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The effects, in sheep, of dietary plant species and animal live weight on the faecal recovery rates of alkanes and the accuracy of intake and diet composition estimates obtained using alkanes as faecal markers

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SUMMARY

Alkanes can be used as natural markers for estimating diet composition, but a factor should be used to correct for incomplete recovery in faeces. Faecal alkane recovery rates may be influenced by diet and animal factors. However, little research has been conducted to evaluate the effects of herbage species and live weight of animals on faecal alkane recoveries. In the current study, faecal recoveries of alkanes were determined in sheep in four different live weight groups (from 20 to 40 kg) fed three plant species (*Elymus sibiricum*, *Leymus chinensis* and *L. dasystachys*). In a second experiment, the accuracy of intake and diet composition estimates, using alkanes as faecal markers, was assessed by feeding known amounts of the same plant species as a three-component mixture. The results showed that faecal alkane recoveries were influenced significantly by herbage species ($P<0{\cdot}01$), but no effect of live weight of animals was observed. Total dry matter intake was estimated correctly based on either C31:C32 or C29:C32 alkane pairs. With respect to estimators of *E. sibiricum* intake, reasonable results could only be obtained if the faecal alkane concentration was corrected based on diet-specific faecal recovery. More accurate estimations were obtained only if the alkanes found in relatively higher concentrations were used in diet composition estimates instead of using all available alkanes. Due to lower alkane concentrations or similar alkane patterns of *L. chinensis* and *L. dasystachys* in the diet, estimates of diet composition of these two herbage species were significantly different from the actual ones ($P<0{\cdot}05$), implying that other markers need to be used for accurate estimation.

INTRODUCTION

The alkane technique has been shown to be an effective method for the estimation of animal intake and dietary composition (Mayes *et al.* 1986; Dove & Mayes 1991, 1996; Mayes & Dove 2000). When faecal alkane concentrations are used to estimate diet composition, corrections for incomplete faecal recovery of alkanes might be necessary (Dove & Mayes 1991, 1996).

Previous studies have indicated that the faecal alkane recovery rates were unaffected by diet (Mayes *et al.* 1986; Brosh *et al.* 2003) or feeding level (Mayes *et al.* 1986; Dove & Oliván 1998; Dove *et al.* 1989*a*; Elwert *et al.* 2004). However, Dove *et al.* (1989*a*) found differences in faecal alkane recovery between experiments that offered different diets. Additionally, the dietary treatments in the studies of Mayes *et al.* (1986) contained the same herbage species (grasses and white clover) but different feeding levels, or an altered component (pelleted concentrate) containing a negligible quantity of alkanes.

More research is needed to evaluate possible effects of animal factors such as species, breed, age and physiological and reproductive states, in addition to diet factors such as n-alkane source, or natural

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n-alkane chain length (even or odd), on n-alkane recovery in faeces (Brosh *et al.* 2003). Estimates of alkane recovery in cattle have been more variable than in sheep (Dove & Mayes 1991). The incomplete recovery of alkanes is due to absorption from the small intestine. The effect of live weight on alkane recovery should be evaluated before further evaluation of other factors. Meanwhile, live weight is important for breed selection at the same stage of physiological development of an animal in breeding systems. However, there is little published information on the effects of herbage species and animal live weight on faecal alkane recovery rates.

The present experiment was designed to: (1) investigate the effects of the dietary herbage species and animal live weight, and n-alkane chain length on faecal alkane recovery rates; (2) evaluate the accuracy and precision of the alkane technique for estimating herbage intake and dietary composition of sheep.

MATERIALS AND METHODS

Animals and diets

All the animal experiments received approval from the China Agricultural University Laboratory Animal Care Advisory Committee.

Experiment 1

Thirty-six young wethers (Inner Mongolia fine wool sheep × Mongolia sheep), 6–8 months of age, were housed indoors and tethered individually with neck chains. The sheep were allocated to four groups (nine sheep per group) on the basis of live weight (I, 21·2 ± 1·2 kg; II, 26·7 ± 0·5 kg; III, 32·1 ± 1·1 kg; IV, 37·5 ± 1·5 kg). Each group was randomly offered *L. chinensis*, *L. dasystachys* or *E. sibiricum* (three sheep per herbage species).

