Comparison of Techniques for Estimating Herbage Intake of Grazing Dairy Cows

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ABSTRACT

For estimating herbage intake during grazing, the traditional sward cutting technique was compared in grazing experiments in 2002 and 2003 with the recently developed n-alkanes technique and with the net energy method. The first method estimates herbage intake by the difference between the herbage mass before and after grazing and the regrowth between the 2 points in time. The second technique estimates herbage intake by the ratio of a dosed even-chain synthetic n-alkane (C_{32}) and a naturally occurring odd-chain n-alkane (C_{31} or C_{33}) in the herbage and feces. The third technique calculated the intake from the animal's energy requirements for milk production and maintenance. The sward cutting technique estimated herbage intake with the highest coefficient of variation and had different results in the 2 experimental years. The n-alkanes method yielded less variable results, whereas the net energy method gave the least variable results. In 2002, the estimates of the alkane ratio $C_{32}:C_{33}$ were best related with estimations of the net energy method. In 2003, the estimates of the alkane ratio $C_{32}:C_{31}$ were best related. The estimate based on the alkane ratio $C_{32}:C_{33}$ had a lower coefficient of variation than the one based on the alkane ratio $C_{32}:C_{31}$. Therefore, the $C_{32}:C_{33}$ alkane method was considered to be a better direct estimator for herbage intake by grazing lactating dairy cows.

(Key words: dairy cow, herbage intake, sward cutting, n-alkanes)

Abbreviation key: FPCM = fat- and protein-corrected milk, LINGRA = light interception and utilization simulator for grasslands.

INTRODUCTION

Limited herbage intake is considered one of the main constraints for ruminant production (milk, meat, and wool) (Forbes, 1995). The measurement of DMI during grazing is, however, still not very accurate. The classical method to determine intake is the so-called sward cutting method. A measured proportion of the area allotted to the animals is harvested, and the total herbage offered to the animal can be calculated. The residual herbage after grazing is determined in a similar manner. The difference between these 2 herbage masses and a correction for the regrowth provides an estimate of the herbage consumed in the area grazed (Meijs, 1981; Macoon et al., 2003). The sward cutting method can provide reliable estimates of intake when short grazing periods are applied and when a large part of the offered herbage is consumed (Walters and Evans, 1979; Meijs, 1981). However, this method has large variation (Meijs et al., 1982; Reeves et al., 1996) and is mainly used to determine herbage intake for groups of animals.

In the late 1980s and early 1990s, a new method for herbage intake was developed, the n-alkanes method (Mayes et al., 1986; Dove and Mayes, 1991; Dillon, 1993). The n-alkanes are long-chain (C_{25} to C_{35}) hydrocarbons present in the cuticular wax of plants. In grassland species, the odd-numbered chain length alkanes (especially C_{29} , C_{31} , and C_{33}) are present in much greater amounts than the even-numbered chain length (Tulloch, 1976; Dove and Mayes, 1991). Herbage intake could be estimated by using the n-alkanes as fecal markers. Animals are dosed with a synthetic even-numbered alkane and consume herbage with a certain content of naturally occurring odd-numbered alkane. Herbage intake can be calculated from the alkane dose, the alkane content in the herbage, and the ratio of the dosed and natural alkanes in the feces. Although the fecal recovery of alkanes might not be complete, alkanes of adjacent chain length (e.g., C_{32} and C_{33}) have similar recoveries (Mayes et al., 1986; Stakelum and Dillon,

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Figure 1. Experimental set-up of the grazing trial.

1990), and it was shown that herbage intake of dairy cows could be estimated accurately (Dillon, 1993; Lipke, 2002).

The aims of this paper were to measure DMI of grazing dairy cows using the 2 methods and compare their estimates. Furthermore, the estimates of the 2 methods were compared with a calculated DMI based on the energy requirements for lactation and maintenance ($NE_{L, \text{required}}$) of the cows and the net energy content (NE_L) of the herbage.

MATERIALS AND METHODS

Experimental Set-Up

During the summers of 2002 and 2003, similar grazing experiments were conducted. Twelve dairy cows were used in each experiment. Two paddocks were sown with 4 perennial ryegrass cultivars in a randomized block design with 3 replicates. Each paddock consisted of 12 strips that were 22 m wide and 84 m long. The strips were divided into 14 plots that were 22×6 m each (Figure 1). The experiment was designed as a strip-grazing system, and each cow was allowed to graze individually a plot during 24 h. A mobile fencing system

was used, and each cow was moved daily to a new plot at 1200 h. In total, each experiment consisted of 4 periods of 14 d.

Animals

Twelve multiparous Holstein Friesian dairy cows were used. In 2002, cows were 67 ± 4.2 DIM, and in 2003, cows were 114 ± 3.7 DIM. The BW was recorded every week. Pre-experimental BW was 528 ± 2.0 and 549 ± 4.2 kg in 2002 and 2003, respectively. Animals were milked twice a day at 0600 and 1600 h. Milk yield was recorded after every milking. Daily samples of milk were analyzed for fat and protein content. Milk production is expressed as fat- and protein-corrected milk (FPCM). The Institutional Animal Care and Use Committee of Wageningen University approved the experiment.

