic in those suckler cow farms which keep their cattle out through the whole growing season, noticing the weight loss of cows only at the end of the grazing season (Huuskonen, 2011; Junnonaho, 2017). At the same time, the weight gain of calves has already been impaired (Huuskonen, 2011; Junnonaho, 2017). Overgrazing does not usually occur on dairy farms because the result of it is quite rapidly seen as a decrease in the daily milk yield (Virkajärvi *et al.*, 2002, 2003). However, it is more typical for the total grazing area on dairy farms to be excessive. The acreage is too big at the beginning of the grazing season and the rotation is too slow. This, in turn, leads to rapidly senescing pasture swards (Virkajärvi *et al.* 2002 and 2003) and to the need of cleaning cuts. Nevertheless, if only dry cows and heifers are grazed on a dairy farm, the management of pastures may be insufficient: constant grazing is favoured for its low labour intensity, pastures are not fertilised adequately and weeds are not controlled sufficiently.

It is estimated that a significant proportion of grazing cattle farms in Finland have substantial losses in their pasture yields due to poor grazing practices. A-Farmers Ltd. and Natural Resources Institute Finland co-operated in the project 'Productive Grassland for Cattlefarms' and created a grazing management (GM) tool. The main aims of the tool are to improve the profitability of grazing, to encourage farmers into grazing, to help Finnish cattle farmers and advisers to utilise the entire grazing season with the right management practices and to disseminate facts on good grazing practices. The GM tool is addressed primarily to the needs of suckler cow farms, but it can also be utilised on other grazing cattle farms.

Materials and methods

The tool was created with Microsoft Excel. Calculation formulae were computed using results from Finnish studies on grassland pastures. The utilised results contained data on growth rhythm and crop physiology of grass species (Virkajärvi, 2003; Virkajärvi *et al.*, 2012) and good grazing management practices (Virkajärvi, 1999; Virkajärvi *et al.*, 2002). Some parts of the data on good grazing practices were not based on scientific studies but on practical experiences and observations. As the aim of the GM tool was to predict the progression of the pasture rotation under given terms, the input data consist of the number of grazing animals, rate of average daily herbage intake at pasture, total grazing area, density of the grass sward, tolerable proportion of left-over patches, initiation time of the grazing period, typical rate of grass growth during the planning period, the height of grass sward during the initiation of grazing, and the preferred stubble height of the sward after each grazing cycle. The initiation time and amount of buffer feeding were incorporated in the prediction.

The tool was tested during thematic education days during the winter season of 2019 in local discussion groups of cattle farmers. In total, nine discussion groups were formed by 59 individual farms. Each of these farms was given a farm-specific grazing plan for the 2019 growing season. Then, the GM tool was re-adjusted based on the observations that were discussed during these thematic days. At the testing phase of the tool we found out that it is difficult to estimate the intake of pasture herbage by cattle during the grazing period. To solve this problem, we have been developing an additional tool. Eventually, the GM tool was officially released in April 2019 and is openly available in Finnish at https://tinyurl.com/vpft55g.

Results

The tool gives a prediction for the pasture herbage yield and for the sufficiency of grazing area for the whole grazing season and each rotational cycle. The tool gives an estimation of the grazing time for each plot based on the predicted amount of available pasture herbage and the size of the herd. As the estimate for the sward regrowth of is based on the data for the preferred stubble height and timing of grazing, the GM tool gives feedback on the size of grazing plots based on the herd size and expected time of stay per plot, giving remarks if the grazing time would extend beyond the recommended time. With this prediction, the farmer can avoid overgrazing by modifying the calculation parameters, for example by allocating more grazing acreage, giving buffer feed when it is needed or by modifying the size or timing of grazing for each pasture plot. Furthermore, the tool can take notice of the availability of annual pastureland and it can be used as a field book for pasture bookkeeping.

The GM tool has been downloaded 36 times from the website. Most of the downloaders were farmers but advisers and workers in education and business organisations were included also. Farmers have warmly welcomed the tool. It has been demonstrated in an important national seminar by a farmer in one of the discussion groups. This farmer has also made improvements to the GM tool.

Discussion

When planning rotational grazing, it is essential to know the amount of available feed, herd size and feed intake rate during the grazing period. In a conventional grazing plan, the basic idea is to reserve a certain acreage of pasture for each animal for the whole grazing season. This model is poorly suited to Finnish conditions, as the rate of grass growth is highly variable, e.g. 50-240 kg DM ha^{-1} d^{-1} (Virkajärvi *et al.*, 2012) during the growing season. The conventional model does not include the optimal plot sizing of the total pasture acreage.