Experiment 2

Six wethers of the same breed as above with an average live weight of 38·5 kg were housed indoors and tethered individually with neck chains. The sheep were fed a three-component mixed diet consisting of *L. chinensis*, *L. dasystachys* and *E. sibiricum* in equal proportions on a DM basis (1:1:1).

In both experiments, fresh herbage was harvested daily and divided into three approximate equal portions and offered at 09:00, 12:00 and 16:00 h. The amount fed to each sheep was adjusted before the lead-in period, so as to leave less than 5% refusals (Brosh *et al.* 2003). The material was chopped into approximately 20 mm lengths from which length it was assumed that the animals are unable to select, even for leaves or stem (Hameleers & Mayes 1998); refusals were therefore not evaluated for their botanical composition.

Sampling procedures

In Expt 1, the experimental period consisted of a 10-day lead-in period followed by a 7-day faecal and herbage collection period. On day 5, faecal collection bags were fitted to each sheep for adaptation. Experiment 2 contained a 10-day adaptation period, a 6-day build-up phase, and a 7-day sampling period. Faecal collection bags were adapted for sheep at the beginning of the build-up period. On day 10, sheep were dosed with a controlled release device capsule containing 1 g of dotriacontane (C_{32}) and 1 g of hexatriacontane (C_{36}) (Captec^{TM}; New Zealand) designed for 25–80 kg live weight sheep. The expected mean release rates of alkanes documented by the company were 52·3 and 52·0 mg/day for C32 and C36 respectively.

In both experiments, total daily faecal collections were carried out, using faecal collection bags, which were emptied daily at 08:00 h during the 7-day collection period. The weight of the faeces was recorded and a representative sample of 20% was taken (Elwert *et al.* 2004).

Refusals were weighed daily at 18:00 h to estimate the actual DM intake. Herbage samples were taken on a daily basis 2 days ahead of the respective faecal sample.

Sample preparation

Samples of the herbage and faeces were immediately dried on the day of collection, using a forced-air oven at 65 °C for 48 h, and ground through a 1 mm screen and stored at room temperature until alkane analysis.

Analysis

Alkanes were extracted and analysed according to the method of Mayes *et al.* (1986), modified by Zhang *et al.* (2002). The samples (1·0 g) were placed in Pyrex bottles (IWAKI glass, Japan) with two replicates. Two internal standards (0·2 mg n-Docosane (C22) + 0·2 mg n-Tetratriacontane (C34)) and 10 ml ethanolic KOH of 1·5 M were added into the samples. The bottles were capped tightly; the contents were then well mixed and heated at 90 °C for 4·5 h. Alkane extraction from herbage samples was performed by adding 7 ml of heptane and 5 ml of distilled water, shaking and heating in a water bath at 65 °C for 10 min (Chen *et al.* 1998; Oliván & Osoro 1999), and then centrifuging them at 2000 rpm for 5 min before transferring the upper solvent layer to a glass vial and repeating the extraction with another 7 ml of heptane. The pooled extracts were evaporated with dry air and the residue was re-dissolved in 2 ml of heptane before applying to 2 g Silica Gel 60 column (230–400 mesh, Merck, Germany) with a 5 ml bed volume, and eluted with an additional 8 ml of heptane. The eluent was evaporated by air pump,

re-dissolved in 1 ml of heptane, and then analysed in a gas chromatograph.

Gas chromatography

Alkane extracts were taken up in 1 ml of heptane, and 1 μl of final re-dissolved alkane solution was injected into GC-2010 gas chromatography (Shimadzu Company, Japan) fitted with a flame ionization detector. A TC-1 high-resolution capillary column, 0·25 mm in inner diameter and 30 m long, 0·25 μm in film thickness was used with split autojector (split ratio 50:1). Helium was used as a carrier gas at a constant flow of 0·98 ml/min. The temperature for the injector and detector ovens was maintained at 350 °C. The column oven temperature was programmed to 200 °C, maintained for 30 s and then increased by 6 °C/min to 300 °C, which was then maintained for 10 min.