Sward Cutting Method

Herbage allowance. On d 10, 11, 12, and 13 of each experimental period, fresh herbage yield, DM percentage, and DM yield were measured before the cows were allowed grazing (pregrazing). Fresh herbage yield was measured by cutting at least 5% of the total area with a mowing machine (Agria 3200; Agria-Werke, Möckmühl; cutter bar, 1.25 m) at a stubble height of 4 cm. In 2002, in periods 1 and 2, in total 7 m^2 was cut in one strip, and in periods 3 and 4, in total 14 m^2 was cut in 2 strips of 7 m^2 each. In 2003, in all periods, in total 7 m^2 was cut in 2 strips of 3.5 m^2 each. The cut herbage was collected, weighed, and sampled for DM determination. Duplicate core samples of approximately 200 g of fresh material were taken and dried at 70°C for 24 h.

Herbage residual. On d 11, 12, 13, and 14 of each period, the residual herbage (postgrazing) was measured as described for the herbage allowance, but now twice the amount of strips was cut (10% of the area) (Green, 1949; Meijis, 1981). Herbage samples were collected and processed as described for the herbage allowance.

Herbage accumulation. Herbage accumulation was calculated using the light interception and use simulator for grasslands (LINGRA) (Schapendonk et al., 1998), which calculates daily regrowth of perennial ryegrass swards using the meteorological data [daily photosynthetic radiation ($\text{MJ}/\text{m}^2/\text{d}$) and temperature ($^\circ\text{C}$)] of the Haarweg meteorological station, located 500 m from the experimental fields. The LINGRA successfully predicted growth and development of perennial ryegrass in a vegetative stage at the level of potential and water-limited production (Barrett et al., 2004). Herbage

accumulation was incorporated in the herbage intake calculation using the Linehan equation (Linehan et al., 1952; Meijjs, 1981).

Dry matter intake (kg DM/d) was calculated as follows:

$$DMI = (allowance - residual) \times \frac{\log(allowance + \Delta regrowth) - \log(residual)}{\log(allowance) - \log(residual)} \quad [1]$$

Dry matter intake was measured during 4 d; the mean of these 4 d was calculated and considered representative for the whole period. As 12 cows were measured during 4 periods, there were potentially 48 recordings in each year. However, in the third period of 2002, one cow was removed from the data set because of health problems; so, in total, 47 recordings were made. In 2003, one cow was removed from the complete experiment because of health problems, as was another cow in the last period; so, 43 recordings remained.

n-Alkanes Method

Field procedure. Each cow received twice daily during milking 1.5 kg of concentrates containing an added C₃₂ alkane. To prepare the concentrates, the even-chain n-alkane C₃₂ (Fernz Health & Science Ltd., Auckland, New Zealand) was dissolved over cellulose powder (arbolcel) (1:10 ratio) using a Rotavapor (Büchi, Flawil, Switzerland) (90°C); then, the mix was cooled, sieved, and added to the concentrates in the required quantity (0.3 g/kg) before pelleting in the feed mill. Each cow was daily dosed with 839.3 mg and 709.9 mg C₃₂-alkane in 2002 and 2003, respectively. The alkane dosing started 1 wk prior to the experiment and continued throughout the experiment to obtain a stable excretion pattern. The concentrates were fed in small portions to prevent spilling by the cows; care was taken to ensure each cow consumed everything.

Feces were sampled from each cow individually by sampling each dung patch present in each plot with a spoon. Each day, 12 feces samples were gathered and stored in the freezer. Feces samples were freeze-dried and milled to pass a 1-mm screen.

Grass samples (100 g of fresh material) were taken daily on d 8 to 14 of each experimental period at 3 time points (1400, 2000, and 0700 h), which corresponded to the main 3 grazing bouts of the animals (Taweel et al., 2004). Every sample was taken by walking with the cows for 5 min and taking hand-plucked samples in every spot where the cow was grazing. Grass was oven-dried at 60°C for 48 h and milled to pass a 1-mm screen.

The daily samples of grass and feces were pooled on equal dry weight basis for each cow in each period.

Concentrates were pooled for each period. In total, 48 grass samples, 48 feces samples, and 4 concentrate samples were collected each year and were analyzed for n-alkanes. Because of the health problems mentioned earlier, some cows were removed from the data set. In 2002, one cow showed reluctance to consume the alkane-marked concentrates; therefore, these data were also removed from the data set.

Analysis. Samples were analyzed according to Mayes et al. (1986). Samples of grass (1 g), concentrates (0.25 g), and feces (0.5 g) were weighed, in duplicate, into a screw-cap vial (25 mL; Schott, Mainz, Germany). Vials were checked on leakage with chloroform before usage. Internal standard (250 µL) was added to the sample using a glass syringe with an adapter. Overnight, these samples were saponified with 10 mL of alcoholic KOH solution (1.5 M) in an oil bath at 90°C. The alcoholic KOH solution was prepared daily to prevent discoloration (8.42 g/100 mL of ethanol). After cooling, 8 mL of heptane and 5 mL of demineralized water were added, and the tubes were shaken vigorously. The samples were placed in a water bath at 45°C for 5 min and centrifuged. The nonaqueous top liquid layer was removed with a Pasteur pipette. Three further extractions of these samples were carried out by the addition of 5 mL heptane. The samples were evaporated to dryness, redissolved in 2 mL of heptane, and applied to the top of a small column containing silica gel (3 cm) and glass fiber (0.5 cm). This eluate was again evaporated to dryness, and 250 µL of heptane was added; 1 µL was injected in a gas chromatograph with N₂ as carrier gas at a flow of 30 mL/min. Peak areas of n-alkanes were determined using Chrom Card Data System 2.2 (Thermo Finnigan, Waltham, MA).

