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The use of dosed and herbage n-alkanes as markers for the determination of herbage intake

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SUMMARY

The recovery in the faeces of the n-alkanes of herbage (odd-chain, C_{27}–C_{35}) and of dosed artificial alkanes (even-chain, C_{28} and C_{32}) was studied in twelve 4-month-old castrated male lambs. The lambs received three levels of cut, fresh perennial ryegrass or a mixed diet of perennial ryegrass (0·70) and a barley-based concentrate (0·30) (500–900 g D.M./day). C_{28} and C_{32} n-alkanes (130 mg each), absorbed onto shredded paper, were given once daily for 17 days to test whether the recoveries of herbage and dosed alkanes were similar to enable their use as markers for determining the herbage intake of grazing sheep. Stearic and palmitic acids (130 mg each) were given with the dosed alkanes to half of the animals with the objective of facilitating emulsification of the dosed alkanes within the digestive tract.

With the exception of C_{27} n-alkane, the faecal recoveries of all alkanes were unaffected by diet, feeding level or emulsifying agent. Faecal recovery of odd-chain herbage n-alkanes increased with increasing C-chain length. The recovery of the dosed C_{28} n-alkane was slightly greater than the recoveries of both C_{27} and C_{29} n-alkanes of herbage. The recoveries of the dosed C_{32} n-alkane and the herbage C_{33}-alkane were the same.

The mean herbage intake estimated using C_{33} and C_{32} n-alkanes was identical to the actual herbage intake. Other alkane pairs gave slight underestimates of herbage intake ranging from 3·5% for the C_{28}–C_{29} pair to 7·6% for the C_{27}–C_{28} pair. No cyclical pattern of n-alkane excretion throughout the day was observed. Examination of daily variations in faecal alkane concentrations indicated that the start of alkane dosing should precede the sampling of faeces by at least 6 days.

These results suggest that accurate estimation of herbage intake in grazing sheep is possible from the simultaneous use of dosed C_{32} and herbage C_{33} n-alkanes as markers. The method may be particularly useful in enabling unbiased estimates of herbage intake to be made in animals receiving supplementary feed.

INTRODUCTION

The use of indigestible plant components as markers for digestibility determinations (internal markers) in grazing ruminants offers potential advantages over other methods, such as *in vitro* or index techniques, in that digestibility can be directly estimated *in vivo*. With a knowledge of faecal output unbiased estimates of herbage intake can also be obtained in grazing animals. However, the majority of internal markers tested to date cannot be analysed as discrete compounds and incompatibility in their analysis in herbage and faeces can lead to errors in the estimation of digestibility and intake (Langlands, 1975). Long-chain fatty acids of chain length C_{19}–C_{32}, which are present in plant cuticular wax, have been suggested as internal markers as they are discrete compounds and are apparently indigestible (Grace & Body, 1981); however, their analysis is slow and difficult. Also present in the cuticular wax are long-chain n-alkanes (see Tulloch, 1976) which are relatively easily analysed and are apparently absorbed to a very limited extent in monogastrics (Kolattukudy & Hankin, 1966).

The n-alkanes of herbage species are predominantly odd-chain in the range C_{25}–C_{35} with nonacosane (C_{29}), hentriacontane (C_{31}) and tritriacontane (C_{33}) being the most abundant. Using lambs fed fresh perennial ryegrass (*Lolium perenne*) Mayes & Lamb (1984) reported that the proportion of ingested alkane which was recovered in the faeces increased as the C-chain length increased. The faecal recovery of pentatriacontane (C_{35}) was

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almost quantitative (0·98) but its relatively low concentration in herbage (12 mg/kg D.M.) may limit the accuracy of digestibility and intake estimations. Whilst the concentrations in herbage of C_{29}, C_{31} and C_{33} are 5–9 times higher than that of C_{35} the use of these alkanes as digestibility markers is dependent upon the faecal recovery of these alkanes being known and universally constant and this may not be the case.

Errors in digestibility and intake estimation due to between-animal variations in recovery of alkanes in faeces could be eliminated if such faecal recoveries could be concurrently estimated in the same animals. This could be achieved by dosing with n-alkanes of similar chain lengths to those of herbage as long as the faecal recoveries of dosed and natural alkanes are always the same and faecal output is determined. Suitable alkanes to use as dosed markers could be octacosane (C_{28}) or dotriacontane (C_{32}) as they can be easily obtained in pure form at low cost and are present in herbage at very low concentrations. Herbage intakes (without digestibility estimates) could be directly determined using dosed alkanes without the need for estimation of faecal output.

The purpose of the experiment described in this paper was to assess the use of dosed and herbage alkanes as a means of estimating herbage intake. The faecal recoveries of dosed C_{28} and C_{32} were compared with those of herbage alkanes in an indoor experiment in which the effects of level of intake, supplementary feeding and method of dosing were also investigated.