The prediction of the actual rate of pasture herbage intake for a suckler cow can be challenging, since the feed intake capacity of the cow differs from the actual energy need. The maximum feed intake can be 150% of the energy intake needed for basic maintenance (Natural Resources Institute Finland, 2017). The maximum intake rate is affected by the quality of the feed and the size, breed and production phase of the cow. The GM tool solves those planning problems of rotational grazing that are connected to the variation in the rate of grass growth during the growing season. However, it contains some challenges, as the actual rate of intake at pasture by cattle is difficult to estimate. In order to make more accurate predictions with the tool, the farmer needs data of the weight and body condition score of cattle to estimate the average herbage intake, but these data are often unavailable. If the rotation of pastures is well implemented, it is possible to use maximum intake rates in the calculation because the quality of the pasture sward is always optimal, and cattle tend to eat more than they need. The additional tool that is being developed will calculate the difference between the maximum intake potential and the energy need of the herd. In the additional tool, the energy intake will be converted into kilograms of dry material of pasture, which, in turn, can be used to estimate pasture yields. It will help the user to outline the temporal variation in the consumption of the pasture grass and support the use of the original GM tool.

Conclusions

It is not yet clear if the suggestions given by the GM tool have changed grazing practices of farmers and if these changes are significant. Results will be seen at the end of the project 'Productive Grassland for Cattlefarms' in May 2021. It seems that advisers and farmers have found the GM tool on the website and are interested to improve their grazing practices.

References

Huuskonen A. (2011) Towards more efficient beef production II. *MTT Kasvu 14.* 183 p.

Junnonaho L. (2017) *Grazing strategies and their impact on production and economy of suckler cow farms* (in Finnish). BSc Thesis, Savonia University of Applied Sciences. 61 p.

Natural Resources Institute Finland (2017) Protein requirements of suckler cows. *Feed tables and feeding requirements 2017.* Available at: www.luke.fi/feedtables

Virkajärvi P. (2003) Effects of defoliation height on regrowth of timothy and meadow fescue in the generative and vegetative phases of growth. *Agricultural and Food Science in Finland* 12, 3-4, 177-193.

Virkajärvi P. (1999) Comparison of three indirect methods for prediction of herbage mass on timothy-meadow fescue pastures. *Acta Agriculturae Scandinavica, Section B Soil & Plant Science*, 49, 2, 75-81.

Virkajärvi, P., Sairanen, A., Nousiainen, J.I. and H. Khalili, H. (2002) Effect of herbage allowance on pasture utilization, regrowth and milk yield of dairy cows in early, mid and late season. *Animal Feed Science and Technology*, 97, 1-2, 23-40.

Virkajärvi, P., Sairanen, A., Nousiainen, J.I. and Khalili, H. (2003) Sward and milk production response to early turnout of dairy cows to pasture in Finland. *Agricultural and Food Science in Finland* 12, 1, 21-34.

Virkajärvi P., Pakarinen K., Hyrkäs M., Lemettinen J.-P., Rinne M., Manninen O. and Seppänen M. (2012) Crop physiology of grasses as a tool for higher quality of silage. *MTT Science* 56. 123 p.

Soil signals of grassland: a practical guide with a checklist for soil quality assessment

Van Eekeren N.[1] and Philipsen B.[2]
[1]*Louis Bolk Institute, Kosterijland 3-5, 3981 AJ Bunnik, the Netherlands;* [2]*Wageningen Livestock Research, De Elst 1, 6708 WD Wageningen, the Netherlands*

Abstract

Soil quality is an important factor for the stability of grassland production, and the profitability of farming. Moreover, the soil delivers ecosystem services such as carbon storage, water regulation and maintenance of biodiversity. For dairy farmers soil quality often remains a black box and difficult to assess. Knowledge transfer around this theme is not easy since it is not always the primary interest of dairy farmers. A practical guide has been developed with many photos and illustrations, which follows in consecutive chapters the six pillars of soil quality. The guide and the checklist are discussed.

Keywords: soil quality, soil organic matter, roots, soil structure and soil biota

Introduction

For dairy farmers soil quality often remains a black box. Knowledge transfer around this theme is not easy since it is not always the primary interest of dairy farmers and no simple tools are available for an integral assessment of soil quality. A practical guide has been developed for dairy farmers, advisers and industry. Like the guides 'Soil Signals' (Koopman *et al.*, 2015) and 'Grassland Signals' (Klein-Swormink *et al.*, 2010), it contains many photos and illustrations, which follows in consecutive chapters the six pillars of soil quality; soil organic matter, soil chemical, water characteristics, roots, soil structure and soil biota (Figure 1) (Van Eekeren *et al.*, 2019; www.roodbont.nl)). These elements are linked and need an integral assessment. The guide and checklist for assessment of soil quality are discussed.