Calibration

A standard solution containing a mixture of synthetic alkanes from C20 to C36 was prepared with five different concentrations. The response factors for each alkane were calculated from peak areas and the known concentrations with linear calibration curve method. Extraction rate was calculated from two internal standards (C22 and C36). Alkane concentrations in samples were calculated by reference to the difference of the extraction rates of two internal standards.

Calculations

Actual herbage intakes were recorded daily. Intakes were also calculated daily using the equation proposed by Mayes *et al*. (1986) and expressed below in a simplified version (Ferreira *et al*. 2004):

$$\text{Herbage intake (kg DM/day)} = \frac{\text{Dj}}{\frac{\text{Fj}}{\text{Fi}} \times \text{Hi} - \text{Hj}}$$

Where Dj is the release rate of the CRC for C32 (mg/day), Fi and Hi are the respective concentrations (mg/kg DM) of C29, C31 or C33 in faeces and herbage, and Fj and Hj are the concentrations (mg/kg DM) of C32 in faeces and herbage, respectively.

Diet proportions were estimated using a non-negative least square algorithm as supplied by the software EatWhat (Dove & Moore 1995). The results of the EatWhat were then transformed into the estimated intake of different dietary components according to the following equation:

$$\text{Dietary component intake (g/day)} = \text{P} \times \text{I}$$

where P is the estimated proportion of the components in the diet, I is the above estimated daily herbage intake.

The faecal concentration of individual alkanes was corrected either by the mean recovery rate of the six animals in Expt 2 (R1), or by its mean recovery rate across all animals given *L. chinensis* (R2). Two combinations of the alkanes were tested, either all seven alkanes (C25, C27, C28, C29, C30, C31 and C33) (A1) or only the four alkanes (C27, C29, C31 and C33) that were found in relatively higher concentrations (A2).

Therefore, the combination of factors resulted in a series of three estimations (E1 = R1 + A1; E2 = R1 + A2; E3 = R2 + A2).

Statistical analysis

All statistical analysis was performed using SPSS11.5 for Windows. The faecal alkane recovery rates in Expt 1 were examined by analysis of variance (ANOVA) to evaluate the effects of herbage species (S) and live weight (LW) of wethers on the faecal recovery rates of individual alkanes. Paired-samples *t*-test was carried out to test the accuracy of estimates.

The accuracy of estimates was also assessed by the Mean Discrepancy (MD) and the Relative Mean Discrepancy (RMD), as follows (Mayes *et al*. 1986; Elwert *et al*. 2004):

$$\text{MD} = \sqrt{1/\text{n} \sum (\text{estimated} - \text{actual})^2}$$

$$\text{RMD} = \sqrt{1/\text{n} \sum ((\text{estimated} - \text{actual})/\text{actual})^2}$$

RESULTS

Experiment 1: effects of herbage species and live weight of wethers on the faecal alkane recovery rates

The patterns of alkane concentrations in the experimental plant species and faeces are shown in Table 1. In general, C27, C29, C31 and C33 alkanes were predominant in the three herbage species as well as in the mixture of herbages and the faeces, constituting 0·847 ± 0·046 of the total C20–C36 alkanes. *E. sibiricum* was characterized by high concentrations of C29 and C31, but *L. chinensis* and *L. dasystachys* had low concentrations of all alkanes. As would be expected, the alkane concentrations in faeces were about twice those in the herbage species offered to the wethers. However, the diet and faeces showed similar alkane patterns (Table 1).