The DMI was calculated with the following equation:

$$DMI = \frac{C_j}{\frac{F_i}{F_j} \times H_i - H_j} \quad [2]$$

where F_i and F_j are fecal concentrations of odd-numbered alkanes and the even-numbered alkanes (mg/kg of DM), respectively; H_i and H_j are the concentrations of the odd- and even-numbered alkanes in the grass (mg/kg of DM); and C_j is intake of dosed alkane in the concentrate (mg/d). In this equation, it is assumed that even- and odd-numbered alkanes with nearly similar chain lengths have a similar fecal recovery, independent of their source (natural occurrence in grass and concentrate or dosed). In this experiment, C₃₂ alkane was used to dose the animals, and this was added to a concentrate with no natural occurring alkanes. Both combinations of C₃₂:C₃₁ and C₃₂:C₃₃ alkanes were examined as potential estimators for DMI.

Net Energy Method

The DMI could also be calculated from the NE_L requirements of the cows and the net energy content of the grass. Daily NE_L requirements for milk production and maintenance were calculated according to the standard energy system used in The Netherlands (van Es, 1978; CVB, 1999a), using the following equation:

$$NE_{L,required} = 6.9 \times [(42.4 \times BW^{0.75} + 442 \times FPCM) \times (1 + (FPCM - 15) \times 0.00165)] \quad [3]$$

where BW = average BW of the cow (kg) during the measurement period, and FPCM was measured in kg/d. Because the animals were grazing, an extra allowance of 20% of their maintenance requirements was assumed (van Es, 1978; CVB, 1999a). The energy value of the grass was calculated from the chemical composition of the grass, which was determined by NIRS in the samples taken for the alkane analysis and assumed to be representative of the quality of grass ingested by the cows. Near infrared reflectance spectroscopy calibration equations were developed with >1000 fresh grass and hay samples by Center de Recherches Agronomiques (Libramont, Belgium) (Biston et al., 1998). Samples were analyzed for ash, CP, crude fiber, and water-soluble carbohydrates. Crude fat was assumed to be 40 g/kg of DM (CVB, 1999b). Nitrogen-free extract was calculated by subtracting ash, CP, crude fiber, and crude fat from 1000 g DM.

Digestible CP and digestible OM expressed as g/kg DM were calculated using the following equations (van Es, 1978; CVB, 1999a), where CF = crude fiber:

$$Digestible\ CP = (0.959 \times CP + 0.04 \times ASH - 40) [4] \\ - 0.1 \times (days\ after\ 1st\ April - 105)$$

$$Digestible\ OM = (1029 - 0.77 \times CF - 1.12 \times ASH - 0.3 \times days\ after\ 1st\ April) \quad [5]$$

The gross energy (GE), metabolizable energy (ME), and NE_L per kilogram DM were calculated using equations 6, 7, and 8. The DMI was calculated using equation 9:

$$GE = 24.14 \times CP + 36.57 \times CFAT + 20.92 \times CF + 16.99 \times NFE - 0.63 \times WSC \quad [6]$$

where CFAT = crude fat and WSC = water-soluble carbohydrates.

Table 1. Herbage allowance, residual, accumulation, and intake (kg DM/d per animal) and standard error in 2002 and 2003.

Item	2002	2003
Allowance	36.4 ± 1.5	32.5 ± 1.0
Residual	21.8 ± 1.4	14.5 ± 1.0
Accumulation	2.2 ± 0.07	0.9 ± 0.06
Intake	16.2 ± 0.3	18.6 ± 0.4

$$ME = 14.2 \times Digestible\ OM + 5.9 \times Digestible\ CP \quad [7]$$

$$NE_L = 0.6 \times \left(1 + 0.004 \times \left(\frac{ME \times 100}{GE} - 57 \right) \right) \times 0.9752 \times ME \quad [8]$$

$$DMI = \frac{NE_{L,required} - NE_{L,concentrate}}{NE_{L,herbage}} \quad [9]$$

Statistical Analyses

The DMI estimates of all methods were compared using a paired *t*-test. Each year was analyzed separately. Correlations were calculated using the 2-tailed Pearson correlation coefficient.

RESULTS

Sward Cutting Method

Table 1 shows the herbage allowance, residual, and intake. In 2002, the herbage allowance was higher ($P < 0.05$) than in 2003. Mean herbage allowance was, on average, almost 35 kg/d DM per animal. The herbage residual in 2002 was much higher ($P < 0.001$) than in 2003. The herbage accumulation in 2002 was 2.2 kg and was higher ($P < 0.001$) than in 2003, because of the hot and dry weather conditions in the latter year. The herbage intake in 2002 was lower ($P < 0.001$) than in 2003. Contrary to what was expected because of the ongoing lactation period, no decrease in DMI with time was observed.

Standard errors in 2002 for herbage allowance, residual, and accumulation were higher than in 2003. The standard error for herbage intake, however, was slightly lower in 2002.

n-Alkanes Method

The results of the n-alkanes method are presented in Table 2. The level of natural occurring n-alkanes in the concentrates was below detection limits. The levels of odd-numbered alkanes (C_{31} and C_{33}) in the herbage

Table 2. Concentration of n-alkanes (mg/kg of DM) and standard errors in herbage and feces in 2002 and 2003.

n-Alkane	Herbage		Feces	
	2002	2003	2002	2003
C ₃₁	144.3 ± 2.6	230.7 ± 5.7	448.2 ± 10.1	695.9 ± 14.8
C ₃₂	12.8 ± 0.6	12.4 ± 0.3	185.0 ± 3.2	158.3 ± 2.9
C ₃₃	134.2 ± 2.4	164.9 ± 3.3	402.4 ± 10.6	487.2 ± 9.5

were considerably higher than those of the even-numbered alkane (C₃₂), which was 12 mg/kg of DM in both years. In contrast, the levels of C₃₃ and especially C₃₁ were much lower ($P < 0.001$) in 2002 than in 2003.

