# N-Alkanes: A technique to measure herbage intake in dairy cows

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# Background

- Herbage intake influences animal performance at grass
- Sward cutting used to estimate herd intake
  - Poor estimation of individual animal intake
- n-alkane method developed and modified
  - Mayes et al., 1986
  - Dillon and Stakelum, 1989

### Introduction

- What are n-alkanes?
  - $\square$  Long-chain ( $C_{25}$  to  $C_{35}$ ) hydrocarbons
  - □ Present in cuticular wax of plants
  - □ In grass odd-numbered chain lengths (C29, C31 and C33) predominate over even-numbered chain lengths
  - □ Used as fecal markers to estimate herbage intake
  - □ Incomplete recovery of alkanes in feces
  - □ Adjacent chain lengths have similar recoveries

# Concentration of n-alkanes in cuticular wax of some temperate pasture species

	C <sub>27</sub>	C <sub>28</sub>	C <sub>29</sub>	C <sub>30</sub>	C <sub>31</sub>	C <sub>32</sub>	C <sub>33</sub>	C <sub>35</sub>
L. Perenne	19	5	73	9	137	9	116	18
L. Multiflorum	105	8	260	11	250	4	43	0
D. Glomerata	20	2	38	2	58	2	21	0
T. Repens	38	7	109	5	67	1	7	0

(Source: Dove and Mayes, 1996)

- This is a review of a number of studies
- Odd-number alkanes predominate
- •Species differences in pattern of alkane concentrations

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#### Method Outline

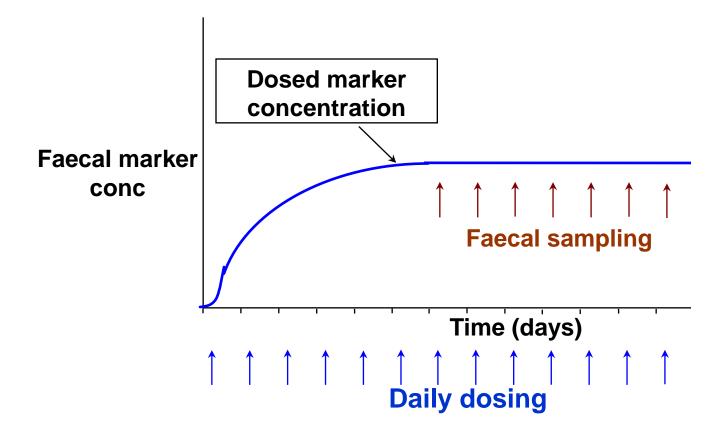
- Method of Mayes et al., (1986), modified by Dillon and Stakelum (1989)
  - Animal dosed with synthetic even-numbered alkane
  - Offered herbage which has been sampled and contains naturally occurring oddnumbered alkane
  - Herbage intake is calculated from the alkane dose, alkane content in the herbage and the ratio of the dosed and natural alkanes in the feces

### Procedure

- Cow dosed twice daily (am and pm)
- For 12 consecutive days
- With paper pellet containing 500 mg of dotriacontane (C<sub>32</sub>-alkane)
- Faecal samples collected from d 6 to d 12
  - In both morning and evening
  - □ In field collect sample when voided
  - □ In holding yard rectal grab samples
  - □ Stored at -20 C
- Faecal sample preparation
  - Thawed
  - □ Bulked by cow (10g/cow per d)
  - □ Dried at 40 C for 48 hrs
  - □ Milled through a 1-mm screen
  - □ Analysed for C<sub>32</sub> and C<sub>33</sub>

# Measuring faecal output

- Dose with a known amount of alkane not in the feed (C<sub>32</sub>)
- Ensure stable excretion in the feces



#### Procedure

- Herbage representative of what cows graze
  - □ sampled from each paddock on days 5 to 11
  - □ sample at similar time each day
  - □ Two samples of 25 individual snips are taken from the grazing area (depending on paddock size)
  - □ Stored at -20 C
- Sample preparation
  - □ Bowl-chopped
  - □ Freeze-dried
  - Milled and analysed for C<sub>33</sub>

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#### Intake calculation

 N-alkane analysis to determine the ratio of C<sub>33</sub> (tritriacontane) concentration in herbage and faeces

Grass DMI (kg) = 
$$\frac{(F_i)(F_j) \times (D_j)}{(H_i - (F_i)(F_j) \times (H_j))}$$

F<sub>i</sub> and H<sub>i</sub> are concentration of natural odd-chain n-alkane in faeces and pasture (mg kg<sup>-1</sup> of DM)

F<sub>j</sub> and H<sub>j</sub> concentration of even-chain n-alkane in faeces and pasture (mg kg<sup>-1</sup> of DM)

D<sub>i</sub> dose rate of even-chain n-alkane (mg d<sup>-1</sup>)

# Comparison to other methods

■ Herbage removed R<sup>2</sup>= 0.85

(McEvoy et al., 2007)

Better than herbage removed or energy calculations

(Smit et al., 2005)

- Time consuming & delay for results
- Cost

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# Benefits of n-alkane technique

- Provides estimates of individual animal intake
- Method can accommodate feeding of supplements
- Can estimate diet composition
  - □ Plant species
  - □ Plant cultivars
  - □ Plant parts
  - □ Plant communities

(Dove and Mayes, 1996)

Single analytical process

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#### Sources of error

- Diurnal pattern of n-alkane excretion
  - □ Dosing twice daily to reduce variation in feces
  - 6-day dosing pattern stabilises excretion
- Herbage sampling
  - □ Animal selection
  - □ Plant species
- Animal consumption of synthetic alkane
  - □ Dose in paper pellet
  - □ Offered in concentrate
- Sample preparation
  - □ Drying method unlikely to affect n-alkane in herbage (Dove and Mayes 1991)
  - Drying method may affect n-alkane in feces



#### Conclusions

- Minimise sources of error throughout procedure
- Ensure accurate sampling of feces and herbage
- N-alkane technique provides an accurate method to estimate individual animal intakes at grass