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R. M. Lewis

Scottish Agricultural College, ron.lewis@unl.edu

A. M. Magadlela

University of Edinburgh

N. S. Jessop

University of Edinburgh

G. C. Emmans

Scottish Agricultural College

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The ability of the n-alkane technique to estimate intake and diet choice of sheep

R. M. Lewis¹†, A. M. Magadela²‡, N. S. Jessop² and G. C. Emmans¹

¹*Animal Biology Division, Scottish Agricultural College, West Mains Road, Edinburgh EH9 3JG, UK*

²*Institute of Ecology and Resource Management, University of Edinburgh, West Mains Road, Edinburgh EH9 3JG, UK*

† Present address: Department of Animal and Poultry Sciences (0306), Virginia Polytechnic Institute and State University Blacksburg, Virginia 24061, USA. E-mail: rmlewis@vt.edu

‡ Present address: Department of Livestock and Pasture Science, Faculty of Agricultural and Environmental Sciences, University of Fort Hare, Private Bag x1314, Alice 5700, Eastern Cape, South Africa.

Abstract

This study assessed the efficacy of the n-alkane technique to estimate intake and diet composition in animals given single foods or a choice of two. In the first experiment intakes of pelleted ryegrass and lucerne, given either alone or as a choice, were measured in lambs housed indoors in individual pens. Each of the three feeding treatments was given to 12 lambs at two degrees of maturity (0.30 and 0.45 of estimated mature sizes). The 12 lambs were constituted as three replicates of the two sexes of each of two breeds. The measured intakes were compared with those estimated using the n-alkanes C₃₁ and C₃₃, found naturally in the foods, and C₃₂, which was given as a dose. On the choice treatment diet composition was estimated using a non-negative least squares procedure and data on C₃₁ and C₃₃ alone. The agreement between actual and predicted intake was good: R² of 0.938 for log-linear regression with a residual standard deviation of 0.0845. Intake of lucerne when offered alone was slightly yet significantly over-predicted. The proportion of ryegrass in the diet was also accurately predicted (R² of 0.950 and residual s.d. of 0.0398). Using the data on C₂₇ and C₂₉, in addition to that on C₃₁ and C₃₃, gave a poorer agreement with the observed diet compositions. The low and similar levels of C₂₇ in the two foods meant that this n-alkane provided little extra information that could be used to estimate diet composition. In a second experiment faecal samples were collected every 4 hours over a 24-h period in six lambs on ad libitum, and in six lambs on a restricted quantity, of pelleted ryegrass. There was no significant diurnal variation in the ratios of either C₃₁ or C₃₃ to C₃₂ on either ad libitum or restricted feeding. The time of faecal collection within a day should not therefore affect the reliability of the predictions. The study confirmed the value of using n-alkanes in methods to determine the intake of forages by sheep, and that the time of faecal collection within the day does not affect the reliability of these predictions. The results also confirm the utility of the n-alkane method for estimating diet choice, at least with two-component mixtures.

Keywords: alkanes, food intake, food preferences, sheep.

Introduction

The nutrient supply to grazing animals, and hence their performance, is determined by the quantity and composition of ingested forage. However, these variables are difficult to measure particularly for animals grazing swards of mixed species. Traditional methods for estimating the composition of consumed herbage are either laborious or imprecise in distinguishing between species in herbage mixtures

(Dove and Mayes, 1991). In the mid-1980s, methodologies based on long-chain n-alkanes (aliphatic hydrocarbons) were introduced to estimate food intake by grazing animals (Mayes *et al.*, 1986).

The compounds n-alkanes are saturated hydrocarbons found in the cuticular wax of most plants. Different dietary plants tend to have different patterns of individual alkanes. Thus the plant-species

composition of a herbivore's diet can potentially be determined from the pattern of alkanes found in the faeces and the individual alkane contents of the component plant species (Dove, 1992; Mayes *et al.*, 1994; Newman *et al.*, 1995). This is achieved mathematically by determining the best match between the n-alkane concentrations in the herbage and in the faeces after correcting for the quantities presumed to be absorbed by the gut (Hameleers and Mayes, 1998). When grazing animals are also dosed with known quantities of another n-alkane, usually with an even chain length, then the concentrations of the natural and the dosed n-alkanes in the herbage and the faeces can be used to estimate dry matter intake (DMI) (Dove and Mayes, 1996).