The odd-numbered alkane levels in the feces were higher ($P < 0.001$) in 2003 compared with 2002, but the levels of C₃₂ in the feces were higher ($P < 0.001$) in 2002, because of the higher dose (839.3 vs. 709.9 mg/d) in 2002 than in 2003. The level of C₃₁ in the feces was much lower ($P < 0.001$) in 2002 compared with 2003 (448.2 vs. 695.9 mg/kg of DM), the level of C₃₃ was also lower ($P < 0.001$) in 2002 compared with 2003, but the increase was less pronounced (402.4 vs. 487.2 mg/kg of DM). This is in line with the variation in the odd-numbered alkane in the herbage.

Results of the DMI estimated by the n-alkanes method are presented in Table 3. The C₃₁ estimates for DMI were 18.2 kg/d of DM in both years. The C₃₃ estimates were 17.2 and 17.5 kg/d of DM, respectively, and did not differ among years ($P = 0.46$). In both years, the lowest DMI values (C₃₂:C₃₁ and C₃₂:C₃₃) were observed in the last period. The standard errors of the C₃₃ estimates of DMI were smaller than in the C₃₁ estimates of DMI.

Net Energy Method

The NE_L value of the grass was calculated with equations 4 to 8, and the chemical composition is presented in Table 4. The NE_L of the grass was lower ($P < 0.001$) in 2002 than in 2003, mainly because of the lower CP concentration ($P < 0.001$) in the grass in 2002 compared with 2003 (180 vs. 201 g/kg of DM). The water-soluble carbohydrate concentration was higher ($P < 0.001$) in 2002 compared with 2003 (134 vs. 109 g/kg of DM). The

Table 3. Dry matter intake from herbage (kg/d) and standard errors estimated with the n-alkanes method using different ratios (C₃₂:C₃₁ and C₃₂:C₃₃) in 2002 and 2003.

n-Alkane ratio	2002	2003
C ₃₂ :C ₃₁	18.2 ± 0.5	18.2 ± 0.5
C ₃₂ :C ₃₃	17.2 ± 0.3	17.5 ± 0.4

Table 4. Chemical composition (g/kg of DM) and energy value (MJ/kg of DM) and standard errors of herbage in 2002 and 2003.

Item ¹	2002	2003
	DM basis	
Ash	97.6 ± 0.8	83.8 ± 0.6
WSC	133.6 ± 9.0	109.8 ± 4.3
Crude fiber	258.6 ± 2.5	255.7 ± 2.0
CP	179.6 ± 3.0	201.8 ± 3.2
Crude fat	40.0 ^a	40.0 ^a
NFE	424.1 ± 4.9	418.8 ± 1.9
DAA	127	135
Digestible CP	134.0 ± 2.7	153.9 ± 2.9
Digestible OM	682.4 ± 2.5	697.9 ± 1.7
GE	18.3 ± 0.04	18.7 ± 0.02
ME	10.5 ± 0.03	10.8 ± 0.03
NE _L	6.1 ± 0.02	6.3 ± 0.02

^a = Assumed value, no variation.

¹ASH = Crude ash, WSC = water-soluble carbohydrates, NFE = nitrogen-free extract, DAA = days after April 1, GE = gross energy, and ME = metabolizable energy.

other chemical characteristics did not differ between years.

The NE_L requirements of the dairy cows used in this experiment were calculated according to equation 3 and are presented in Table 5. The BW of the dairy cows slightly increased during the experiment in 2002 and varied among periods in 2003. The FPCM decreased during the grazing experiments in both years. The NE_L requirement decreased with stage of lactation. The DMI was calculated by subtracting 20.2 MJ from NE_L required because the animals consumed this amount of energy with the concentrates (3.0 kg). The corrected value was divided by the energy concentration in the grass (Table 4). The DMI decreased during the experiment, except in the last period of 2003. This increase in DMI was due to an increased BW and, therefore, higher maintenance costs. The DMI was higher in 2002 (16.8 kg/d of DM) than in 2003 (15.3 kg/d of DM), mainly because of lower milk production in 2003.

Comparison Between Methods

Figure 2 shows the relationship between DMI measured by the sward cutting method, the C₃₂:C₃₃-alkane method, and net energy method, respectively. In both

Table 5. Body weight, fat- and protein-corrected milk production (FPCM¹), net energy requirements (NE_{L,required}), and herbage DMI (with SE) of 12 dairy cows during four 14-d periods in 2002 and 2003.

Trait	2002	2003
BW, kg	534 ± 3.9	549 ± 6.4
FPCM, kg/d	26.7 ± 0.5	24.8 ± 0.3
NE _{L,required} , MJ/d	122.9 ± 1.6	117.3 ± 1.2
DMI, kg/d	16.8 ± 0.2	15.3 ± 0.2

¹FPCM = [(0.337 + 0.116 fat (%) + 0.06 protein (%)) × milk production (kg)].

