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USING n-ALKANES AND KNOWN SUPPLEMENT INTAKE TO ESTIMATE ROUGHAGE INTAKE IN SHEEP

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SUMMARY

In animals consuming known amounts of supplement, total intake of roughage plus supplement could be estimated by dividing known supplement intake by the supplement proportion in the diet, if the latter were estimated using the alkane patterns in roughage, supplement and the faeces of the animals consuming them. The roughage intake could then be obtained by multiplying total intake by the estimated roughage proportion in the diet. This approach was tested using data from a previous study, and the results of a second experiment. In the first experiment, perennial ryegrass chaff and sunflower meal labelled with beeswax alkanes were fed in different proportions to young sheep. Known roughage intake was significantly under-estimated ($P<0.05$) by about 8% using this approach, due mainly to the 12% under-estimate in animals receiving the lowest level of supplement. This reflected the compounding of errors in estimating the dietary proportion of supplement in that treatment. In the other treatments, there was a smaller and non-significant difference between known and estimated roughage intakes. In the second experiment, similar animals were fed either subterranean clover chaff alone or known proportions of chaff and cottonseed meal labelled with beeswax. There was excellent agreement between the known proportions of supplement in the diet and those estimated using alkane patterns in the chaff, meal and the faeces obtained by total collection. As a result, total intakes and thus chaff intakes were estimated very accurately; mean estimated chaff intake was less than 1% different from known mean chaff intake. The results suggest that in animals such as the dairy cow, where daily supplement intake is known or can be controlled, the intake of pasture or its components could be estimated using the approach described, without the need to dose the animals with synthetic alkanes.

Keywords: intake, alkanes, roughage, supplement, beeswax, sheep

INTRODUCTION

The n-alkanes of plant cuticular wax can be used to estimate the diet composition and herbage intake of grazing animals (Mayes *et al*. 1986; Dove and Mayes 1996). Estimates of herbage intake are derived from the herbage and faecal concentrations of long-chain alkanes adjacent in chain length (e.g., C32, C33 alkanes) and the daily dose rate of the even-chain alkane. The odd-chain alkane is derived from herbage, and the method requires that animals be dosed with the even-chain alkane, administered either daily (or more frequently) as gelatin capsules or paper pellets, or administered only once as an intra-ruminal, controlled-release device (Dove and Mayes 1996).

Diet composition can be estimated by relating the alkane pattern in the faeces, adjusted for incomplete alkane recovery, to the alkane patterns of the various species available for consumption by the animal, using least-squares mathematical procedures (e.g., Dove and Moore 1995; Newman *et al*. 1995). If the animals are also consuming supplements, then the proportion of supplement in the diet, and ultimately supplement intake, can be estimated by treating the supplement as one of the ‘species’ in the diet. Dove and Oliván (1998) extended this concept to supplements that contain no alkanes (e.g., solvent-extracted oilseed meals), by labelling them with a source of alkanes such as beeswax.

In some animal production systems, it is either normal or convenient to feed known amounts of supplement to individual animals (e.g., supplementation of individual dairy cows during milking). If the supplement either contains or can be labelled with alkanes, then it is possible to estimate the proportion of supplement in the diet (Dove and Oliván 1998) but, since actual supplement intake is also known, then total and thus herbage intake can also be computed. This approach provides a means of estimating the intake of pasture or its component species, without having to dose animals with alkanes, which in turn may avoid undue stress or perturbations of normal grazing behaviour. We report the results of two experiments that provided an assessment of this approach.

MATERIALS AND METHODS

Experiment 1

Experimental animals, design and diets

The first data set was drawn from the experiment reported by Dove and Oliván (1998), where details of the experimental design and animal management are given. Briefly, 24 young crossbred sheep were offered diets of 720 g DM/day of mixtures of chaffed perennial ryegrass and unpelletted sunflower meal in the proportions 7:1, 6:2, 5:3 and 4:4 (six sheep/treatment). The meal had been sprayed with beeswax alkanes at the rate of 11.5 g beeswax/kg meal, using the procedures described by Dove and Oliván (1998). The original report concentrated on the use of the beeswax alkanes to estimate the proportion of supplement in the total diet, but since actual supplement intake was known, this data set also provides a preliminary assessment of the approach described above.

Experiment 2

The second experiment was conducted as an explicit test of the use of alkane-labelled supplement as a means of estimating roughage intake.