The use of n-alkanes to estimate diet composition and intake has some limitations. For instance, although the alkane profiles of different herbages usually differ, they are seldom orthogonal. This introduces 'noise' when one attempts to delineate diet choice when animals graze a mixed sward or are offered several foods. Another potential constraint is that diurnal variation in the concentrations of dosed even-chain alkanes has been reported. If such variation is present, and the time of faecal sampling varies between days or animals, this could compromise the accuracy of the technique to determine food intake (Dillon and Stakelum, 1990; Stakelum and Dillon, 1990; Dove and Mayes, 1991).

This study was designed to assess the accuracy of the n-alkane technique in estimating DMI and diet choice in animals given two foods. The study was conducted under controlled conditions where diet composition and levels of intake of individual lambs were measured directly and compared with those estimated using the n-alkane method. While there is no direct correspondence to a grazing situation, this approach to testing the technique is a prerequisite to its more widespread use.

Our first objective was to assess accuracy in prediction of DMI in male and female lambs of two breeds at 0.30 and 0.45 of their projected mature weights when offered either pelleted ryegrass (*Lolium* spp) or lucerne (*Medicago sativa*) alone or as a choice. The eight combinations of breed, sex, and maturity level used was to ensure variation in food intake and to test the robustness of the n-alkane technique, as most historic studies to verify the accuracy of the method have been based on small numbers of animals. We also tested the accuracy of our estimate of diet composition for those lambs offered ryegrass and lucerne as a choice treatment. The second objective was to investigate whether diurnal patterns of excretion of n-alkanes in lambs

given food *ad libitum* or a restricted amount of food was sufficient to produce poorer estimates.

Material and methods

Two experiments were carried out indoors at the Scottish Agricultural College, Edinburgh between early June and late July 1998.

Experiment 1

Experiment 1 had two phases. The first began in early June when, on average, the lambs had reached 0.30 of their projected mature weight and the second in late July when, on average, they had reached 0.45 of their projected mature weight. Two breeds, the Suffolk and the Scottish Blackface, which differ markedly in mature weight, were used. The mature weights for females were assumed to be 100.0 kg for Suffolks (Lewis *et al.*, 1998; Lewis *et al.*, 2002) and 69.0 kg for Scottish Blackfaces (Friggens *et al.*, 1997); the male weights were assumed to be 1.3 times the female weights (Hammond, 1932). Different breeds, degrees of maturity and genders were used to generate a wide range of food intakes under which the n-alkane technique could be tested for its ability to predict intake. It was also possible that these factors would generate variation in the composition of the diet selected where a choice was given.

In each phase, 36 lambs were used in a factorial design with two breeds (Suffolk (S) and Scottish Blackface (B)), two sexes (male and female) and three feeding treatments (pelleted ryegrass or pelleted lucerne alone, or both as a choice). Each treatment was replicated three times. The mean weights of the lambs used in phase 1 were 30 kg for S females, 39 kg for S males, 21 kg for B females and 28 kg for B males. Mean weights for the lambs used in phase 2 were 48 kg for S females, 59 kg for S males, 37 kg for B females and 44 kg for B males.

From near birth, the lambs were offered free access to a pelleted creep food. Once a lamb reached 0.20 of its estimated mature weight, or a lamb was 8 weeks of age (whichever came first), it was weaned. Weaned lambs were placed in pens in small groups and gradually introduced to their feeding treatment. For lambs allocated to the choice, equal proportions of the foods were offered as a mixture. The lambs were closely monitored for refusal to eat and ill health. Once lambs were approximately 1 kg heavier than their target weaning weight, they were individually penned on slatted floors. The time period from weaning to individual penning was on average 14 (s.d. 8.3) days.