Figure 2. A) Relationship between DMI from herbage estimated by the sward cutting method and C₃₂:C₃₃ alkanes and B) DMI from herbage estimated by the sward cutting method and net energy method (van Es et al., 1978) in 2002 and 2003.

years, there was a relationship ($P < 0.01$) between sward cutting and the alkanes methods, but the relationship was rather weak ($R < 0.50$) as shown in Table 6. The relationship between estimates of the 2 alkanes methods was high ($R > 0.90$). There was a relationship ($P <$

0.01) between the net energy method and the sward cutting method in 2002, but no relationship was found in 2003. The C₃₂:C₃₁ ratio showed in both years a relationship, whereas C₃₂:C₃₃ showed only a relationship in 2002.

Table 6. Correlation coefficients of the relationship between the sward cutting method, the n-alkanes method ($C_{32}:C_{33}$ and $C_{32}:C_{31}$), and net energy method in 2002 and 2003.

Method	2002			2003		
	$C_{32}:C_{33}$	$C_{32}:C_{31}$	Net energy	$C_{32}:C_{33}$	$C_{32}:C_{31}$	Net energy
Sward cutting	0.433**	0.398**	0.434**	0.413**	0.504***	0.120
$C_{32}:C_{33}$		0.941***	0.503***		0.908***	0.241
$C_{32}:C_{31}$			0.392**			0.358*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

In 2002, the $C_{32}:C_{31}$ alkane method estimate was higher ($P < 0.01$) than all other methods (Table 7). The DMI estimate of the sward cutting method was lowest, but not different from the net energy method. The $C_{32}:C_{33}$ alkane method was also not different from the net energy method in 2002. In 2003, the net energy method was lower ($P < 0.001$) than that determined using the other methods. The DMI estimates of the sward cutting, and the $C_{32}:C_{31}$ alkane methods were both higher ($P < 0.05$) than the $C_{32}:C_{33}$ alkane method.

The sward cutting method showed a large difference between the 2 experimental years, 16.2 and 18.6 kg of DM/d in 2002 and 2003, respectively. The 2 alkane methods were not different ($P > 0.05$) between the 2 experimental yr. The net energy method differed ($P < 0.001$) between years (16.8 and 15.3 kg of DM/d in 2002 and 2003, respectively). Thus, while the sward cutting method estimated the highest DMI in 2003, the net energy method's estimate was lowest in 2003.

The range of the DMI estimated by the $C_{32}:C_{33}$ alkane and the sward cutting method were comparable, 9.0 and 10.8 kg of DM/d in 2002 and 2003, respectively. However, the range of DMI estimated using the $C_{32}:C_{31}$ alkane method was much larger (Table 7).

DISCUSSION

A good method for any scientific research purpose should give values with a small variation and should be highly repeatable. Measuring herbage intake by

grazing dairy cows is a complicated procedure because the herbage intake itself is very variable, too. Even in a controlled stall feeding experiment, a coefficient of variation of 10% in DMI was observed (Taweel, 2004).

Sward Cutting Method

The classical sward cutting method can give a good estimate of herbage intake by grazing animals (Walters and Evans, 1979; Meijs, 1981; Macoon et al., 2003), but often a large variation in the estimation of herbage mass is found. Variation of both pre- and postgrazing measurements are added; hence, the herbage intake values become even more variable. Some experiments described intake measurements in groups of cows (Walters and Evans, 1979; Meijs, 1981; Macoon et al., 2003), but this experiment obtained DMI measurements from individual animals that were kept in individual fields.

Walters and Evans (1979) pointed out that animals (especially sheep) may graze below stubble height of cutting, which would result in an underestimation of the herbage intake. However, in our experiment, the sward surface height after grazing was always above the cutting height. Another point to consider is the herbage accumulation in the period between sward cutting, which might result in an underestimate of the herbage intake. This herbage accumulation has a large influence over longer grazing periods (>3 d) (Walters and Evans, 1979) but is of less importance in short grazing periods when a large difference exists between

Table 7. Descriptive statistics of all DMI methods in 2002 and 2003.

Method	Mean	Minimum	Maximum	Range	SD	CV
2002						
Sward cutting	16.2 ^a	11.0	20.9	9.9	2.36	14.7
$C_{32}:C_{33}$	17.2 ^b	12.4	21.4	9.0	2.31	13.4
$C_{32}:C_{31}$	18.2 ^c	12.5	26.8	14.3	3.27	18.0
Net energy	16.8 ^{ab}	13.5	19.8	6.3	1.64	9.8
2003						
Sward cutting	18.6 ^c	12.7	23.4	10.7	2.75	14.8
$C_{32}:C_{33}$	17.5 ^b	12.6	23.4	10.8	2.58	14.7
$C_{32}:C_{31}$	18.2 ^c	12.7	26.2	13.5	3.17	17.4
Net energy	15.3 ^a	11.6	19.4	7.9	1.36	8.9

^{a,b,c}Means within the same year with different superscripts differ significantly ($P < 0.05$).