MATERIALS AND METHODS

Animals, treatments and feeds

Twelve Scottish Blackface wether lambs, aged 4 months and weighing about 30 kg, were used. For at least 4 weeks prior to the start of the experiment (August 1984) they grazed a perennial ryegrass sward adjacent to the site from which the cut herbage used in the experiment was derived. Two lambs were allocated to each of the dietary treatments shown in Table 1.

Within each treatment a lamb was allocated to one of two dosed alkane treatments: A, 130 mg/day each of C_{28} and C_{32}, and B, 130 mg/day each of C_{28} and C_{32} mixed with palmitic and stearic acids.

Table 1. *Dietary treatments (two lambs/treatment)*

Treatment	Herbage offered (g D.M./day)	Concentrate offered (g D.M./day)
GL	500	—
GM	700	—
GH	900	—
GCL	345	155
GCM	490	210
GCH	630	270

The cutting area comprised predominantly perennial ryegrass species. The herbage was cut from a 6-week regrowth each afternoon with an Allen Scythe, chopped into approximately 3 cm lengths and stored at 4 °C overnight. The herbage was offered the following day allowing a D.M. estimation to be made and the desired weight of herbage to be offered. The herbage contained 0·937 grasses and 0·063 white clover on a D.M. basis. The pelleted concentrate contained 700 g barley, 100 g sugar beet pulp, 60 g soya-bean meal and 60 g white fish meal per kg. The chemical composition of the herbage and concentrate are given in Table 2.

Preparation of pellets containing C_{28} and C_{32} alkanes

In order to prepare 125–130 pellets 13 sheets of Whatman No. 1 filter paper were trimmed to fit a 35 × 49 cm aluminium tray. Whilst the tray with paper was heating in an oven at 100 °C, 17 g each of C_{28} (Sigma London Ltd, Poole, Dorset) and C_{32} (Koch-Light Ltd, Haverhill, Suffolk) (treatment A) and, in addition for treatment B, 17 g each of palmitic and stearic acids (Sigma London Ltd, Poole, Dorset) were dissolved in 500 ml n-heptane by heating to 80 °C. The hot solution was added to the heated tray and the sheets of paper were shuffled in order to achieve uniform absorption of the solution. The sheets were separated and hung up to dry in a well-ventilated room. The dried sheets were individually placed in an oven at 100 °C for 2 min to allow the paper to absorb the alkanes. When cool the paper was shredded with a guillotine. The weighed amount

Table 2. *Chemical composition of herbage and concentrate*

	D.M. (g/kg)	Ash (g/kg D.M.)	N (g/kg D.M.)	Acid detergent fibre (g/kg D.M.)
Herbage	157 ± 3·9*	101·2†	32·2†	248·3†
Concentrate	869	70·5	27·3	66·5

* S.E. of mean of eight daily samples taken over faecal collection period.
† Bulked herbage sample from eight daily samples.

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of shredded paper for each pellet was compacted by squeezing through a tube of 1·2 cm internal diameter and wrapped in a 12 × 7 cm sheet of tissue paper using starch paste as adhesive. The pellets were dried at 40 °C.

Experimental procedures

The lambs were allocated to dietary treatment immediately upon housing. For the first 9 days they were housed in individual pens on slatted floors and thereafter housed in metabolism cages fitted with chutes and separators for excreta collection. Herbage was offered in two meals at 09.30 and 16.30 h each day; concentrate was offered at 09.30 h. From the 10th day each sheep received an alkane pellet administered with a dosing gun immediately before the morning feed. Five days after beginning alkane dosing total daily faeces collections were carried out for 11 days; on the final 8 days subsamples of the daily faecal output were taken and bulked and any feed refusals were also bulked for each sheep. For 24 h after completion of the balance period faeces were collected every 3 h from each sheep. Samples of the herbage offered each day, refusals and faeces were stored at −20 °C until freeze-dried and milled through a 1 mm screen before analysis.