Experimental animals, design and diets

Eighteen young crossbred sheep, approximately 15 months of age and with mean live weight 38.4 (s.e. 0.52) kg were housed in individual metabolism crates to allow total collection of faeces. They were offered once daily 800 g air dry (710g DM) of chaffed clover (*Trifolium subterraneum*) hay (six sheep) or a mixture of the chaffed clover hay and cottonseed meal (CSM; solvent extracted) in the proportions 7:1, 6:2 5:3 and 4:4 (three sheep per treatment). Any feed refusals were collected daily, and water was freely available at all times. Following an adjustment period of seven days the animals were switched from unlabelled CSM to CSM labelled with beeswax as described below.

Preparation of labelled supplement

The CSM (45kg DM) was labelled with 550g beeswax (12.2 g beeswax/kg meal) using a slight modification of the technique described by Dove and Oliván (1998). The beeswax was grated using a domestic kitchen grater and dissolved in 2.5L of n-heptane, with heating. The warm solution was then transferred to a plastic hand-held sprayer and sprayed onto the CSM while it turned in a horizontal mixer. The solution was kept warm by occasional immersion of the spray vessel in hot water. The heptane solvent was allowed to evaporate from the labelled CSM before the meal was fed to animals. No palatability problems were experienced with either the unlabelled or the labelled CSM.

Sampling and analytical procedures

Starting on day seven after the introduction of the labelled CSM supplement, total faecal output was measured for 6 days by standard collection procedures. A sample of 10% fresh weight of the daily faecal output was bulked across days for each animal. Bulked samples of chaff, labelled CSM and faeces were stored at -18°C until being freeze-dried prior to alkane analysis. Alkanes were extracted from samples by direct saponification/solvent separation and quantified by gas chromatography (Dove *et al*. 1996).

Calculation of intake and statistical analysis

The proportions of CSM and clover chaff in the diets were estimated from the patterns of C27, C29, C30 and C31 alkanes in the chaff, CSM and faeces, using the non-negative least squares procedure EatWhat (Dove and Moore 1995). The data required to correct faecal alkane concentrations for incomplete faecal recovery were based on mean recoveries within a treatment. Total intake was calculated as (known supplement intake/estimated supplement proportion), and chaff intake as (estimated total intake*estimated chaff proportion). Known and estimated intakes in both experiments were compared using regression analysis and t-tests for paired comparisons.

RESULTS

Experiment 1

Alkane concentrations in the perennial ryegrass chaff and the beeswax-labelled sunflower meal are reported in detail by Dove and Oliván (1998). Known chaff intakes are compared in Table 1 with those calculated from known supplement intake and the proportion of supplement in the total diet, estimated using alkanes.

Table 1. Comparison of known and estimated perennial ryegrass chaff intakes (g DM/day), Experiment 1

	Ratio of chaff:supplement			
	7:1	6:2	5:3	4:4
Known intake	626	536	448	358
Estimated intake (s.e.)	549 (15.5)	511 (8.9)	416 (9.0)	337 (13.8)

Overall, estimated chaff intake significantly under-estimated the known intake (P<0.05, paired t-test). This was due principally to the 12% under-estimate found with the animals fed the lowest level of supplement (90 g DM/day). The mean under-estimate for the other three treatments was 5.9%, but this was not significant (P>0.05, paired t-test).

Experiment 2

The concentrations of alkanes C27, C29, C30 and C31 differed markedly between the subterranean clover chaff and the labelled CSM, as intended (Table 2). The concentrations of the same alkanes in the sunflower meal used by Dove and Oliván (1998) are shown for comparison. The same batch of beeswax was used to label both oilseed meals and whilst the application rate of beeswax in the present experiment was slightly higher than in the earlier study (see above), an examination of the data in Table 2 indicates that very similar labelled alkane concentrations were achieved in each meal.

Table 2. Alkane concentrations (mg/kg OM) in the subterranean clover chaff and labelled cottonseed meal (CSM) used in Experiment 2, and the labelled sunflower meal (SFM) used by Dove and Oliván (1998)

	Concentration of alkane:			
Feed	C27	C29	C30	C31
Clover chaff	39.5	270.8	14.1	95.0
Labelled CSM	466.0	305.2	8.4	256.8
Labelled SFM	466.6	298.4	7.8	236.2

These alkanes, together with faecal concentrations of the same alkanes (adjusted for incomplete faecal recovery) were used to compute the supplement proportion in the diet. Estimated proportions were extremely close to the actual proportions and did not differ significantly from them by either regression analysis or by a paired t-test (Table 3). Note that since the EatWhat procedure does not permit negative proportions of a dietary component, it is possible that this introduces some bias into the standard errors of estimated proportions (Table 3), particularly for the 8:0 and 7:1 treatments.

