USING SYNTHETIC OR BEESWAX ALKANES FOR ESTIMATING SUPPLEMENT INTAKE IN SHEEP

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SUMMARY

Intakes of perennial ryegrass chaff/sunflower meal (SFM) mixtures were estimated in 24 crossbred young sheep, from the whole-diet and faecal concentrations of C32 and either C31 or C33 alkanes, and the dose rate of C32 from intra-ruminal controlled-release devices. Total intakes were estimated accurately and any differences between known and estimated intakes were attributable to the small differences in faecal recovery between the two alkanes used in the estimates. The SFM, which was free of alkanes, had been labelled by spraying with a mixture of beeswax (15% hydrocarbons) and synthetic C28 and C38 alkanes as possible markers to permit simultaneous estimates of supplement intakes. SFM intakes estimated from C38 concentrations in SFM and faeces were significantly less than known intakes, due to the low faecal recovery (83.9%) of this alkane. As an alternative approach, the patterns of alkane concentrations in the chaff, labelled-SFM and faeces were used in the least-squares package EatWhat to estimate the proportion of supplement in the diet and thence supplement intake. These estimates, with or without the inclusion of C28 in the calculations, were not significantly different from known SFM intakes. This suggests that beeswax can be used as a low-cost marker for estimating supplement intakes, when the supplement itself contains no alkanes.

Keywords: intake, supplement, alkanes, beeswax, sheep

INTRODUCTION

Herbage intakes by individual animals can be estimated accurately using dosed alkanes and those of plant cuticular wax (Mayes *et al*. 1986; Dove and Mayes 1996). Individual supplement intakes have been estimated accurately using markers such as chromic oxide (eg Dove and Coombe 1990), tritiated water (eg Dove *et al*. 1995) or tritiated gypsum (Dove and Coombe 1990). However, all of these methods require chemical analyses in addition to those needed to estimate herbage intake by the alkane method and, in the case of the tritiated markers, have the disadvantage of being radioactive.

Supplement intake could be measured by estimating total intake using the alkane method, and then estimating the proportion of supplement in that total, based on the pattern of alkane concentrations in supplement, herbage and faeces (Dove and Moore 1995; Dove and Mayes 1996). Unfortunately, many supplements (eg legume grains and solvent-extracted oilseed meals) contain little or no alkanes. We therefore evaluated a synthetic alkane (octatriacontane; C38 alkane) and a non-plant alkane mixture (beeswax), as markers for estimating supplement intake in housed sheep. In the first case, supplement intakes were calculated using the C38 alkane as an external marker added to the supplement. In the second, the proportions of perennial ryegrass chaff and supplement in the diet were estimated using the pattern of alkane concentrations in chaff, supplement and faeces (Dove and Moore 1995). Synthetic C28 alkane was also mixed with the beeswax prior to use, to ensure that the alkane pattern in the beeswax-based mix did differ markedly from that of the chaff.

MATERIALS AND METHODS

Animals, experimental design and management

Twenty-four crossbred young sheep (approximate fleece-free liveweight 30kg) were offered (once daily) four dietary treatments consisting of 720 g DM/day of mixtures of chaffed perennial ryegrass and unpelletted sunflower meal (SFM; decorticated, solvent-extracted) in the proportions 7:1, 6:2, 5:3 and 4:4 (six sheep/treatment). Eight animals ('crate sheep'; two/treatment) were housed in metabolism crates to allow total collection of faeces, while the other 16 were housed in individual pens ('pen sheep'). Following a 10-day adjustment period, all animals were dosed with intra-ruminal, controlled-release devices (CRD) delivering 54.3 mg/day of C32 alkane and 49.4 mg/day of C36 alkane, for the estimation of total intake and faecal output, respectively. On the same day as the dosing, animals were switched from unlabelled-SFM to SFM labelled with a beeswax/C28/C38 alkane mixture.

Preparation of labelled supplements

The SFM (100kg DM) was labelled with 40 g C28 alkane, 20 g C38 alkane and 1150 g beeswax by melting these (together with 500mL heptane to aid mixing) in the metal reservoir of a paint-sprayer. The resultant warm solution was sprayed directly onto the SFM in a horizontal mixer, in order to produce SFM containing 400 mg/kg DM C28, 200 mg/kg C38 and 50-400 mg/kg DM of the alkanes derived from the beeswax. Preliminary analyses had shown that the solvent-extracted, unlabelled-SFM contained almost no alkanes and that beeswax contained approximately 15% hydrocarbons, with a pattern of alkane concentrations different from that of the perennial ryegrass (eg large quantities of C27).

Sampling procedures and calculation of intakes

Six days after the introduction of labelled-SFM supplement, faecal output by the crate sheep was estimated by total collection over a further 6 days. Rectal grab-samples of faeces were also obtained from these sheep at 0900 and 1600h on each day of the collection period. Pen sheep were grab-sampled over this same period, but only in the mornings. Faecal output in all sheep was also estimated using C36 alkane (from the alkane CRD) as an external marker.