With respect to the faecal alkane recovery rates, significant effects of herbage species were detected ($P < 0{\cdot}01$) (Table 2), accounting for 0·72 ± 0·109 of the total variance, though the effect of herbage species was not significant for C25 ($P < 0{\cdot}05$). No significant effects of live weight or interactions between live weight and herbage species were found, with one

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Table 1. *Alkane concentrations (μg/g DM) in the herbage species and faeces*

Herbages	Species	C20	C21	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32	C33	C35	C36
	L. chinensis	3		2	2	3	2	11	4	26	4	57	2	15		
	L. dasystachys	4		3	2	10	4	28	4	47	4	46	2	12		1
	E. sibiricum	2		3	2	8	2	16	4	114	7	185	4	25		
	Mixture*	3		2	2	7	3	20	5	61	5	83	3	16		
Faeces	Diet															
	L. chinensis	7		5	4	8	5	24	9	60	11	137	5	37		
	L. dasystachys	7	1	5	5	21	7	59	10	89	7	82	3	23		
	E. sibiricum	6		5	4	16	4	36	9	200	14	346	9	54		
	Mixture	6		5	5	13	6	40	10	123	10	182	202	39		152

* Mixture of *L. chinensis*, *L. dasystachys*, and *E. sibiricum* in the rate of 1:1:1.

Table 2. *Faecal alkane recovery rates in animals of different live weight classes (I–IV) and the results of ANOVA in Expt 1*

	I			II			III			IV			Total			Effects (*P*)		
LW* Species	*LC*	*LD*	*ES*	*LC*	*LD*	*ES*	*LC*	*LD*	*ES*	*LC*	*LD*	*ES*	*LC*	*LD*	*ES*	LW	Species	LW × S†
C25	0·84	0·95	0·90	0·86	0·82	1·00	0·84	0·84	0·86	0·91	0·77	0·74	0·86	0·85	0·89	0·19	0·79	0·11
C27	0·88	0·92	1·03	0·89	0·83	1·14	0·83	0·87	0·94	0·94	0·79	0·87	0·88	0·86	1·01	0·13	0·00	0·10
C28	0·94	0·90	1·13	1·00	0·93	1·11	0·96	0·96	1·03	0·85	0·89	0·97	0·95	0·92	1·07	0·02	0·00	0·32
C29	0·91	0·79	0·86	0·90	0·73	0·80	0·83	0·79	0·80	0·97	0·83	0·75	0·89	0·79	0·81	0·26	0·00	0·19
C30	0·96	0·88	1·06	0·94	0·79	0·97	0·95	0·91	0·95	0·99	0·91	0·89	0·96	0·88	0·97	0·29	0·01	0·20
C31	0·96	0·75	0·92	0·92	0·70	0·83	0·92	0·69	0·87	0·95	0·81	0·79	0·94	0·74	0·86	0·44	0·00	0·65
C33	0·97	0·78	1·04	0·94	0·66	0·97	0·91	0·76	0·97	0·99	0·88	0·89	0·95	0·77	0·97	0·32	0·00	0·24
N	3	3	3	3	2	3	3	3	2	2	2	2	11	10	10			

* LW = live weight.
† LW × S = Interaction between live weight and species.
Group: *LC* = *L. chinensis*; *LD* = *L. dasystachys*; *ES* = *E. sibiricum*.

Table 3. *Mean faecal recovery rates for C25–C36 alkanes measured in the six sheep in Expt 2*

Alkanes	C25	C26	C27	C28	C29	C30	C31	C32	C33	C36
Recovery	0·74	0·83	0·84	0·83	0·85	0·96	0·92	0·90	1·00	0·70
S.D.	0·091	0·052	0·073	0·052	0·059	0·060	0·063	0·093	0·095	0·078

exception: the effect of live weight was significant in C28 ($P < 0·05$) (Table 2).

In general, the faecal recovery rates of C28 and C30 alkanes were higher than the adjacent odd alkanes (C27, C29 and C31) (Table 2). The faecal alkane recovery rates were affected significantly by alkane chain length for *L. dasystachys* and *E. sibiricum*, but not *L. chinensis*.