Table 3. Comparison of actual proportions of supplement in the consumed diet and those estimated using the alkane concentrations in subterranean clover chaff, beeswax-labelled CSM and faeces. Values in parenthesis are standard errors of estimated proportions.

Chaff:supplement ratio offered	8:0	7:1	6:2	5:3	4:4
Known proportion of supplement in consumed diet	0	0.125	0.250	0.375	0.500
Estimated proportion of supplement in consumed diet	0.001 (0.0007)	0.125 (0.0006)	0.250 (0.0039)	0.375 (0.0046)	0.500 (0.0049)

For animals that were offered supplement, dividing the known supplement intake by the estimated proportion of supplement in the diet provides an estimate of total intake, from which chaff intake can be obtained by multiplying by the estimated chaff proportion. Estimates of chaff intake so derived are compared with known chaff intakes in Figure 1. Estimated chaff intakes were related to known intakes (both g DM/day) by the following expression, which did not differ from the line of equality.

Estimated = 1.008 (s.e. 0.0377)*Known + 0.718 (s.e. 18.8242) (r^2 = 0.986; RSD = 12.6; P<0.001)

DISCUSSION

Although Experiment 1 was never intended as a test of the proposal that roughage intake could be estimated from the intake of supplement containing alkanes, the results presented in Table 1 indicate the feasibility of this approach. Overall, the intake of perennial ryegrass chaff was significantly under-estimated, but this was due mainly to the effect of a major under-estimate (12%) at the lowest level of supplement offered. This under-estimate was due, in turn, to errors in the estimated proportion of supplement in this diet, which are exacerbated when known supplement intake is divided by estimated supplement proportion, to calculate total intake. Supplement intakes were more accurately estimated in the other treatments, with the result that estimated roughage intakes did not differ significantly from actual intakes. Nevertheless, there was still a tendency to under-estimate known roughage intake.

No such trend was obvious when the method was directly tested in Experiment 2. Estimated proportions of supplement in the diet agreed very closely with known proportions (Table 3), with the result that, on average, estimated roughage intakes differed by less than 1% from known intakes and did not differ significantly from them (Figure 1).

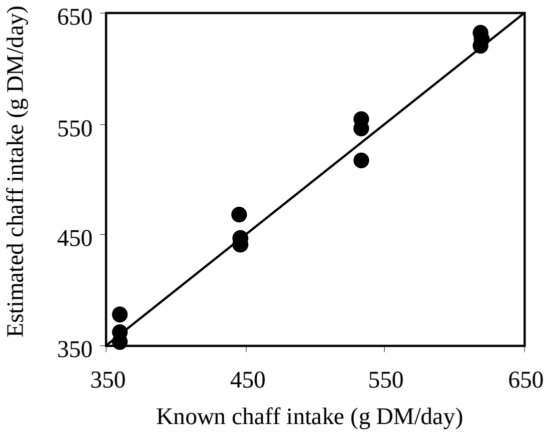

Figure 1. Comparison of subterranean clover chaff intake (g DM/day) estimated from the known intakes of beeswax-labelled cottonseed meal, with known chaff intakes. The solid line is the line of equality.

Taken together, the results suggest that in animals which are consuming known amounts of supplement in addition to roughage, such as the dairy cow, roughage intakes could be estimated by either taking advantage of the natural alkane pattern of the supplement, or by labelling the supplement with a source of alkanes, as in the present study. Although there were only two dietary components in our experiments, the approach is equally applicable to multi-component diets (e.g. supplement, pasture species 1, pasture species 2…). We have yet to establish how low natural alkane concentrations have to fall before it is necessary to label the supplement. However, it is of interest in this regard that in the work of Dove *et al*. (1995), oat grain with low alkane concentrations (C27 5.1, C29 8.5, C31 10.7 mg/kg OM) was used as a supplement for sheep receiving lucerne chaff (C27 15.3, C29 83.3, C31 311.5 mg/kg OM). When the proportion of supplement in the diet was estimated using these greatly differing concentrations, the estimated mean proportion (0.308) was very close to the actual mean proportion of 0.305, with the result that estimated mean chaff intake was only 1.3% (6.4 g DM/day) less than known mean chaff intake. This suggests that the present method could be used in circumstances in which the supplement alkane concentrations are much lower than those of the roughage component, but further work is required to confirm that this is the case.

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