Total intakes (chaff+SFM) were estimated using either the C31/C32 or the C32/C33 alkane pairs, based on the whole-diet and faecal concentrations of these alkanes and the C32 release rate from the alkane-CRD (Mayes *et al.* 1986, Dove and Mayes 1996). Supplement intakes were estimated either directly (from the concentrations of C38 alkane in labelled-SFM and faeces), or indirectly by estimating the proportions of chaff and SFM in the total intake, using the computer package EatWhat (Dove and Moore 1995). This uses a least-squares procedure to find the combination of the alkane patterns of diet components (in this case, chaff and SFM) which best matches the pattern of alkane concentrations observed in faeces. The latter calculations were performed with and without the inclusion of the C28 alkane added to the beeswax. However, the use of C28 in these calculations did not improve the estimates and will not be discussed further. Calculations of the proportion of supplement in the consumed diet required the adjustment of faecal alkane concentrations for incomplete faecal recovery of alkanes; the required estimates of recovery were obtained from the total faecal collection made with the crate sheep. For the reasons discussed elsewhere (Mayes *et al.* 1986, Dove and Mayes 1996), alkane-based estimates of total intake do not require such corrections.

Analytical procedures and statistical analyses

Samples of faeces, chaff, unlabelled-SFM, labelled-SFM and any feed refusals were freeze-dried prior to chemical analysis (in triplicate). Procedures for the extraction of alkanes from these samples and from beeswax, and subsequent quantification by gas chromatography, have been described elsewhere (Mayes *et al*. 1986; Dove and Mayes 1996). Known and estimated total and supplement intakes were compared by linear regression or by using Student's *t*-test for paired comparisons.

RESULTS

Alkane concentrations in chaff and SFM supplement

The predominant alkanes in the ryegrass chaff were those with odd-numbered carbon chains, as in previous studies (Table 1; Dove and Mayes 1996). There were only small quantities of the two alkanes with which the animals were dosed (C32 and C36). The labelling procedure adopted for the SFM achieved the desired minimum concentrations of the alkanes of beeswax, and the synthetic C28 and C38 alkanes (Table 1).

Faecal recovery of alkanes

Mean faecal recoveries of alkanes in the crate sheep did not differ significantly between the four dietary treatments and pooled means (±s.e.) are shown in Table 2. Recoveries of the natural alkanes increased with increasing carbon-chain length, in a curvilinear fashion similar to that described previously (Mayes *et al*. 1986; Dove and Mayes 1996). The faecal recovery of C32 and C36 alkanes from the intra-ruminal CRD conformed closely to the pattern noted for the natural alkanes and, for C36 alkane, was essentially complete. By contrast, the recoveries of C28 alkane and especially C38 alkane, sprayed onto the SFM with the beeswax, were lower than those found for adjacent natural alkanes.

Estimation of total intake

In the crate sheep, mean DM intake estimated using the C31/C32 alkane pair under-estimated known intake by 2% (706 v. 720 g/day; Table 3). The estimate of DM intake based on the C32/C33 alkane pair was only 0.8% different from the known mean intake (726 v. 720 g/day). In the 16 pen sheep, total DM intake estimated using C31/C32 was very close to known intake, but the mean intake estimated using C32/C33 was 44 g/day (5.8%) higher than known intake (766 v. 722 g/day). The standard errors associated with the estimated intakes in pen sheep were higher than those found with crate sheep.

Faecal DM output estimated using C36 alkane was 5% less than known faecal output (269 v. 282 g/day) despite the almost complete faecal recovery of this alkane. As a result, whole-diet digestibility was slightly over-estimated (63.0 and 61.9% v. 60.8%). In pen sheep, true faecal outputs and digestibilities were by definition unknown, but estimates of faecal output based on faecal grab samples taken only in the morning, were similar to those found with the crate sheep.

Estimation of supplement intake

Mean supplement intakes in crate and pen sheep were 229±38.7 and 230±26.6 g DM/day respectively. These were significantly under-estimated ($P<0.05$) when C38 was used as the supplement marker (crate sheep 185±32.7; pen sheep 205±23.6 g DM/day respectively). By contrast, the dietary proportions of chaff and SFM estimated from the pattern of alkane concentrations in each and in faeces (using EatWhat), were not

Table 1. Concentrations of alkanes (mean values ± s.e.) in perennial ryegrass chaff and SFM labelled with C38 alkane and a beeswax/C28 alkane mixture

Alkane (mg/kg DM)	Ryegrass chaff	Labelled-SFM
C25	51.1±0.54	107.3±2.32
C26	3.7±0.20	11.3±0.72
C27	113.9±1.10	432.6±10.90
C28	10.7±0.11	420.1±10.20
C29	270.5±2.87	276.6±6.99
C30	11.9±0.08	7.2±0.24
C31	256.3±2.27	219.0±6.56
C32	5.3±0.07	2.5±0.20
C33	35.8±0.02	33.8±1.51
C35	3.7±0.25	0.8±0.79
C36	5.5±0.13	5.1±0.29
C38	0	206.8±8.18