Once individually penned, a pre-weighed amount of food sufficient to last 1 week was prepared for each

lamb. A sufficient amount was transferred from the reservoir to the individual feeders twice daily at 08:30 and 15:30 h so that it was always available through the period of 24 h. This ensured that feeding was *ad libitum* to simulate as closely as possible the continuous access to food of grazing animals. Refusals were removed from each pen weekly and weighed. On average 2166 (s.d. 1371) g of food was weighed as the refusal, with less than 100 g remaining as the refusal on less than 0.01 of occasions.

The pelleted ryegrass was acquired from Vitagrass Farm (Holker) Ltd., Cumbria, England and the pelleted lucerne from Drygrass (Northern) Ltd., Cumbria, England. Each food was derived from a single cutting of the respective sward with sufficient quantity provided in bulk to last the entire trial. The pellets were stored in upright silos and, as needed, placed in 500 kg tote sacks for feeding within the slatted shed. The chemical composition of the two foods is provided in Table 1.

Each lamb was dosed twice daily over a period of 12 days at 08:00 h and 16:00 h with an intended 100 mg of C_{32} impregnated into a tissue-paper bung designed for use with laboratory glassware [cellucotton stoppers (Carl Roth GmbH and Co. KG, Karlsruhe, Germany)]. Faecal grab samples were collected twice daily at 08:00 h and 16:00 h from each lamb from day 8 to 12. The samples were oven dried at 60°C for 48 h and then ground. For each lamb, the two faecal samples from each day were bulked and an aliquot extracted as described by Mayes *et al.* (1986), with the modifications that duplicate 0.5 g samples were directly heated in ethanolic potassium hydroxide and 7 ml of heptane were used as the solvent.

Duplicate 1 g samples from each of the two foods were extracted in the same way as for the faeces

Table 1 Chemical composition of the food used

	Ryegrass	Lucerne
Dry matter (DM; g/kg)	958	939
Crude protein (g/kg DM)	135	182
NDF (g/kg DM)†	493	449
AHEE (g/kg DM)†	32	36
Ash (g/kg DM)	103	103
NCGD (g/kg)†	654	576
Metabolizable energy (MJ/kg DM)‡	9.5	8.3

† NDF = neutral-detergent fibre; AHEE = acid hydrolysed ether extract; NCGD = neutral cellulase gamanase digestibility.

‡ Predicted from $0.154 \text{ NCGD} - 0.59$ (Agricultural and Food Research Council, 1993).

except that about 50% more solvent and water than in faecal extraction were used. The final eluates were injected onto a capillary column (HP, 5 Crosslinked Phenyl Methyl Silicone, 50 m $\times$ 0.32 mm $\times$ 0.17 μm) in a model HP6890 gas chromatograph fitted with a flame ionization detector and linked to a PC run by HP Chemstation software. The oven temperature programme was initially 65°C and then rose to 300°C at 10°C/min where it was held for 20 min. The temperatures of the injection and detector ports were 325°C and 350°C, respectively. The flow rate of the carrier gas (N_2) through the column was 1.1 ml/min. The ratios of the peak areas of the analysed n-alkanes to that of the internal standard (tetratriacontane, C_{34}) were used to calculate n-alkane amounts in the samples. Identification of the different n-alkanes was made based on the relative retention times of known standards.

The average amount of C_{32} that was adsorbed onto each cellucotton stopper (bung) was measured on nine bungs. The bungs were boiled for 1 h under reflux in 100 ml of heptane after adding 100 mg of internal standard (C_{34}), (R. W. Mayes, unpublished protocol). After partial cooling to about 60°C, an aliquot was taken and analysed by gas chromatography in the same way as the faecal and food samples. On average there were 118.7 (s.d. 3.47; CV 2.9%; s.e. 1.16) mg of C_{32} per bung; this value was used to estimate food intake. The measured content of C_{32} on the bungs was higher than the 100 mg intended as a consequence of some volatilization of the heptane as bungs were prepared.