Analysis of n-alkanes

Samples of freeze-dried herbage (3 g), refusals (3 g) and concentrate (10 g) were extracted for 6 h with petroleum spirit (B.P. 40–60°) in a Soxhlet extraction system after prior addition to the extraction thimble of 0·6 mg tetratriacontane (C_{34}, Sigma London Ltd, Poole, Dorset) as an internal standard. Faeces samples (1 g) were extracted, together with 0·6 mg C_{34} internal standard, with the same solvent for 40 min in a Soxtec system 1040 extraction unit (Tecator Ltd, Bristol). The lipid extracts were transferred to 100 × 20 mm thick-walled screw-topped Pyrex test-tubes. After removal of residual solvent 5 ml 1 M alcoholic KOH solution was added and the tubes were stoppered with PTFE-lined caps. The tubes were heated overnight in a dry-block heater at 90 °C. After partial cooling 5 ml hexane and 2 ml water were added and the tubes were shaken vigorously. The top (non-aqueous) liquid layer was removed, evaporated to dryness, re-dissolved in 1·5 ml hexane and applied to the top of a small column (disposable polypropylene column, Supelco Inc., Bellafonte P.A., U.S.A.) containing silica gel (Kieselgel 60, 70–230 mesh, Merck, Darmstadt, W. Germany) with a bed volume of 5 ml. The hydrocarbons were eluted with 10 ml hexane. The eluate was concentrated to 150–300 μl; 1 μl was injected onto a 1·6 m × 4 mm glass column containing 3% SE-30 on 100–120 mesh Supelcoport (Supelco Inc., P.A., U.S.A.) in a Model 104 gas chromatograph fitted with a flame ionization detector (Pye Unicam Ltd, Cambridge). The chromatograph oven was maintained at 275 °C and the carrier gas, N_2, had a flow rate of 30 ml/min. Peak areas of the n-alkanes were determined using a Pye Unicam CDPI computing integrator. The carbon chain lengths of the n-alkanes were deduced by their retention times relative to known alkanes (Jones, 1970).

The alkane contents of the dosed pellets were determined by extraction of complete pellets for 10 h in a Soxhlet extractor using petroleum spirit (B.P. 40–60 °) after weighing 120 mg C_{34} internal standard into the extraction thimble and perforating the paper wrapper of the pellet. A portion of the extract was then submitted to the same analytical procedures as the dietary and faecal extracts.

Other chemical analysis

Organic-matter contents were determined by ashing samples overnight in a muffle furnace at 550 °C. Acid detergent fibre in herbage was estimated by the method of Van Soest (1965). Herbage N was determined by a macro-Kjeldahl digestion followed by determination of ammonia by an automated procedure.

Calculations

The herbage intake was calculated from a pair of a natural dietary alkane, i, and a dosed alkane, j, as follows:

$$\text{herbage intake (kg D.M./day)} = \frac{\frac{F_i}{F_j}(D_j + I_c.C_j) - I_c.C_i}{H_i - \frac{F_i}{F_j}.H_j},$$

where H_i, C_i and F_i are the respective concentrations (mg/kg D.M.) of the natural odd-chain alkane in herbage, concentrate and faeces; H_j, C_j and F_j are the respective concentrations (mg/kg D.M.) of the even-chain alkane in herbage, concentrate and faeces; I_c is the intake of concentrate (kg D.M./day) and D_j is the amount of alkane j, dosed by pellet (mg/day).

The intake of herbage was also calculated from the C_{35} contents of herbage, concentrate and faeces and from the faecal D.M. outputs using the following equation:

$$\text{herbage intake (kg D.M./day)} = \frac{F_o.F_i - I_c.C_i}{H_i},$$

where F_o represents faecal D.M. output (kg/day).

Statistical methods

Measurements of *in vivo* digestibility, and concentrations and recoveries of n-alkanes in the faeces, were statistically analysed as a 2 × 3 × 2

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Table 3. *n-alkane (C_{27}–C_{35}) contents in herbage and concentrate (mg/kg* D.M.*) and of alkane pellets (mg/pellet)*

	n-alkane							
	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{35}
Herbage (mg/kg D.M.)	19*	5	73	9	137	9	116	18
S.E.	0·4	0·4	1·3	0·3	2·6	0·3	1·7	0·3
Concentrate (mg/kg D.M.)	2	1	5	1	7	1	1	0
Pellets A (mg/pellet)	—	139†	—	—	—	130	—	—
S.E.	—	2·9	—	—	—	1·1	—	—
Pellets B (mg/pellet)	—	137†	—	—	—	131	—	—
S.E.	—	1·4	—	—	—	1·0	—	—

* Mean of eight daily herbage cuts taken over faecal collection period.
† Means of six pellets per treatment.

Table 4. *Mean daily outputs of faecal* D.M. *and concentrations of C_{27}–C_{35} n-alkanes in faeces of lambs during the 8-day collection period*

Lamb	Treatment	Faecal D.M. output (g/day)	n-alkane (mg/kg D.M.)							
			C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{35}
1	G LA	125	56	836	217	28	463	923	408	66
2	G LB	132	51	846	205	28	445	933	386	63
3	G MA	197	50	600	195	25	410	649	367	60
4	G MB	185	44	547	179	23	381	601	338	55
5	G HA	251	51	471	198	25	417	506	367	60
6	G HB	264	48	427	190	24	403	475	357	58
7	GCLA	115	42	920	172	23	383	1070	331	53
8	GCLB	124	37	869	159	22	361	1028	314	51
9	GCMA	178	41	593	154	19	315	635	269	43
10	GCMB	180	36	615	149	20	320	702	282	46
11	GCHA	188	36	522	147	19	323	611	283	46
12	GCHB	227	36	445	147	19	323	511	285	46

factorial design, assuming the third-order interaction (diet × feeding level × pellet type) to represent the residual term. The accuracy of estimations of herbage intake using various alkanes as markers was assessed by summing the squares of discrepancies between actual and estimated herbage intake for each method of intake estimation. Evidence of bias in intake estimation was assessed by testing the significance of the difference between the mean discrepancy and zero.