Experiment 2: estimates of herbage intake in wethers

Table 3 shows the mean faecal recovery rates for C25–C36 alkanes measured in the six sheep in Expt 2, which increased with longer carbon chain length, except for C36. The recovery rates of dosed C32 were similar to C31 and C29.

Daily dry matter intakes of the six lambs in Expt 2, estimated using the alkane pairs C29:C32, C31:C32 and C33:C32, are shown in Table 4. No significant differences were found between actual and estimated DM intake from alkane pairs C29:C32 or C31:C32 ($P > 0·05$), but the estimates differed significantly from actual intake using the alkane pair C33:C32 ($P < 0·05$). The C31:C32 alkane pair gave the lower MD (46·7) and RMD (8·2%) than the C29:C32 pair (MD 48·7; RMD 8·9%).

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Table 4. *Actual daily DM intake, and intakes estimated using different alkane pairs in the six sheep used in Expt 2*

Animals	1	2	3	4	5	6	Average	MD*	RMD (%)†	*t*-test (*P*)
Actual mean intake (g DM/day) ($n=7$)										
	590	620	623	525	607	482	574			
Estimated intake (g DM/day)										
C29:C32	625	585	587	428	590	469	548	48·7	8·9	0·185
C31:C32	686	612	625	491	638	516	595	46·7	8·2	0·324
C33:C32	726	638	687	540	690	584	644	87·1	15·5	0·016

* $MD=\sqrt{1/n\sum(\text{estimated}-\text{actual})^2}$.

† $RMD=\sqrt{1/n\sum((\text{estimated}-\text{actual})/\text{actual})^2}$.

Table 5. *Actual daily DM intakes and intakes estimated using alkane markers, of* E. sibiricum (*ES*), L. chinensis (*LC*), L. dasystachys (*LD*) *and* L. chinensis (*LC*) + L. dasystachys (*LD*), *in the six sheep used in Expt 2*

Animals	1	2	3	4	5	6	MD*	RMD (%)†	*t*-test (*P*)
Actual mean daily intake (g DM/day) ($n=7$)									
ES, LC or LD	197	207	208	175	202	161			
LC+LD	393	414	415	350	405	321			
Estimated daily intake (g DM/day)‡									
E1									
LC	90	0	41	132	65	97	133·2	66·0	0·005
LD	392	392	388	233	375	285	160·0	81·5	0·001
ES	205	220	196	125	198	134	24·6	14·1	0·275
LC+LD	482	392	429	365	440	382	47·9	12·9	0·099
E2									
LC	79	0	29	124	52	91	140·7	69·9	0·004
LD	398	392	394	237	382	287	164·3	83·7	0·001
ES	209	220	202	130	204	138	22·1	12·6	0·447
LC+LD	477	392	423	361	434	378	44·3	12·0	0·131
E3									
LC	161	30	110	176	132	149	88·6	43·0	0·060
LD	346	365	342	205	332	250	123·3	62·4	0·002
ES	179	218	172	110	174	117	37·9	21·3	0·036
LC+LD	507	395	452	381	464	399	64·9	17·4	0·042

* $MD=\sqrt{1/n\sum(\text{estimated}-\text{actual})^2}$.

† $RMD=\sqrt{1/n\sum((\text{estimated}-\text{actual})/\text{actual})^2}$.

‡ Methods of estimation:
E1 = all seven alkanes (C25, C27, C28, C29, C30, C31, C33); corrected by the mean recovery rates of the six animals in Expt 2.
E2 = like E1; but only the four alkanes (C27, C29, C31, C33).
E3 = like E2; but corrected by its mean recovery rate across all animals given *L. chinensis*.