Food intake. The intakes of the penned lambs on ryegrass or lucerne alone, and those given the choice (free and continuous access to both foods in separate containers), were estimated from the concentrations of C_{31} , C_{32} and C_{33} in the faeces and the consumed food (Mayes *et al.*, 1986). For the choice treatment the composition of the diet selected was estimated first. The intakes were estimated using the n-alkane pairs C_{33} and C_{32} , and then C_{31} and C_{32} , in turn; this was done on the assumption that the recovery rate of C_{32} was the mean of that of the other two. The food intake was predicted as the mean of the two estimates. Mayes *et al.* (1986) suggested that this approach should give 'an estimate of $\times$ intake close to the true value'; it was also used by Vulich *et al.* (1991). The observed intakes used for comparison with the predicted values were the average daily intakes over the period of n-alkane dosing for each animal.

The estimated and the observed mean daily DMIs for each lamb were compared by regressing the observed on the predicted intake, with both

expressed as natural logarithms because of the wide range in measured food intake. The hypotheses tested were that the regression coefficient of the log of observed food intake on the log of predicted food intake was not different from unity and that the intercept was not different from zero. The residual s.d. was used to describe the proportional error of a single observation. The ratio of the observed to the predicted intake was also compared with unity. Intakes were analysed using ANOVA to test whether the factors of breed, sex, degree of maturity and food, and their interactions, had the expected effects. The Genstat statistical package was used for the analyses (Genstat 5 Committee, 1998).

Diet composition. The amounts of ryegrass and lucerne in the diets of animals offered choice of both foods were estimated from the heptacosane (C_{27}), nonacosane (C_{29}), hentriacontane (C_{31}) and tritriacontane (C_{33}) n-alkane contents of the foods and faeces, and subsets of these n-alkanes. Estimates were obtained using a non-negative least squares algorithm called EatWhat (Dove and Moore, 1995). The algorithm is based on searching for the best set of forage species to include in the estimated diet at non-zero levels (Lawson and Hanson, 1974; Dove and Moore, 1995). Faecal recovery rates of 0.713 (C_{27}), 0.745 (C_{29}), 0.854 (C_{31}), and 0.891 (C_{33}) were used for the calculations (Mayes *et al.*, 1986). The hypotheses, tested by regressing the observed on the predicted proportion of ryegrass selected in the diet, were that the slope was unity and the intercept zero. Diet compositions were also analysed using ANOVA to test whether the factors of breed, sex and degree of maturity, and their interactions, had any effect. Genstat was used for all analyses (Genstat 5 Committee, 1998).

It was expected that including information on the C_{29} and C_{27} n-alkanes, in addition to that on C_{33} and C_{31} , would improve the accuracy of the predictions of diet composition. However, this was found not to be the case and, for all final analyses, diet composition predictions were based on C_{31} and C_{33} alone.

Experiment 2

Twelve Suffolk lambs at 0.45 their mature size were given either a daily allowance of 1000 g of pelleted

Table 2 Concentrations (mg/g dry matter) of odd-chain n-alkane in the diets

Diet	n-alkane			
	C_{27}	C_{29}	C_{31}	C_{33}
Lucerne	0.0328	0.1289	0.2645	0.0174
Ryegrass	0.0334	0.1729	0.2164	0.0674

ryegrass or this same food *ad libitum*. The aim of the feeding restriction was to ensure that the daily food allowance was eaten in 1 to 2 h. One lamb did not consume all its daily allowance so that over the experimental period it consumed only about 750 g/day. After 1 week, they were dosed with C_{32} as described for experiment 1. Faecal samples were also collected as described for experiment 1, except that on the 3rd day of the faecal collection period, a faecal sample was obtained from each lamb at 4-h intervals over a 24-h period.