Guide

In each chapter of the guide the backgrounds of the pillars of soil quality are discussed followed by visual assessment (above- and belowground), what it means and what you can do. Soil Signals of Grasslands provides tools for optimising soil management. It contains plenty of practical advice, management measures and techniques that are directly applicable for farmers. This also means more return in product

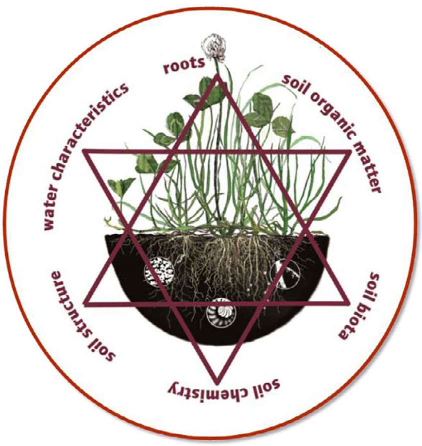

Figure 1. Six pillars of soil quality.

and money per hectare of grassland; for example on sandy soils a 1% soil organic matter increase in the top 0-10 cm soil layer means an increase in production of 1,320 kg DM ha^{-1} on unfertilised grasslands (Van Eekeren *et al.*, 2010). Calculating with an estimated value for grass in the field on basis of energy and protein of € 0.11 kg DM this means +€ 145 ha^{-1} yr^{-1}.

Checklist

The guide contains a checklist for soil assessment which takes into account the mineral analysis of the spring silage, the soil analysis and a visual soil assessment in the field (Table 1).

Table 1. Checklist of soil quality.

	Indicator	Unit	Target
Step 1. Production and efficiency	DM-production	kg DM ha^{-1}	>12.000
	soil N-efficiency	%	>70
	soil P-efficiency	%	>100
Step 2. Mineral analysis spring silage	K-content	g kg ds^{-1}	25-35
	P-content	g kg ds^{-1}	>3.5
	S-content	g kg ds^{-1}	2.2-4.0
	Fe-content	mg kg ds^{-1}	<1000
Step 3. Soil analysis (0-10 cm)	Soil organic matter	sand %	>5
		clay %	>10
	pH-KCl	sand	4.8-5.5
		clay	4.8-5.5
		peat	4.6-5.2
	P-$CaCl_2$	mg P kg^{-1} dry soil	>1.5
	P-Al	mg $P_2 0_5$ 100 g^{-1} dry soil	>25
	K-$CaCl_2$	mg P kg^{-1} dry soil	
	S-soil delivery	kg S ha^{-1}	>20
Step 4. Visual soil assessment[1]	**poor**	**moderate**	**good**
Grass cover	<80%	80-90%	>90%
Signs of ponding or flooding	a lot of	limited	none
Signs of tracks or trampling	a lot of	limited	none
Maximum rooting depth (cm)			
Sand	<20 cm	20-40 cm	>40 cm
Clay	<30 cm	30-70 cm	>70 cm
Peat	<20 cm	20-40 cm	>40 cm
Root intensity at 20 cm (number 10×10 cm)			
Sand	<10	10-40	>40
Clay	<40	40-80	>80
Peat	<10	10-50	>50
Soil resistance	a lot of	limited	none
Soil structure in 10-20 cm layer	75-100% angular blocky elements	>25% crumbs	>50% crumbs
Earthworms			
Sand	<10	10-40	>40
Clay	<40	40-80	>80
Peat	<10	10-50	>50

[1] Inspired by Peerlkamp (1959), Shepherd (2000).

References

Klein-Swormink B., Philipsen B. and van Eekeren N. (2010) *Grassland Signals: Practical guide for getting the most out of grassland*. Roodbond, Zutphen, the Netherlands, 96 p.

Koopmans C.J., Bokhorst J.G., ter Berg C. and van Eekeren N. (2015) *Soil Signals: A practical guide to a fertile soil*. Roodbond, Zutphen, the Netherlands, 96 p.

Peerlkamp P.K. (1959) *A visual method of soil structure evaluation*. Instituut voor Bodemvruchtbaarheid, Groningen, the Netherlands.