RESULTS

Over the 8-day balance period daily herbage refusals were very small, less than 0·05 of herbage offered, except for two sheep whose refusals were 0·07 and 0·19 of herbage offered. There were no concentrate refusals. *In vivo* apparent D.M. and organic-matter (OM) digestibility was not affected by feeding level but the diet including concentrate had higher ($P < 0·05$) mean D.M. (0·753, S.E. 0·0050) and OM (0·776, S.E. 0·0059) digestibilities than the herbage alone (D.M. 0·721, S.E. 0·0069; OM 0·743, S.E. 0·0066).

The n-alkane contents (C_{27}–C_{35}) of herbage, concentrate and of alkane pellets are given in Table 3. C_{31} was the most abundant alkane in the herbage followed by C_{33} and C_{29}. Even-chain alkanes constituted only about 0·06 of the total C_{27}–C_{35} alkanes in herbage. The between-day coefficients of variation (C.V.) of the odd-chain alkanes in herbage ranged from 4·1% (C_{33}) to 5·6% (heptacosane, C_{27}); the C.V.s of the even-chain alkanes were higher (mean 14·1%). C_{31} was also the most

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Table 5. *Faecal recoveries (proportion of ingested recovered in faeces) of C_{27}–C_{35} n-alkanes over the 8-day collection period*

Lamb	Treatment	n-alkane C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{35}
1	G LA	0·742	0·744	0·745	0·825	0·843	0·859	0·876	0·912
2	G LB	0·731	0·799	0·766	0·880	0·883	0·908	0·904	0·945
3	G MA	0·772	0·835	0·781	0·848	0·875	0·937	0·922	0·966
4	G MB	0·657	0·723	0·695	0·748	0·788	0·813	0·820	0·860
5	G HA	0·755	0·829	0·766	0·804	0·856	0·920	0·888	0·932
6	G HB	0·741	0·798	0·761	0·812	0·856	0·890	0·894	0·938
7	GCLA	0·700	0·752	0·746	0·844	0·886	0·919	0·922	0·960
8	GCLH	0·665	0·778	0·753	0·882	0·912	0·951	0·956	0·998
9	GCMA	0·776	0·751	0·756	0·803	0·823	0·841	0·843	0·863
10	GCMB	0·697	0·796	0·745	0·844	0·855	0·935	0·903	0·951
11	GCHA	0·662	0·695	0·714	0·784	0·839	0·851	0·889	0·928
12	GCHB	0·656	0·721	0·706	0·760	0·828	0·849	0·877	0·918
	General mean	0·713	0·768	0·745	0·820	0·854	0·889	0·891	0·931

abundant n-alkane in the concentrate. However, the total C_{27}–C_{35} n-alkane content of concentrate was less than one-twentieth of that of herbage.

The daily amounts of faecal D.M. and the C_{27}–C_{35} n-alkane concentrations in faeces from the lambs over the 8-day collection period are shown in Table 4.

The presence of concentrate in the diet did not affect the faecal concentrations of dosed alkanes (C_{28} and C_{32}) but, as would be anticipated, significantly ($P < 0·01$) reduced the concentrations of all other alkanes. Increasing the level of feeding significantly ($P < 0·05$) decreased the faecal concentrations of all alkanes except for C_{35}. The only alkane concentrations apparently affected by pellet type were those of C_{27} ($P < 0·01$) and C_{29} ($P < 0·05$) alkanes; pellet A gave higher concentrations than pellet B. With the exception of the diet × feeding level term for faecal C_{27} concentration, no interaction effects were statistically significant.