Samples were oven dried and ground as described in experiment 1, but each sample was analysed individually (as opposed to bulking the daily samples as in experiment 1). The concentrations of C_{32} and the ratios of C_{33} and C_{31} to C_{32} in the faeces of the restricted and *ad libitum* feeding lambs were compared for the 4-h intervals over the 24-h period of sampling.

It was planned to analyse the data as a split-plot design using ANOVA with animal as the main plot and sampling time as the subplot (Genstat 5 Committee, 1998). The hypothesis to be tested was that there would be no effect of sampling time on the $C_{33}:C_{32}$ and $C_{31}:C_{32}$ ratios. The values of the ratios collected per animal were expressed relative to the animal's mean values. This was done to allow comparison of temporal changes across the two feeding regimes as the ratios were expected to differ given variation in food intake between animals. With six samples collected on each animal over time, the statistical property of compound symmetry (i.e. the correlations between all pairs of time points are equal) was needed to analyse the experiment as a true split-plot design. To test whether there was compound symmetry, we used the AREPMEAS procedure in Genstat (Genstat 5 Release 4.1, 1998). Where such was not the case, the Greenhouse-Geisser epsilon factor was used to adjust degrees of freedom (Greenhouse and Geisser, 1959). This procedure enabled a more conservative statistical test.

Results

The concentrations of the odd-chain n-alkanes of the foods used are shown in Table 2. There were differences in the concentrations of C_{29} , C_{31} , and C_{33} in the two foods. There was 3.95 times as much C_{33} in the ryegrass as in the lucerne, with 0.82 and 1.35 times as much of C_{31} and C_{29} , respectively. The concentration of C_{27} was similar for both foods at 0.033 mg/g DM (Table 2). In addition, the proportion of C_{27} was only 0.065 of the total n-alkane in each foodstuff.

Figure 1 Observed *versus* predicted dry matter intakes (g/day) for lambs offered *ad libitum* access to pelleted ryegrass (■) or pelleted lucerne (●) alone, or both as a choice (▲). The solid line shows where observed and predicted intakes are equal.

Experiment 1

Food intake. The total food dry matter intake ranged from 698 to 4293 g/day. The regression of log of observed food intake [$\ln(\text{FI})$] on the log of predicted food intake [$\ln(\text{PFI})$] was:

$$\ln(\text{FI}) = -0.220 \text{ (s.e. 0.239)} + 1.025 \text{ (s.e. 0.0315)} \ln(\text{PFI}) \quad (1)$$

with residual s.d. of 0.0845 (equivalent to a proportional error of 8.45%) and R^2 of 0.938. The data are shown in Figure 1. The regression coefficient was not significantly different from unity and the

intercept was not significantly different from zero ($|t \text{ value}| < 1$). These results show, on this test, that the n-alkane method used predicted intake accurately and with no bias.

Table 3 provides simple means for observed and predicted DMIs, and their ratio, for the combinations of breed, sex and maturity level for each feeding treatment. Measures of variation for these means are shown in the footnote. As expected there were effects ($P < 0.001$) of the four main factors (breed, sex, food and degree of maturity) on intake. The only two interactions significant at $P < 0.05$ reflected scaling effects as they were not significant when the natural logarithm of intake was used as the response variable.

The mean ratio of actual to predicted intake was 0.9732 (s.e. 0.00953), which was less than unity ($P < 0.01$). This indicates a small, but systematic, overestimation of intake that was not detected by the regression method. The ANOVA showed that maturity level, breed, and sex of the lamb had no significant effect on the ratio of actual to predicted intake ($P > 0.2$). However, there was an effect of food treatment on the ratio. For ryegrass, the mean ratio of actual to predicted was 0.987 (s.e. 0.0186; $t \text{ value} < 1.0$), which did not differ significantly from unity. For lucerne, the mean ratio of actual to predicted was 0.936 (s.e. 0.0141; $t \text{ value} = 4.57$; $P < 0.001$), which did differ from unity. Where ryegrass and lucerne were given as a choice, the mean ratio of actual to predicted was 0.993 (s.e. 0.0138; $t \text{ value} < 1$), which did not differ significantly from unity.