pre- and postherbage yield (Linehan et al., 1952; Meijs, 1981). In this study, the herbage accumulation was not measured because only 1 d elapsed between the pre- and postgrazing cut in this experiment, and the decline in herbage yield was large (on average 1000 kg of DM/ha). Nevertheless, the herbage accumulation, calculated from the LINGRA growth model (Schapendonk et al., 1998), was significant (2.2 and 0.9 kg of DM/d in 2002 and 2003, respectively). Using the Linehan equation, the herbage accumulation was responsible for 9% of the total herbage intake in 2002 but only for 3% in 2003. The herbage accumulation, also in short grazing periods, should not be considered as negligible when growing conditions are favorable for grass growth. The sward cutting method is vulnerable to variation because of machine conditions, e.g., sharpness of the knives. Also, changing operators during the measurement period could induce additional variation (Meijs, 1981). In this experiment, the machine was regularly cleaned, and the same operator performed the sampling throughout the experimental period. The largest variation was due to spatial variation in the sward. The estimation of herbage yield, especially the postgrazing yields were very difficult to estimate. The residual herbage was very irregularly distributed over the field, especially when there was a large availability of herbage. This irregular distribution could be due to selection, as cows try to avoid patches of feces (Bosker et al., 2002). However, when the available herbage was depleted, selection became less important, and cows grazed closer to dung patches. Furthermore, cows did not graze regularly in horizons (Wade et al., 1989), but grazed deep in certain patches while other patches were left untouched. Dung patches were avoided during harvesting the residual because including dung would overestimate the postgrazing yield, but avoiding such patches could overestimate the intake.

Green (1949) argued that taking more samples per field would considerably reduce the variability of grazed residual herbage mass. Therefore, the number of samples taken from the residual was twice as many as that from the initial herbage mass. Meijs (1981) mentioned that locating the strips for sward cutting next to each other would improve the measurement. Therefore, in this experiment, where possible, the 2 postcut strips were placed adjacent to the precut strips.

n-Alkanes Method

The n-alkanes method has been a good estimator of DMI during grazing (Malossini et al., 1994; Reeves et al., 1996; Dove et al., 2000). Nevertheless, the n-alkanes method is also associated with sources of variation.

Diurnal patterns of n-alkanes excretion have always been a major concern for variation in marker studies (Dove and Mayes, 1991; Dove et al., 2002; Lippke, 2002). The fecal concentrations of the natural odd alkanes tend to be relatively constant, but because of the dosing schedule, diurnal variation in excretion of the dosed alkane can occur (Stakelum and Dillon, 1990; Dove and Mayes, 1991). The animals in the present experiment were dosed twice daily, which was an attempt to reduce the diurnal variation in alkane ratios in the excreted feces (Stakelum and Dillon, 1990; Dove et al., 2000). In addition, in this experiment, no rectal feces were sampled at one time point, but all dung patches left by the cow in each plot were sampled, which ensured a representative fecal sample over 24 h. This method of sampling will not be applicable in other grazing studies with several cows grazing in one group.

Also, the sampling of herbage could be a source of variation. Animals can select for certain plant species or plant parts that have a different n-alkane level than the average field sample (Dove et al., 1996). The paddocks used in this experiment were monocultures of perennial ryegrass with a very low infestation level of weeds. To ensure that the herbage sample was not different from what the cow consumed, the sampling took place 3 times a day. In the used strip grazing system, cows grazed down the sward in 1 d, and the 3 sampling points ensured that the herbage was representative of what the cow consumed (Taweel et al., 2004). The herbage samples were oven-dried at 60°C for 48 h, because of lack of freeze-drying capacity to dry >900 samples each year. Freeze-drying of herbage and feces is recommended (Dove and Mayes, 1991; Sandberg et al., 2000). However, the drying method is thought not to affect the n-alkanes concentration of herbage, especially perennial ryegrass (Dove and Mayes, 1991) and oven-drying of herbage was done by others (Smith et al., 2001; Martins et al., 2002). N-alkanes concentration in the feces has been affected by the drying (Sandberg et al., 2000); therefore, all feces samples were freeze-dried.

Dosing of the synthetic alkane could be considered a major source of variation of the alkanes method, so the dosing should be very precise. In this case, the alkanes were dissolved, followed by mixing the solution with a premix of cellulose that was subsequently mixed with the other ingredients of the concentrates, so that an equal distribution of the alkanes over the concentrates was ensured. The dosing of the alkane-marked concentrate to the animals might be the largest source of variation. Care was taken that each cow consumed all of the concentrates; nevertheless, at times, some concentrates could be spilled. In addition to the synthetic C₃₂ that the animals were dosed, there

was also a small amount of natural C_{32} present in the herbage. The concentrations of odd n-alkanes exceeded this small amount by far, but in comparison with the daily dose, it could be a considerable amount (e.g., in 2003, the C_{32} level in the herbage was 12.4 mg/kg DM) (Table 2). The estimated herbage intake ($C_{22}:C_{33}$) was 17.5 kg of DM (Table 3), resulting in a daily intake of natural C_{32} alkane of 217 mg, which was 31% of the synthetically dosed amount of C_{32} alkane. A change in the concentration of C_{32} in the herbage can have a large influence on the DMI (e.g., 1-mg change in the C_{32} concentration of the herbage gives an average change in DMI of 0.5 kg of DM). This would suggest a need for a larger dose of synthetic C_{32} alkane than the 700 to 800 mg/d given in this experiment. The doses used by others in experiments with cattle were, however, in the same range (600 to 1000 mg) (Ohajuruka and Palmquist, 1991; Dillon, 1993; Malossini et al., 1994; Reeves et al., 1996). The average C_{32} alkane concentrations were 12.8 and 12.4 mg/kg DM in 2002 and 2003, respectively (Table 2). Lower values ranging from 5 to 10 mg/kg of DM were found by Dove and Mayes (1991), Dillon (1993), and Malossini et al. (1994). In 2003, concentrations of all odd n-alkanes in the herbage were higher than values reported in the literature. This might be related to the high temperatures and drought stress. Plant waxes that contain alkanes play a major role in defense mechanisms against water losses (Kolattukudy, 1976; Tulloch, 1976). The values of C_{31} (144 vs. 230 mg/kg of DM) were much more variable than the values of C_{33} (134 vs. 164 mg/kg DM).