From the intakes and amounts of faeces excreted over the 8-day collection period, and the alkane

Table 6. *Estimates of daily herbage* D.M. *intake (g) using n-alkanes*

Lamb	Treatment	Actual herbage intake	Herbage intake estimates* C_{27}–C_{28}	C_{29}–C_{28}	C_{31}–C_{32}	C_{33}–C_{32}	C_{35}-faeces output
1	G LA	504	502	505	495	515	460
2	G LB	485	443	465	472	484	459
3	G MA	676	624	632	629	665	654
4	G MB	657	594	630	634	664	565
5	G HA	896	813	826	830	863	835
6	G HB	909	842	866	862	902	853
7	GCLA	354	328	352	341	355	340
8	GCLB	351	298	340	336	353	350
9	GCMA	488	505	492	477	490	421
10	GCMB	483	420	451	439	466	460
11	GCHA	515	488	530	507	539	478
12	GCHB	631	573	621	618	656	582
Discrepancy† (actual intake – calculated intake):							
		Σ (discrepancy)²	32012	11374	12324	2788	27705
		Mean discrepancy	44	20	26	0	41

* First four columns represent intake estimates using alkane pairs: natural alkane–dosed alkane, equation in text p. 163. Fifth column represents intake estimate using C_{35} as an indigestible internal marker, equation in text p. 163.

† See text, p. 164 for explanation.

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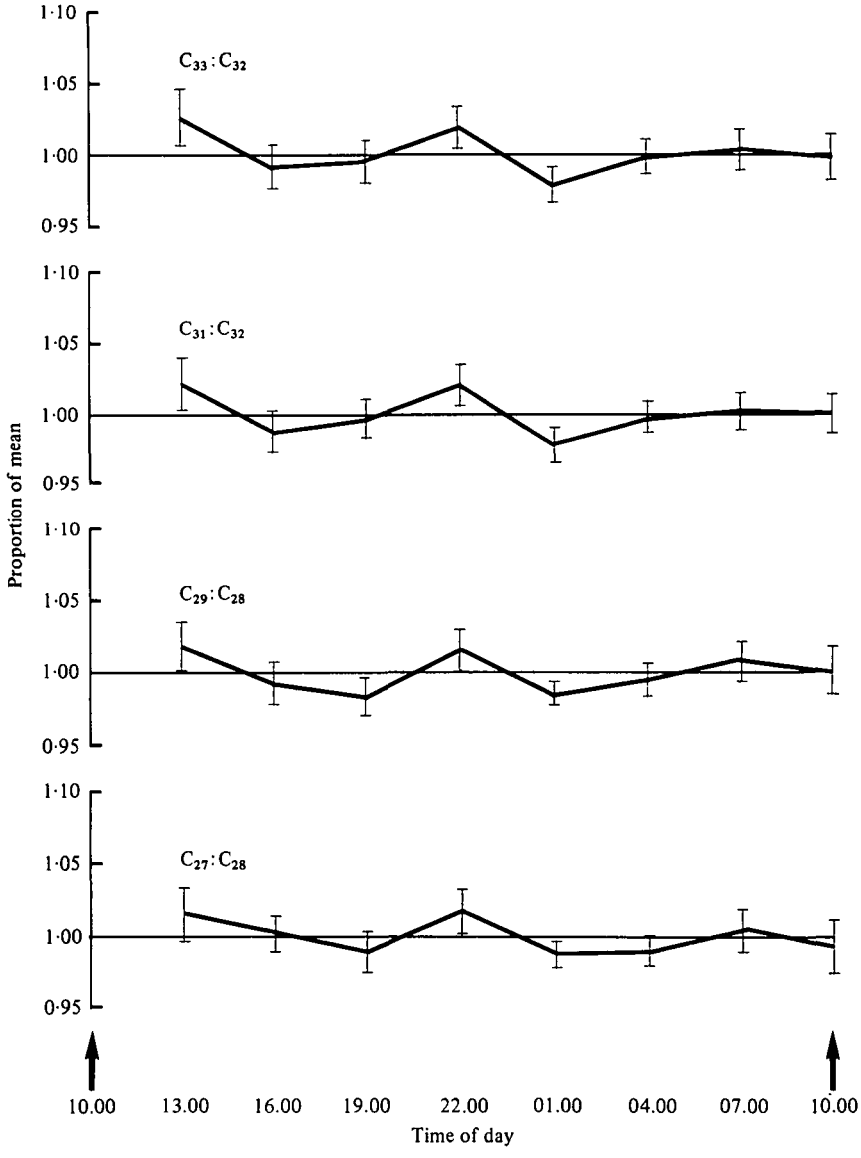

Fig. 1. Mean variations throughout the day in ratios in faeces of odd-chain (herbage) n-alkane concentrations to even-chain (dosed) n-alkane concentrations expressed as proportions of the mean value for each lamb ($\pm$S.E.; $n = 12$). ↑, Dose.

contents of herbage, concentrate, alkane pellets (Table 3) and faeces (Table 4) the faecal recoveries of the C_{27}–C_{35} alkanes (proportion of ingested alkane recovered in the faeces) for each lamb were calculated and are shown in Table 5.