Table 2. Faecal recoveries (mean values ± s.e.) of alkanes in ryegrass:SFM mixtures fed to sheep

	C25	C26	C27	C28	C29	C30	C31	C32	C33	C36	C38
Recovery	47.2	56.4	66.2	65.6	84.1	89.9	92.8	96.0	95.2	99.1	83.9
s.e.	±2.48	2.50	2.34	1.41	2.20	2.08	2.10	1.43	1.06	3.13	1.20

Table 3. Comparison of known total intakes (g DM/day), faecal outputs (g DM/day; crate sheep only) and DM digestibilities (%; crate sheep only) with estimates based on the C31/C32 or the C32/C33 alkane pairs (intake) or C36 alkane (faecal output). Mean values±s.e.

	Crate sheep			Pen sheep		
	Known	C31/C32	C32/C33	Known	C31/C32	C32/C33
Intake	720±1.9	706±11.7	726±5.6	722±0.9	724±22.8	766±22.4
Faecal output	282±2.5	269±7.8	269±7.8	-	260±6.7	260±6.7
Digestibility	60.8±0.35	61.9±1.01	63.0±0.87	-	63.8±1.03	65.9±0.94

significantly different from known proportions (Table 4). The resultant estimates of supplement intake (Table 4) were related to known intakes by the following equation, which did not differ in either slope or intercept from the line of equality (P>0.05):

$$\text{Estimated intake} = 0.990*(\text{known intake}) + 10.662 \quad (P<0.001;\ r^2 = 0.991).$$

Table 4. Comparison of the known proportions of supplement (SFM) in the diet and those estimated from the pattern of alkane concentrations in faeces and dietary components (using the least-squares package *EatWhat*), and comparison of resultant estimated supplement intakes with known intakes (means ± s.e.)

	Crate sheep		Pen sheep	
	Known	Estimated	Known	Estimated
Estimated SFM: Proportion	0.32±0.053	0.34±0.056	0.32±0.036	0.33±0.039
Intake (g DM/day)	229±38.7	244±40.5	230±26.6	238±26.4

DISCUSSION

Our results confirm the usefulness of the alkane procedure for estimating total intake in animals consuming a mixture of forage and supplement. The small differences observed between known and estimated total intakes by crate sheep (Table 3) were almost identical to the differences in recovery of the alkane pairs used to estimate intake (see Table 2). Dove and Mayes (1996) recently showed that a difference in faecal recovery of two alkanes used to estimate intake will result in an equivalent proportional error in intake. With the exception of C28 alkane and particularly C38 alkane, faecal alkane recoveries were similar to published values (see Dove and Mayes 1996) and increased with carbon-chain length. The faecal recoveries for C32 and C36 alkanes (Table 2) can be considered to relate to the pure alkanes derived from the intra-ruminal CRD, since the data in Table 1-3 indicate that over 95% of the input of these alkanes came from this source. The lower recoveries of pure C28 and C38 are thus difficult to explain but may suggest that, in contrast to the alkanes of beeswax or the C32 and C36 released gradually from the CRD, pure alkanes sprayed onto dietary components may behave differently during passage through the gut. The low recovery of C38 alkane was the major cause of the significant under-estimates of supplement intake found with this alkane; when allowance was made for the incomplete faecal recovery of C38, estimated supplement intakes in the crate and pen sheep were 221 and 244 g DM/day respectively, close to the known intakes of 229 and 230 g DM/day respectively.

The use of beeswax as an alkane-bearing marker allows the alkane procedure to be used for the simultaneous estimation of total and supplement intake, in a situation where the supplement itself does not contain alkanes. The proportion of supplement in the diet was accurately estimated, using the non-negative least-squares procedure in the EatWhat programme. Since total intakes were accurately estimated using either the C31/C32 or C32/C33 alkane pairs, the product of these and the supplement proportion in the diet of individual animals resulted in accurate estimates of supplement intake, over a four-fold range of supplement intakes. The absence of any benefit from adding C28 alkane to the beeswax suggests that beeswax on its own would be a useful marker, and it has the added advantages of being cheap (approximately $5/kg) and easily applied to supplements. By contrast, the high cost and poor performance of C38 alkane suggest it offers no advantage over beeswax.

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REFERENCES

DOVE, H. and COOMBE, J.B. (1990). *Proc. Aust. Soc. Anim. Prod.* **19**, 239-41.
DOVE, H. and MAYES, R.W. (1996). *J. Nutr*. **126**, 13-26.
DOVE, H. and MOORE, A.D. (1995). *Aust. J. Agric. Res*. **45**, 1535-44.
DOVE, H., MAYES, R.W. and FREER., M. (1995). *Ann. Zootech.* **44** (Suppl.), 237.
MAYES, R.W., LAMB, C. S. and COLGROVE, P.M. (1986). *J. Agric. Sci., Camb*. **107**, 161-70.