Comparison Between Methods

The n-alkanes method has been mainly validated under stall-feeding conditions. In a grazing situation, the sward cutting method and the n-alkanes method have not been compared with our knowledge. Reeves et al. (1996) concluded that herbage intake estimates from the pre- and postgrazing mass, estimated with the rising plate meter, were not acceptable because of large errors in estimating tropical grass intake. In the present study, a large difference in herbage intake estimated with the sward cutting method was found between 2002 and 2003 (16.2 vs. 18.6 kg of DM, respectively). This difference was not expected because a similar group of dairy cows was used in 2002 compared with 2003, in terms of milk production (26.7 and 24.8 kg FCPM) and BW (534 and 549 kg). Based on their milk production, cows required an even higher DMI in 2002 than in 2003, as can be seen in Table 5. However, the sward cutting method estimate was lower in 2002. The cows did increase in BW; the estimation by the

sward cutting technique seemed to underestimate DMI in 2002.

The n-alkanes method gave similar estimates for DMI in both years (18.2 and 18.2 kg of DM and 17.2 and 17.5 kg of DM for $C_{32}:C_{31}$ and $C_{32}:C_{33}$, respectively). Although the mean of the C_{31} estimate gave more constant results over years, its coefficient of variation was considerably higher than the C_{33} estimate (Table 7), which is similar to results found by others (Mayes et al., 1986; Stakelum and Dillon, 1990; Reeves et al., 1996). However, in pastures with a high clover content, the concentration of C_{33} is much lower (Dove et al., 1996; Lee and Nolan, 2003), and the C_{31} estimate might be a better option. The n-alkanes could give direct and precise estimates of herbage intake from pasture and overcome also the major problems in the Cr_2O_3 and net energy method (Malossini et al., 1996; Reeves et al., 1996; Dove et al., 2000) because the n-alkanes method is independent of the digestibility, while other methods are dependent on an *in vitro* digestibility value, which can differ among individual animals. The estimations by the n-alkanes techniques covered the net energy requirements; especially in 2003, the cows ate over 2 kg of DM more than needed according to their requirements.

Practical Considerations

There are also some practical considerations involved in choosing a certain method. The sward cutting method was more labor intensive in the field than the n-alkanes method. The sward cutting method gave fast results; 24 h after the postgrazing cut, herbage intake could be calculated. The n-alkanes method was more time consuming, and it took more than 1 mo before the data for herbage intake were available. In addition, the n-alkanes method needs expensive equipment for measuring and analyzing materials, whereas the sward cutting method is much less expensive.

CONCLUSIONS

It was concluded that, for herbage intake estimations of individual grazing animals, the n-alkanes technique is the best technique to use. It is recommended to use the $C_{32}:C_{33}$ alkane ratio in pastures dominated by perennial ryegrass, because the herbage intake estimations were less variable with this ratio than with the $C_{32}:C_{31}$ alkane ratio. However, the estimations by the n-alkanes overestimated herbage intake compared with the energy requirements of the animals. The sward cutting technique gave highly variable results and is therefore not recommended for herbage intake estimations of individually grazing dairy cows.