The faecal recoveries of n-alkanes were not significantly affected by diet, feeding level, pellet type or interactions with one exception: the faecal recovery of C_{27} was lower when concentrate was given ($P < 0{\cdot}05$) and lower with pellet B than A ($P < 0{\cdot}05$). Mean faecal recoveries of alkanes increased as the C chain length increased. The recovery of C_{28}, a dosed alkane, was slightly higher than the recoveries of both C_{27} and C_{29} natural alkanes. The recovery of the other dosed alkane, C_{32}, was similar to that of the natural alkane, C_{33}.

Estimates of daily herbage D.M. intakes of the lambs over the 8-day collection period, from pairs

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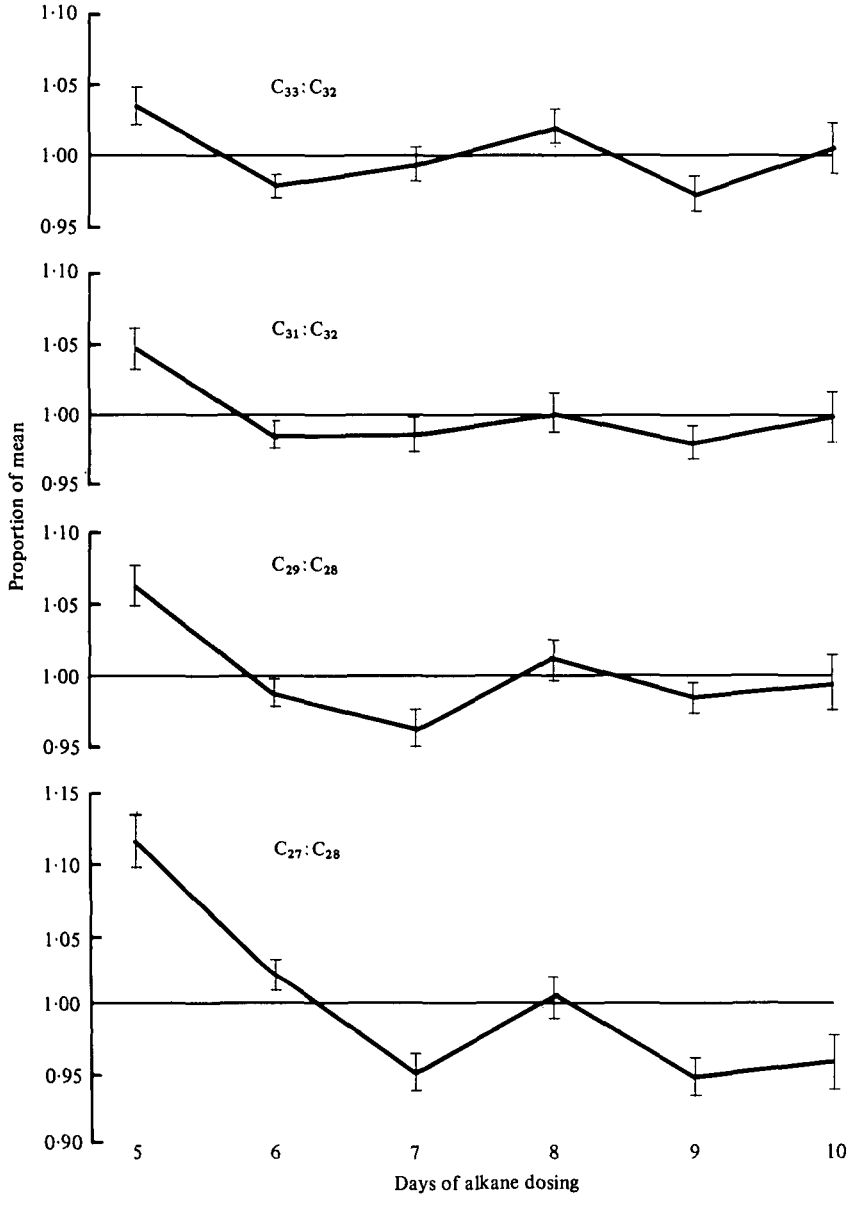

Fig. 2. Changes in mean ratios in faeces of odd-chain (herbage) n-alkane concentrations to even-chain (dosed) n-alkane concentrations with time (days) after commencement of alkane dosing. Values are expressed as proportions of the mean for each lamb ($\pm$ S.E.; $n = 12$).

of dosed and adjacent natural alkanes and from C_{35} natural alkane and actual faecal output, using the formulae given on p. 163 are shown in Table 6.