Diet composition. The diet selected contained 0.3728 (s.d. 0.178; s.e. 0.0363) of ryegrass. This proportion is less than 0.5 ($P < 0.01$), which is that expected on the basis of a random choice between ryegrass and lucerne. There were no significant effects of breed, sex or degree of maturity on the proportion of

Table 3 Simple means for observed and predicted food intakes (g dry matter per day), and their ratio, by treatment factor in experiment 1†

Breed	Sex	Maturity	Ryegrass			Lucerne			Choice		
			Observed	Predicted	Ratio	Observed	Predicted	Ratio	Observed	Predicted	Ratio
Suffolk	Male	0.30	1830	1848	0.994	2285	2439	0.942	2020	1883	1.064
		0.45	2734	2532	1.078	3274	3734	0.881	3749	3550	1.059
	Female	0.30	1358	1383	0.983	1851	1872	0.988	1756	1748	1.003
		0.45	2159	2205	0.981	2804	2927	0.961	2446	2471	0.996
Blackface	Male	0.30	1231	1381	0.889	1622	1682	0.967	1460	1523	0.962
		0.45	2019	1924	1.051	2421	2413	1.001	2140	2198	0.974
	Female	0.30	1168	1314	0.893	1240	1380	0.896	1281	1318	0.973
		0.45	1848	1797	1.031	1813	1977	0.907	1872	2046	0.920

† The CVs of observed and predicted values were on average 0.124 and 0.128, respectively. The average standard error of the ratio of observed to predicted intake was 0.0182.

Figure 2 Observed *versus* predicted proportions of ryegrass in the diet selected by lambs offered a choice of pelleted ryegrass and pelleted lucerne. The solid line shows where observed and predicted proportions of ryegrass in the diet are equal.

ryegrass selected. The major source of variation in diet choice was thus between animals.

The relationship between the actual proportion of ryegrass in the diet selected, and that estimated using the data on C_{31} and C_{33} , is shown in Figure 2. The regression of the observed (PG) on predicted (PPG) proportion of ryegrass selected in the diet was:

$$PG = -0.0233 \text{ (s.e. } 0.0206) + 1.010 \text{ (s.e. } 0.0484) \text{ PPG} \quad (2)$$

with residual s.d. of 0.0398 and R^2 of 0.950. The residual s.d. estimates the absolute error for a single observation. The method was effective in estimating the composition of the diet selected with no bias as

the intercept was not significantly different to zero and the slope was not significantly different to unity.

When information on C_{29} was included the prediction was poorer. The R^2 value was reduced to 0.934 and the residual standard deviation was increased to 0.0457. The value of the intercept of -0.1180 (s.e. 0.0287) became different from zero ($P < 0.01$). Including information on C_{27} did not change the estimates of the proportion of ryegrass in the diet. This was not surprising as both foods had low and similar concentrations of C_{27} (Table 2).

Experiment 2

In Table 4, the ratios of $C_{33} : C_{32}$ and $C_{31} : C_{32}$ collected at 4-h intervals over a 24-h period for lambs offered free access or a restricted quantity (1000 g) of pelleted ryegrass are shown. The ratios were expressed relative to an animal's mean value. With no temporal variation the relative value would be expected to be unity.

The assumption of compound symmetry was rejected for the $C_{33} : C_{32}$ ratio ($P < 0.01$) and for $C_{31} : C_{32}$ ratio ($P < 0.05$). However, it was not rejected for C_{32} concentration ($P > 0.05$). Even before correcting degrees of freedom using the Greenhouse-Geisser epsilon value for our analyses of $C_{33} : C_{32}$ and $C_{31} : C_{32}$ ratios, the null hypothesis of no effect of sampling time was not rejected ($P > 0.2$). There was also no significant effect of sampling time on C_{32} concentration ($P > 0.5$).