Shepherd T.G. (2000) *Visual soil assessment. Field guide for pastoral grazing and cropping on flat to rolling country Vol. 1* Horizons Regional Council & Landcare Research, Palmerston North, New Zealand.

Van Eekeren N., Philipsen B., Bokhorst J.G. and ter Berg C. (2019) *Bodemsignalen grasland* (Dutch edition): Praktijkgids voor optimaal bodemmanagement op melkveebedrijven. Roodbont, Zutphen, the Netherlands, 120 p.

Van Eekeren N., De Boer H.C., Hanegraaf M., Bokhorst J., van Nierop D., Bloem J., De Goede R.G. and Brussaard L. (2010) Ecosystem services in grassland associated with biotic and abiotic soil parameters. *Soil Biology and Biochemistry*, *42*(9), 1491-1504.

What is a good perennial sown pasture? – analysis of farmers' perceptions in a western France network

Vertès F.[1], Tanguy N.[1,2], Falaise D.[3], Woiltock A.[4], Pierre P.[5] and Couvreur S.[2]
[1]INRAE, Agrocampus Ouest, UMR SAS, 29000 Quimper, France; [2]ESA Angers, 49000 Angers, France; [3]RAD F-35 Cesson-Sévigné, France; [4]INRAE, Agrocampus Ouest, UMR Pegase, 35590 Saint-Gilles, France; [5]IDELE, 49105 Angers, France

Abstract

Increasing protein self-sufficiency by increasing the use and duration of grassland on cattle farms is an efficient agro-ecological lever to improve agriculture sustainability. In the SOSProtein-4AgeProd-PERPeT programme, which aimed to study conditions for good persistency of sown grasslands, we created an observatory of 83 young grasslands (35 farms) which biophysical and management characteristics were observed during 4 years. To understand the subject beyond its technical and economic aspects and improve future use of results, we aimed to examine farmers' perceptions about grasslands, as this can either be a driving force or a barrier to move toward more grass-based systems thanks to long term grasslands. Semi-structured interviews were carried out with 22 farmers representing the diverse range of participants. They mentioned 5 major themes relating to economy, zootechnics, agronomy environment and working conditions. Despite a relatively homogenous population we could highlight 4 different farmers' perceptions around functions and persistency of sown grasslands in their farming system. Combined with technical analysis these new elements on farmer's expectations will help to support farmers to improve persistency of their grassland.

Keywords: grassland persistency, enquiries, farmer's perceptions

Introduction

Increasing protein self-sufficiency by increasing the use and duration of grassland on cattle farms is an efficient agro-ecological lever for enabling reduction of soybean imports and improving the sustainability of production systems (Pfimlin *et al.*, 2019). The SOSProtein-4AgeProd-PERPeT is a transdisciplinary programme involving researchers, advisers and farmers (from a 'Sustainable Agriculture Network'), that aims to study the conditions for good persistency of sown grasslands and ways to improve it. Though this is an old topic, there is only limited literature available to give an objective account of the evolution of sown grasslands and how to extend grassland sward productivity. We created a dynamic observatory of 83 young grasslands for which the biophysical (flora, production, rooting and soil structure) and management characteristics were studied for 4 years to understand the main drivers of yields and flora evolution.

The mind set and perception of grassland by farmers can either be a driving force or a barrier to the decision making process, leading to an increase or decrease of grass in the ruminant diet and grasslands in the fodder area (Petit *et al.*, 2019). We aimed to investigate the variability of their perceptions on 'good persistent grassland', why and how they want to maintain (or not) long-term grasslands in their farming system. We assumed that decision making process could involve several issues (zootechnics, agronomy, economy, environment and working conditions) and be linked to their farm characteristics, career paths, and characteristics of their grassland plots.

Materials and methods

Semi-structured interviews were carried out with 22 farmers, chosen to be representative of the 35 farms, located in the 4 main climate contexts of Brittany and Pays de Loire regions. They included questions about farm structure, grasslands, fodder systems and personal trajectory. Their responses were fully transcribed for analysis, focusing on how farmers spoke on grassland qualities and benefits expected. Discourses were then analysed according to the 5 issues mentioned above (Tanguy, 2019). Classifications of farmers' perceptions of grasslands were realized according to the graphical method developed by Bertin (2011). We then identified if perceptions were related to grassland persistency and other structural factors.