The accuracy of prediction of herbage intake was assessed for the different estimates by consideration of the discrepancies between actual intakes and intakes estimated from n-alkanes. The C_{33} natural alkane–C_{32} dosed alkane pair gave the lowest discrepancy sum of squares followed by the C_{29}–C_{28} natural–dosed alkane pair. The poorest estimators of intake were the C_{27}–C_{28} alkane pair and C_{35}-faeces output internal marker method. With the exception of the C_{33}–C_{32} alkane pair all methods underestimated actual intake, albeit to a small extent. Percentage underestimates were as follows: C_{27}–C_{28}, 7·6; C_{29}–C_{28}, 3·5; C_{33}–C_{32}, 4·5; C_{33}–C_{32}, 0·0;

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C_{35}-faeces output, 7·1. The discrepancies in intake estimates from actual intakes were not affected by diet, feeding level or type of alkane pellet administered.

The faeces samples collected every 3 h over a 24 h period following the 8-day balance period were used to assess the variability in alkane excretion throughout the day, so that the potential of using faecal grab samples for intake estimation at pasture could be assessed. As intake estimations require the ratio of faecal concentrations of a natural and a dosed alkane, within-day variations in such ratios are more relevant to consider than variations in individual faecal concentrations. Figure 1 shows the mean variations throughout the day in the ratios of concentrations of natural to dosed alkanes, expressed as proportions of the mean ratio for each lamb, such that the estimates at each sample time can be normalized across animals. As no effects of diet, feeding level or type of alkane pellet were evident each point in Fig. 1 represents a mean of 12 observations. There was no evidence of systematic variation throughout the day as none of the points was significantly different from unity.

Day-to-day variation in ratios of faecal concentrations of natural to dosed alkanes from 5 to 10 days after the start of alkane dosing is shown in Fig. 2.

The values were expressed as proportions of the mean. For the first day of faeces collection (day 5 after dosing began) ratios of C_{27}:C_{28} and C_{29}:C_{28} were significantly higher than the mean values for days 5–10. There was no evidence of any effect of diet, feeding level or type of alkane pellet upon the between-day variation in faecal concentration ratios of dosed and natural alkanes.

DISCUSSION

Faecal recoveries of n-alkanes

Whilst the occurrence of n-alkanes in forage plant species has been reasonably well documented (Tulloch, 1976), their potential as internal markers had not been considered until recently (Mayes & Lamb, 1984; Gosden & Moseley, 1984). Little is known of their fate in the ruminant digestive tract. It is not known if dietary alkanes remain attached to particulate material or enter the liquid phase of digesta. Although some bacterial species have been shown to utilize n-alkanes (McKenna & Kallio, 1965; Hankin & Kolattukudy, 1968) and others to synthesize n-alkanes (Albro, 1976), the general similarity in relative proportions of individual alkanes in the diet and faeces of ruminants (Oró, Nooner & Wikström, 1965; R. W. Mayes, unpublished) suggests that the microbial population of the ruminant digestive tract has little influence upon the metabolism of herbage alkanes. Both monogastrics and ruminants (McCarthy, 1964; Savary & Constantin, 1966) can absorb and metabolize hexadecane, a medium chain-length alkane (C_{16}) and rats can utilize the plant wax alkane, C_{29}, to a limited extent (Kolattukudy & Hankin, 1966). It is thus probable that ruminants can utilize herbage alkanes to some degree.

The observed increase in faecal recovery values of odd-chain herbage alkanes with increase in C chain-length is in agreement with previous reports (Mayes & Lamb, 1984; Gosden & Moseley, 1984). Reduction in utilization of alkanes as chain length increases has been observed in other organisms, for example *Aspergillus versicolor* (Hopkins & Chibnall, 1932).

If odd- and even-chain alkanes behave similarly in the digestive tract, the faecal recoveries of even-chain alkanes may be expected to be intermediate between the recovery values of adjacent odd-chain alkanes. Identical faecal recoveries of herbage (odd-chain) and dosed (even-chain) alkanes are a prerequisite for accurate herbage intake estimates. The use of a dosed alkane and an adjacent shorter odd-chain herbage alkane would be expected to give a slight underestimate of herbage intake, whereas the use of the longer odd-chain adjacent alkane should slightly overestimate intake. Thus a mean of the two estimates should give an estimate of herbage intake close to the true value. However, in practice, owing to the recovery of dosed C_{28} being higher than the recoveries of both C_{27} and C_{29}, and that of C_{32} being the same as C_{33}, the best estimates of herbage intake were found to be from C_{28}–C_{29} and C_{32}–C_{33} alkane pairs, rather than from the mean estimates from C_{28}–C_{27} and C_{28}–C_{29} and of C_{32}–C_{31} and C_{32}–C_{33} alkane pairs. Further work is necessary to show whether the higher faecal recoveries of even-chain alkanes relative to odd-chain alkanes is a general phenomenon. Gosden & Moseley (1984) found that the faecal recoveries of even-chain alkanes occurring in herbage were higher than those of odd-chain alkanes of similar chain length. However, the quantities of naturally occurring even-chain alkanes ingested are much less than the odd-chain alkanes whereas the lambs in the experiment described here received C_{28} and C_{32} in amounts similar to the predominant odd-chain alkanes of herbage. It is possible that the method of administration of C_{28} and C_{32} may explain their higher recoveries relative to the odd-chain alkanes. Herbage alkanes are distributed in the thin film of wax on the plant surface whereas the dosed alkanes were absorbed into the matrix of the shredded paper; this may render the dosed alkane less available for absorption. The purpose of incorporating stearic and palmitic acids with C_{28} and C_{32}-dosed alkanes (pellet treatment B) was to facilitate micelle formation, should emulsification be an important