The ratios of $C_{31} : C_{32}$ and $C_{33} : C_{32}$ observed over the 24-h sampling period in the faeces of the lambs given *ad libitum* and restricted levels of food are shown in Figure 3. The ratios, as expected, were much lower ($P < 0.001$) in the animals given a restricted quantity of food. The concentration of C_{31} in ryegrass was substantially more than C_{33} and thus the average $C_{31} : C_{32}$ ratio (Figure 3a) was higher than the $C_{33} : C_{32}$ ratio (Figure 3b).

Table 4 Least-squares means for the ratios $C_{33} : C_{32}$ and $C_{31} : C_{32}$ (expressed relative to animal mean ratio) collected at 4-h intervals over 24 h for either *ad libitum* or restricted (1000 g) access to pelleted ryegrass

Ratio	Time (h)					
	08:00	12:00	16:00	20:00	00:00	04:00
$C_{33} : C_{32}^\dagger$						
<i>Ad libitum</i>	0.853	0.860	1.049	1.093	1.089	1.056
Restricted	1.056	1.047	0.930	0.946	0.953	1.068
$C_{31} : C_{32}^\dagger$						
<i>Ad libitum</i>	0.833	0.855	1.029	1.100	1.107	1.077
Restricted	1.053	1.023	0.919	0.956	0.972	1.077

† Standard errors of the cell means were 0.0689 ($C_{33} : C_{32}$) and 0.0723 ($C_{31} : C_{32}$).

Figure 3 Ratio of (a) C_{31} to C_{32} n-alkane and (b) C_{33} to C_{32} n-alkane at 4-h samplings over a 24-h period in lambs on *ad libitum* (—) or restricted (-----) amounts of pelleted grass.

Discussion

The concentrations of C_{27} , C_{29} , and C_{31} in the ryegrass used in this study were about 50 percent higher than those reported by Hameleers and Mayes (1998) but similar to those reported by Newman *et al.* (1995). The concentration of C_{33} was much lower than those found in either of the above studies. The

concentrations of all the n-alkanes in the lucerne were different to those obtained by Dove (1992). There were 1.5 times more C_{27} and C_{29} found in this study than in Dove (1992), and the C_{31} and C_{33} concentrations were more than two times higher in this study. These differences may well reflect variation between laboratories in methods of alkane analysis.

Experiment 1

Food intake. We tested the ability of the technique to estimate total DMIs over a large range of observed intakes. There was a close relationship between the observed and the estimated total DMI as shown in Figure 1. This result is consistent with the findings of similar studies that investigated the reliability of the n-alkane method to estimate herbage DMI (see reviews by Dove and Mayes (1991) and Mayes and Dove (2000)).

For ryegrass alone and the choice treatment, the ratio of observed to predicted intake was not significantly different from unity. However, this was not the case for lucerne where observed intake was only 0.936 of that predicted. This may have been because we predicted food intake using both the $C_{33}:C_{32}$ and $C_{31}:C_{32}$ ratios rather than only the $C_{33}:C_{32}$ ratio as suggested elsewhere (e.g. Dove and Mayes, 1991). The extent of the over-prediction of lucerne intake in fact increased slightly for all animals on this food when the $C_{33}:C_{32}$ ratio was used alone. The ratio of observed to predicted intake decreased from 0.936 to 0.907 ($P < 0.001$).

Our results are also broadly consistent with those reported by Vulich *et al.* (1991). They predicted the DMI of a cut herbage (predominantly *Lolium perenne*) offered *ad libitum* to individually penned lambs using the $C_{33}:C_{32}$ and $C_{31}:C_{32}$ ratios separately or together. Their actual intakes were 0.97 of the predicted when the $C_{33}:C_{32}$ ratio was used alone and were 1.08 of the predicted when the $C_{31}:C_{32}$ ratio was used alone. The biases were both in the direction expected (Mayes *et al.*, 1986). Vulich *et al.* (1991) found that the bias in the estimate was least when the average of the two ratios was used for prediction.