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REFERENCES

- Barrett, P. D., A. S. Laidlaw, and C. S. Mayne. 2004. Development of a European herbage growth model (the EU Grazemore project). Pages 653–655 in Proc. 20th General Mtg. Eur. Grassl. Fed., Luzern, Switzerland.
- Biston, R., P. Dardenne, G. Sinnaeve, and E. Agneessens. 1998. NIR-NIT Calibration List. Centre de Recherches Agronomique, Station de Haute Belgique, Libramont, Belgium.
- Bosker, T., N. J. Hoekstra, and E. A. Lantinga. 2002. The influence of feeding strategy on growth and rejection of herbage around dung pats and in studies of digesta kinetics with dairy cows. Ph.D. Diss., Natl. Univ. Ireland, Dublin, Ireland.
- CVB. 1999a. Manual of Calculating the Feeding Value of Roughages. Centraal Veevoeder Bureau, Lelystad, The Netherlands (in Dutch).
- CVB. 1999b. Book of Tables of Animal Nutrition 1999. Centraal Veevoeder Bureau, Lelystad, The Netherlands.
- Dillon, P. G. 1993. The use of n-alkanes as markers to determine herbage intake, botanical composition of available or consumed herbage and in studies of digesta kinetics with dairy cows. Ph.D. Diss., Natl. Univ. Ireland, Dublin, Ireland.
- Dove, H., M. Freer, and J. Z. Foot. 2000. The nutrition of grazing ewes during pregnancy and lactation: A comparison of alkane-based and chromium/in vitro-based estimates of herbage intake. Aust. J. Agric. Res. 51:765–777.
- Dove, H., and R. W. Mayes. 1991. The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores—A review. Aust. J. Agric. Res. 42:913–952.
- Dove, H., R. W. Mayes, and M. Freer. 1996. Effects of species, plant part, and plant age on the n-alkane concentrations in the cuticular wax of pasture plants. Aust. J. Agric. Res. 47:1333–1347.
- Dove, H., R. W. Mayes, C. S. Lamb, and K. J. Ellis. 2002. Factors influencing the release rate of alkanes from an intra-ruminal, controlled-release device, and the resultant accuracy of intake estimation in sheep. Aust. J. Agric. Res. 53:681–696.
- Forbes, J. M. 1995. Voluntary Food Intake and Diet Selection in Farm Animals. CABI, Wallingford, UK.
- Green, J. O. 1949. Herbage sampling errors and grazing trials. J. Br. Grassl. Soc. 4:11–16.
- Kolattukudy, P. E. 1976. Introduction to natural waxes. Chap. 1 in Chemistry and Biochemistry of Natural Waxes. P. E. Kolattukudy, ed. Elsevier, Amsterdam, The Netherlands.
- Lee, G. J., and J. V. Nolan. 2003. Sources of variation in n-alkane concentrations in the cuticular wax of two species of pasture plants. Aust. J. Agric. Res. 54:21–26.
- Linehan, P. A., J. Lowe, and R. H. Stewart. 1952. The output of pasture and its measurement. Part III. J. Br. Grassl. Soc. 7:73–98.
- Lippke, H. 2002. Estimation of forage intake by ruminants on pasture. Crop Sci. 42:869–872.
- Macoon, B., L. E. Sollenberger, J. E. Moore, C. R. Staples, J. H. Fike, and K. M. Portier. 2003. Comparison of three techniques for estimating the forage intake of lactating dairy cows on pasture. J. Anim. Sci. 81:2357–2366.
- Malossini, F., S. Bovolenta, E. Piasentier, and M. Valentinotti. 1994. Variability of n-alkane content in a natural pasture and in feces of grazing dairy-cows. Anim. Feed Sci. Technol. 50:113–122.
- Malossini, F., S. Bovolenta, E. Piasentier, C. Piras, and F. Martillotti. 1996. Comparison of n-alkanes and chromium oxide methods for estimating herbage intake by grazing dairy cows. Anim. Feed Sci. Technol. 61:155–165.
- Martins, H., D. A. Elston, R. W. Mayes, and J. A. Milne. 2002. Assessment of the use of n-alkanes as markers to describe the complex diets of herbivores. J. Agric. Sci. 138:425–434.
- Mayes, R. W., C. S. Lamb, and P. M. Colgrove. 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. J. Agric. Sci. 107:161–170.
- Meijs, J. A. C. 1981. Herbage intake by grazing dairy cows. Ph.D. Diss., Wageningen Univ., Wageningen, The Netherlands.
- Meijs, J. A. C., R. J. K. Walters, and A. Keen. 1982. Sward methods. Ch. 2 in Herbage Intake Handbook. J. D. Leaver, ed. Br. Grassl. Soc., Hurley, UK.
- Ohajuruka, O. A., and D. L. Palmquist. 1991. Evaluation of n-alkanes as digesta markers in dairy cows. J. Anim. Sci. 69:1726–1732.
- Reeves, M., W. J. Fulkerson, R. C. Kellaway, and H. Dove. 1996. A comparison of three techniques to determine the herbage intake of dairy cows grazing kikuyu (*Pennisetum clandestinum*) pasture. Aust. J. Exp. Agric. 36:23–30.
- Sandberg, R. E., D. C. Adams, T. J. Klopfenstein, and R. J. Grant. 2000. N-alkane as an internal marker for predicting digestibility of forages. J. Range Manage. 53:159–163.
- Schapendonk, A. H. C. M., W. Stol, D. W. G. van Kraalingen, and B. A. M. Bouman. 1998. LINGRA, a sink/source model to simulate grassland productivity in Europe. Eur. J. Agron. 9:87–100.
- Smith, D. G., R. W. Mayes, and J. G. Raats. 2001. Effect of species, plant part, and season of harvest on n-alkane concentrations in the cuticular wax of common rangeland grasses from southern Africa. Aust. J. Agric. Res. 52:875–882.
- Stakelum, G., and P. G. Dillon. 1990. Dosed and herbage alkanes as feed intake predictors with dairy cows: The effect of feeding level and frequency of perennial ryegrass. In Proc. 7th Eur. Grazing Workshop, 8–11 October, 1990, Wageningen, The Netherlands.
- Taweel, H. Z., B. M. Tas, J. Dijkstra, and S. Tamminga. 2004. Intake regulation and grazing behavior of dairy cows under continuous stocking. J. Dairy Sci. 87:3417–3427.
- Taweel, H. Z. 2004. Perennial ryegrass for dairy cows: Grazing behaviour, intake, rumen function and performance. Ph.D. Diss., Wageningen Univ., Wageningen, The Netherlands.
- Tulloch, A. P. 1976. Chemistry of waxes in higher plants. Ch. 7 in Chemistry and Biochemistry of Natural Waxes. P. E. Kolattukudy, ed. Elsevier, Amsterdam, The Netherlands.
- van Es, A. J. H. 1978. Feed evaluation for ruminants. 1. Systems in use from May 1977 onwards in the Netherlands. Livest. Prod. Sci. 5:331–345.
- Wade, M. H., J. L. Peyraud, G. Lemaire, and E. A. Comeron. 1989. The dynamics of daily area and depth of grazing herbage intake of cows in five day paddock system. Pages 1111–1112 in Proc. 16th Int. Grassl. Congr., Nice, France.
- Walters, R. J. K., and E. M. Evans. 1979. Evaluation of a sward sampling technique for estimating herbage intake by grazing sheep. Grass Forage Sci. 34:37–44.