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factor affecting faecal recovery. Although Savary & Constantin (1967) showed that oleic acid increased micellar solubilization of C_{16} in rats the lack of any effect of the presence of palmitic and stearic acids on n-alkane recoveries (with the exception of C_{27} recovery) in the present study suggests that additional emulsification agents are not necessary for accurate intake estimation. However, this does not imply that the accuracy of intake estimation cannot be further improved by modification of the method of administration.

The practical application of dosed and herbage alkanes to estimate herbage intake in grazing animals

The results of the experiment described above suggest that good estimates of herbage intake can be obtained with little bias by using C_{32} as the dosed alkane and C_{33} as the herbage alkane. Reasonable estimates of intake can also be obtained using C_{28}–C_{29} as the dosed alkane–herbage alkane pair, which may be useful for forages such as brassicas which contain C_{29} as the predominant alkane (Kolattukudy, 1965). The validity of herbage intake estimates from C_{28}–C_{29} and C_{32}–C_{33} alkanes is apparently unaffected by feeding level or by the presence of concentrate in the ration; thus unbiased estimation of herbage intake should be possible in animals receiving supplementary feed or in suckling lambs.

Errors in estimation of herbage intake by grazing animals, using alkanes as markers, may be expected to be larger than those found in this study, as there are likely to be more sources of variation. It is necessary to obtain truly representative samples of ingested herbage. Oesophageal fistulated animals should provide such samples from extrusa collections. Salivary contamination of the extrusa sample should have little effect upon its alkane content. However, if some alkanes pass into the saliva during eating and the ratio of solid to liquid in the sample differs from the ratio in swallowed material, the extrusa sample may not be representative in respect of alkane concentration. Investigations are in progress to establish whether such problems exist. It may be possible under some circumstances to use clipped or hand-plucked samples where variations in alkane concentration between plant components are low within the same species. In common with external markers such as chromium sesquioxide (Cr_2O_3) (Langlands, 1975), diurnal variations in faecal excretion of alkanes could increase errors in herbage intake estimation as the composition of faecal grab samples is assumed to be representative of that of the total faecal output over the collection period. There is thus a need to define a strategy for frequency of alkane dosing and faecal sampling and to establish the length of time animals are dosed prior to faecal sampling. Whilst the conditions of the indoor experiment described here may not truly represent those of grazing experiments the results suggest that diurnal variation in faecal excretion of alkanes is small and dosing and faecal sampling once each day should be adequate. At least 6 days of dosing with alkanes should be allowed before commencing faecal sampling.

Although the results reported here are encouraging, there are areas which require further examination. It is possible that the relative faecal recoveries of alkanes may vary with age, physiological state and species of animal due to differences in conditions within the digestive tract. Also, the concentrations of different alkanes in herbage can vary greatly among species and, within species, variations in alkane concentrations with plant age and with different parts of the plant have been observed (R. W. Mayes, unpublished data). It is not yet known if faecal recoveries of n-alkanes are affected by their concentrations in the forage.

Other possible applications of alkanes as markers

The concurrent dosing with C_{28} or C_{32} alkanes and Cr_2O_3 enables both herbage intake and diet digestibility to be estimated in the same animal. Hexatriacontane (C_{36}) is an alkane not present in herbage and, if it were to behave according to the pattern of recovery of other herbage alkanes, it should be completely recovered in the faeces. If so, it could substitute for Cr_2O_3 and, if dosed together with C_{28} or C_{32}, would allow the measurement of intake and digestibility from alkane determinations in faeces and herbage with a knowledge of alkane dose rates.

Different plant species differ widely in the relative proportions of individual n-alkanes. It may be possible to establish the proportions of different plant species ingested, from the patterns of alkanes found in the faeces relative to the alkane patterns of the plant species in the diet. Faecal recoveries of the various herbage alkanes may be established by dosing with a mixture of even-chain alkanes ranging from tetracosane (C_{24}) to C_{36}, and interpolation of the resultant relationship between recovery and C-chain length. Although in theory the number of dietary components can be as large as the number of herbage alkanes estimated, perhaps the most useful application may be in simpler situations such as in determining the ratios of clover to grass in the diet of sheep or cattle grazing such a mixed sward.

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