The method assumes that a particular n-alkane recovery rate is the same in all foods; in particular for our case, that it is the same for both ryegrass and lucerne. The observed wide variation in recovery rate for a given n-alkane (see Figure 2 of Dove and Mayes (1991)) suggests that this strong assumption is not justified, although some of the apparent variation could be due to differences between laboratories in analytical methods. While the recovery rate of the dosed C_{32} is unlikely to be affected by the food with

which it is mixed in the rumen, the recovery rates for C_{31} and C_{33} could be different for our two foods. This could account for the effect of food on the accuracy of our prediction of intake (Newman *et al.*, 1998). As we have no direct measurement of the recovery rates of the n-alkanes in these two foods this hypothesis remains untested.

Diet composition. The method we used successfully predicted the composition of the diet selected from a choice of ryegrass and lucerne (Figure 2). Others have also successfully used n-alkane techniques to predict diet composition (Dove and Mayes, 1991; Salt *et al.*, 1994; Hamelers and Mayes, 1998; Mayes and Dove, 2000). We used information only from the natural alkanes C_{33} and C_{31} to do this. We had anticipated that the information that we had on the concentrations of C_{27} and C_{29} could be used to further improve the accuracy of our estimates (Dove and Moore, 1995; Newman *et al.*, 1995). We were surprised that this was not the case.

With hindsight there was no reason to expect that the inclusion of the information on C_{27} would help, as the concentrations of this n-alkane were low and similar in both foods given as the choice (Table 2). However, we expected that the information on C_{29} , for which the concentrations differed substantially in the two foods, would be helpful. Newman *et al.* (1998) pointed out that any bias in the n-alkane recovery estimates will probably have large effects on the accuracy of estimation of diet composition. It is likely that the reason for the information on C_{29} failing to improve accuracy was because our assumed recovery rate of 0.745 was incorrect, at least within the conditions of this study.

Recovery rates. Where intake and diet composition measurements are made under grazing conditions, recovery rates for the different n-alkanes, whether naturally present or given as a dose, are assumed. As pointed out by Newman *et al.* (1998) 'until it can be satisfactorily demonstrated that the method of estimating recoveries is unbiased ... results reported using this technique should be considered suspect.'

In the current study we found that a poorer prediction of diet composition was obtained when C_{29} data were included in addition to those of C_{33} and C_{31} . We have already hypothesized that this was because the recovery rate assumed for this n-alkane of 0.745, taken from Mayes *et al.* (1986), was unsuitable for this experiment. In Figure 4 the recovery rates estimated by Mayes *et al.* (1986) are plotted against the C-chain length, together with their standard errors, which have been calculated from the original data (see their Table 5). The

recovery rate for C_{29} would appear to be anomalous. Using a paired *t* test, the C_{29} recovery rate was less ($P < 0.001$) than the mean of the recovery rates for C_{28} and C_{30} . With this exception, recovery rate changed smoothly with C-chain length (Figure 4).

Experiment 2

There were no significant effects of time of sampling on the $C_{31}:C_{32}$ or $C_{33}:C_{32}$ ratios as shown in Figure 3a and b. The ratios were lower on the restricted treatment because of the fixed dose of C_{32} and the lower food intake of sheep on this treatment. It appears that a single faecal sample in a day provides a satisfactory estimate of the ratio and thus food intake in agreement with Sibbald *et al.* (2000).

Conclusions

The study confirmed the value of using n-alkanes in methods to determine the intake of forages by sheep. The n-alkane technique gave good prediction of DMI in lambs that were given either ryegrass alone or lucerne and ryegrass as a choice. However, the lambs' intake of lucerne when offered alone was slightly yet significantly over-predicted. Still the method accurately predicted diet choice, at least with a two-component mixture. The reliability of the predictions was also not affected by the time of day that faecal samples were collected. However, accurate and relevant estimates of recovery rates of n-alkanes are needed for the technique to give reliable results.

Figure 4 Recovery rate (■) of n-alkanes of different C-chain lengths as reported by Mayes *et al.* (1986). The standard errors have been calculated from the original data in their Table 5. The possible anomalous value for C_{29} is indicated as (□).

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