Results and discussion

Farmers did not talk about grassland persistency all in the same way: 'more than 5 years' is convenient for some farmers while others expect never to plough and reseed their grassland plots. Nevertheless, they all considered that long term grasslands provide economic and labour benefits compared with leys sown every 3-5 years. The farmers perceive that good long-term grasslands contain high biodiversity (flora, insects, soil organisms) and allow more carbon storage than short term grasslands. They expect that root systems develop with time, increasing the resilience to climatic hazards and grazing animal pressure. Some mention another service related to floristic biodiversity, its contribution to better animal health with a larger variety of nutrients and micro-nutrients. The richness of the language and the precision of the topics mentioned by farmers was a remarkable result.

Farmer's indicators to assess grassland quality were: (1) flora (91%), i.e. species diversity; the % good fodder species and legumes ('good value' varying with farmers as well as the way to assess it); (2) animal behaviour (a good grassland is 'invisible', animals are well, eat well) and grassland production (86%) with annual grass production of 5-8 Mg ha^{-1} dry matter according to farmers; (3) ability to maintain/regenerate by itself after summer drought or a very wet winter (27%) and (4) good biological activity (18%).

We could highlight 4 different farmer's perceptions around the function and persistency of sown grasslands summarized in Figure 1. Self-sufficiency, low production costs and 'cow designed to graze' are the most shared drivers. Environmental issues, animal welfare and health, decision-making autonomy and pleasure at work were highlighted differently according groups. No clear farm physical factors (farm size, climatic area, stocking rates per worker, career path, age) explained the different types though it

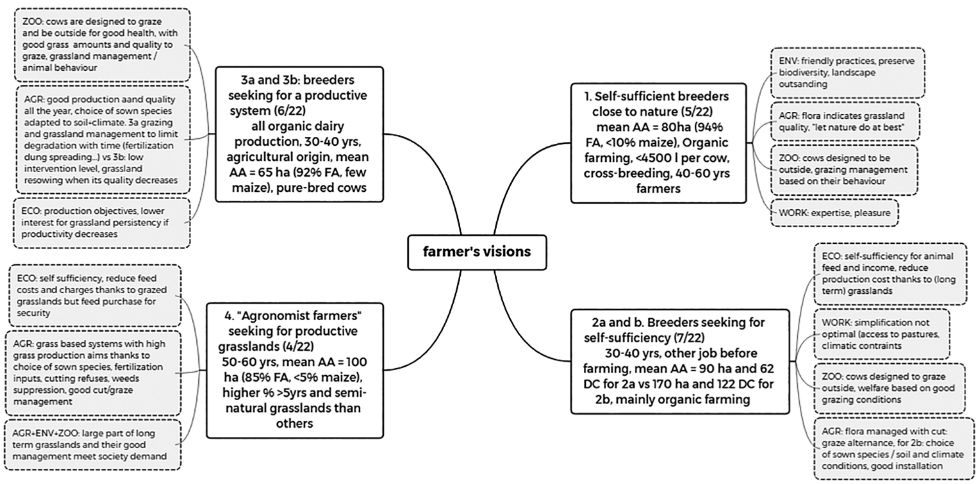

Figure 1. The main components of the farmers' perceptions on 'good persistent grasslands' (AA = agricultural area, FA = fodder area, DC = dairy cows, ZOO = zootechnics, AGR = Agronomy, ECO = economy, ENV = environment).

contributed part of the farmer's perceptions. Some plot characteristics vary between groups (e.g. yields, mean age of plots, flora and yield stability, quality of grazing management, soil depth, soil compaction, etc.) as cause or consequences of farmers' perceptions and practices (on-going study).

Conclusions

Despite a relatively homogenous population (Civam-GAB networks, this study highlighted 4 main type of farmers' perceptions on 'good sown grassland persistency'. Combined with the full technical analysis it will give keys to support farmers to improve their grassland persistency in different bio-physical contexts and provide various adapted supports.

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References

Bertin J. (2011) *Graphics and graphic information processing.* Walter de Gruyter. 289 pp, ISBN 9783110854688.

Petit T., Martel G., Vertès F. and Couvreur S. (2019) Long-term maintenance of grasslands on dairy farms i associated with redesign and hybridisation of practices, motivated by farmers' perceptions. *Agricultural Systems*, 173, 435-448.

Pfimlin A., Rieu M., Magdelaine P., Meynard J.-M., Bazin G. and Allo C. (2019) Available at: https://www.academie-agriculture.fr/publications/publications-academie/points-de-vue/lelevage-europeen-peut-il-se-passer-du-soja.

Tanguy N. (2019) *L'analyse des conceptions de la prairie longue durée chez les éleveurs herbagers du grand Ouest: quelles prairies pour quells objecifs?* Mémoire fin d'étude d'ingénieur, ESA Angers, 67 p.

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