Based on the estimated daily DM intake using C31:C32 alkane pair (Table 4) and the estimated dietary composition, the DM intake of *E. sibiricum*, *L. chinensis* and *L. dasystachys* were calculated. There were no significant differences between the actual and estimated DM intake of *E. sibiricum* and *L. chinensis* + *L. dasystachys* using method E1 and E2 instead of E3 (Table 5). The results of the MD and RMD of *E. sibiricum* and *L. chinensis* + *L. dasystachys* showed that using only the four alkanes (C27, C29, C31 and C33) (E2) instead of all seven alkanes (C25, C27, C28, C29, C30, C31 and C33) (E1) in the estimation resulted in more accurate results (Table 5). Across all methods of estimation, the estimated DM intakes of *L. chinensis* and *L. dasystachys* were all significantly different from the actual ones ($P<0·01$) with one exception: the estimated DM intake of *L. chinensis* using method E3 did not differ significantly from the actual one ($P>0·05$) (Table 5).

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DISCUSSION

Faecal alkane recovery rates are possibly influenced by diet factors such as herbage species, feeding level, and animal factors such as species, age, live weight and physiological and reproductive states (Dove & Mayes 1991; Brosh *et al.* 2003; Elwert *et al.* 2004). However, few reports were found to evaluate the effects of herbage species and live weight of sheep on faecal alkane recoveries. As Elwert *et al.* (2004) discussed, introducing a new species or a labelled concentrate to an existing diet might have an impact on faecal alkane recovery rates. In the present study, the results of ANOVA indicated that faecal alkane recovery rates (C27–C33) were significantly affected by herbage species ($P<0{\cdot}01$) (Table 2). Since plant cuticular wax morphology is determined mainly by the composition of wax exudates which vary with the plant species and the age of the tissue (Baker 1982), the possible explanation for this is that wax morphology may differ among plant species influencing the degree to which the alkane can be removed from plant fragments in the gut, and hence affect its potential absorption. However, more investigations on particular plant species and faecal alkane recovery should be carried out. Table 2 showed that there were no significant effects of live weight or interactions between live weight and herbage species on the faecal alkane recoveries. It is therefore suggested that the faecal alkane concentrations in outdoor studies should be corrected according to the faecal alkane recoveries of animals given the similar diets, or at least the same dietary components.

Many previous studies indicated that alkane chain lengths affected the recovery rates. Most have documented that the faecal recovery rates of alkanes increased in a curvilinear fashion with longer chain length (Mayes *et al.* 1986; Dove *et al.* 1989*a*; Vulich *et al.* 1991; Dove & Mayes 1991, 1996; Dove & Oliván 1998). However, Brosh *et al.* (2003) found that the recovery rates of the even-chain alkanes were not affected significantly by chain length in two of the three trials reported. In the present experiment, the faecal alkane recovery rates were affected significantly by the alkane chain length in *L. dasystachys* and *E. sibiricum*, but not in *L. chinensis*. The faecal recovery rates of C28 and C30 alkanes were higher than the adjacent odd alkanes. This finding is in accordance with the observations of Mayes *et al.* (1986), Brosh *et al.* (2003) and Elwert *et al.* (2004).

In the present study, mean faecal alkane recovery rates increased with longer C chain length, but the recovery of dosed C36 (0·70), in agreement with C36 (0·86) of Vulich *et al.* (1991), was significantly less than those of other alkanes (Table 3) and C36 ($0{\cdot}947 \pm 0{\cdot}0139$) of Dove & Mayes (1991). The discrepancies may arise from the release rates of dosed alkanes. Ferreira *et al.* (2004) reported an apparent deviation between the calculated release rate of dosed alkanes and those indicated by the manufacturer, finding that the mean release rate of C32 indicated by the manufacturer (317·2 mg/day) was larger than the calculated release rate (278·3 mg/day). Therefore, they suggested that, where possible, release rates should be calculated within individual experiments. However, in the present experiment, the faecal recovery rate of C36 was calculated based on the manufacturer's documented release rate (52·0 mg/day). This may underestimate the recovery rate of C36.

For the estimation of intake, the C32:C33 alkane pair is more commonly used (Mayes *et al.* 1986; Dove & Mayes 1991; Dove *et al.* 1989*b*). However, where C33 concentrations are low, other pairs may need to be considered (Mayes *et al.* 1986; Malossini *et al.* 1996; Hameleers & Mayes 1998; Unal & Garnsworthy 1999). The alkane concentration of C33 in the diet of Expt.2 was low (16 μg/g DM), whereas the diet contained much higher concentrations of C29 and C31 alkanes (61 and 83 μg/g DM respectively). Therefore, in the present study, C33:C32, C29:C32 and C31:C32 alkane pairs were used to calculate the total DM intake. The result showed that there were no significant differences between the actual and estimated intake based on either C31:C32 or C29:C32 alkane pairs ($P<0{\cdot}05$). The C31:C32 pair provided the best estimates of herbage intake. The poorest estimator of intake was the C33:C32 pair (Table 4). This result supported the previous findings that the concentration of natural alkanes in the forage needs to exceed 50 mg/kg DM in order to obtain precise estimates of forage intake (Dove & Mayes 1991). The MD and RMD between the actual and estimated daily DM intake were similar to the results in Elwert *et al.* (2004), but higher than in previous studies (Mayes *et al.* 1986; Dove *et al.* 2002) (Table 4). A possible explanation is that the release rate of C32 from the controlled release capsule quoted by the manufacturer, which was used to calculate intake, may have differed from the actual C32 release rate (Ferreira *et al.* 2004).

Estimates of intake of *L. chinensis* and *L. dasystachys* were almost all significantly different from the actual intake ($P<0{\cdot}01$) (Table 5). However, reasonable estimates of *L. chinensis* + *L. dasystachys* were obtained. There were two possible explanations for this. Firstly, the alkane contents of *L. chinensis* and *L. dasystachys* were all lower than 50 μg/g DM (except C31 in *L. chinensis*) (Table 1), which may lead to unreasonable estimates (Mayes & Dove 2000; Brosh *et al.* 2003; Elwert *et al.* 2004). Secondly, the calculation of diet composition from the alkane content of faeces is based on the assumption that the alkane profiles differ markedly among the diet components (Dove & Mayes 1991, 1996; Brosh *et al.* 2003), whereas *L. chinensis* and *L. dasystachys*, belonging to the same genus, exhibited similar patterns of alkanes.

Since the estimates of *L. chinensis* and *L. dasystachys* proved to be unreasonable (Table 5), the MD and RMD of *L. chinensis* and *L. dasystachys* are not further discussed.

The results of the experiment described in Table 5 suggested that good estimates of *E. sibiricum* and *L. chinensis* + *L. dasystachy* intake could be obtained using the mean recovery rates of the six animals in Expt 2 (E1 and E2), but estimates using the mean recovery rate across the animals given *L. chinensis* (E3) differed significantly from the actual intake ($P < 0{\cdot}05$). These results were in agreement with the previous report (Elwert *et al*. 2004).

Dove & Mayes (1996) indicated that it is preferable to use a greater number of alkane markers than the number of ingredients in the diet. However, Brosh *et al*. (2003) recently reported that the accuracy of the alkane analysis increased with higher alkane concentrations and the even-chain alkanes that are always present in low concentrations in herbages would cause an upward bias in the determination of their concentrations. Therefore, they advised that it was preferable not to use the even-chain alkanes in the estimation of botanical composition. Elwert *et al*. (2004) also indicated that it is appropriate not to use all available alkanes for diet proportion estimates but to discard the ones with a high coefficient of variation in their faecal recovery rate. The present study agreed that the use of the alkanes with higher concentration instead of all available alkanes increased the accuracy of the estimates of *E. sibiricum* and *L. chinensis* + *L. dasystachy* (E1 *v*. E2) (Table 5).

CONCLUSIONS

It can be concluded that, under the conditions of the present study, faecal alkane recoveries were influenced significantly by herbage species ($P < 0{\cdot}01$), meaning that the faecal alkane concentration correction should be based on diet-specific faecal recovery rates. Using the alkane technique, accurate and precise estimations of DM intake could be achieved, but the estimators of diet composition were less reliable, especially when the diet included two or more components with a similar pattern of alkanes.

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