A COMPARISON OF METHODS FOR ESTIMATING SUPPLEMENT INTAKE AND DIET DIGESTIBILITY IN SHEEP

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SUMMARY

Housed crossbred wethers were offered diets of chaffed lucerne hay supplemented with pelletted sunflower meal (SFM) labelled with 0.3% Cr_2O_3 and 1.0% tritiated-gypsum. The intake of SFM was esti-mated either from total faecal Cr excretion or from the accumulation of tritium in the body water pool. Both methods accurately estimated SFM intake and were not significantly different. The digestibilities of the consumed diets were estimated *in vivo*, and were also estimated using indigestible acid-detergent fibre (IADF), lignin or plant wax alkanes (C_{31} and C_{33} alkanes) as internal markers. The IADF and alkanes accurately estimated digestibility and thence lucerne chaff intake, but digestibilities were significantly over-estimated when lignin was used as a marker.

(Keywords.= Intake, digestibility, alkanes, lignin, IADF.

INTRODUCTION

The response of grazing animals to supplements is complex. Substitution between supplement and herbage frequently occurs, but is difficult to measure because the estimate of supplement intake is usually only the average intake of a group of animals. The interaction between supplement and herbage intake is even harder to define if the herbage intake is based, as it often is, on a single *in vitro* estimate of digestibility, since this accommodates neither between-animal differences in herbage digestion nor possible influences of the supplement on herbage digestion.

The intake of supplement by individual animals can be estimated from total faecal collection if the supplement is labelled with an inert marker such as Cr_2O_3 (Lobato *et al.* 1980), or from the accumulation of tritium in the body water pool if the supplement is labelled with a radioactive tracer such as tritiated-gypsum (TOHG; Dove 1984). These two approaches have not been compared directly. Theoretically, the intake of the herbage component of the diet can be calculated indirectly by subtracting individual supplement intake from total intake, if the whole-diet digestibility is estimated by monitoring an internal marker in the diet and the faeces. In this paper, we report a direct comparison of Cr_2O_3 and TOHG as markers for estimating supplement intake. We also discuss the relative merits of lignin (Christian *et al.* 1970), indigestible acid detergent fibre (IADF; Penning and Johnson 1983) and plant cuticular wax alkanes (see Dove and Mayes 1991) as possible internal markers for estimating the digestibility of the whole diet. The recovery of plant alkanes in faeces is incomplete but is closely related to carbon-chain length (see Dove and Mayes 1991). Moreover, the alkanes are chemically discrete and relatively easy to analyse. Hence, the correction of faecal alkane concentrations for incomplete recovery can be made with more confidence than for markers such as lignin, which is neither chemically discrete nor predictably recoverable in faeces (see Kotb and Luckey 1972).

MATERIALS AND METHODS

Animals and their diets

Eight Dorset Horn *x* (Border Leicester *x* Merino) wethers aged 15 months and weighing 34.7 ± 0.67 kg (mean ± s.e.) were housed indoors in metabolism crates and randomly allocated to 4 dietary treatments (A-D) consisting of 600, 500,400 or 300 g/day of air-dry luceme chaff mixed immediately before feeding with 200, 300, 400 or 500 g/day respectively of air-dry, pelletted sunflower meal (SFM; solvent-extracted, decorticated). The animals were fed daily at 0830 hours; water was freely available except during the period of estimation of total body water contents.

Estimation of supplement intakes and diet digestibilities

The SFM pellets contained 0.3% (w/w) Cr_2O_3 and 1.0% (w/w) of either gypsum or TOHG, prepared as described by Dove (1984). At the start of the measurements, all sheep were given an oral priming dose of 1 g of Cr (as Cr_2O_3) in a gelatin capsule, after which they consumed the chaff plus SFM containing Cr_2O_3 and non-radioactive gypsum for a preliminary period of 12 days. Total faecal output was then collected for a period of 7 days, during which the SFM contained TOHG. At the beginning and end of this faecal collection period, jugular blood samples were taken to determine, respectively, the background level of tritium in the body water pool and the tritium accumulation from the labelled SFM. The size and fractional turnover rate of the body water pool were then estimated as earlier described (Dove 1984).

The digestibilities of diets A-D were estimated *in vivo* by standard methods and were also estimated using the diet and faecal concentrations of lignin (Christian *et al.* 1970), IADF (Penning and Johnson 1983) and the plant wax alkanes hentriacontane (C_{31} alkane) and tritriacontane (C_{33} alkane). For the latter two estimates, faecal alkane concentrations were adjusted using the mean of the published estimates of recovery for each alkane (Dove and Mayes 1991).

Chemical and statistical analyses

Diet and faecal samples were freeze-dried prior to analysis. Tritium specific activities were determined as described by Dove (1984) while Cr was estimated using atomic absorption spectrophotometry. Lignin, IADF and alkane levels were estimated using the methods 'described by Christian *et al.* (1970), Penning and Johnson (1983) and Dove and Mayes (1991) respectively. Known and estimated intakes or digestibilities were compared using either regression analysis or t-tests for paired comparisons.

RESULTS

Comparison of known and estimated supplement intakes

Supplement intakes (g DM/day) estimated using TOHG or based on Cr excretion were related to known intakes by the equations

$$\text{Intake}_{TOHG} = (0.97 \pm 0.021) \times (\text{Intake}_{Known}) + (8.86 \pm 6.94) \quad (r^2=0.997;\ P<0.001)$$

$$\text{Intake}_{Cr} = (1.05 \pm 0.085) \times (\text{Intake}_{Known}) - (4.5 \pm 27.9) \quad (r^2=0.963;\ P<0.001)$$

Neither equation differed from the line of equality. Moreover, the 2 estimates of intake were themselves related by an equation which did not differ from the equality line.

Estimation of diet digestibility

The mean faecal recoveries of IADF and lignin were 0.974 ± 0.0100 and 1.092 ± 0.0073 respectively; the recovery of lignin was significantly greater than 1.0 ($P<0.05$). Faecal alkane recoveries (Table 1) were significantly less than 1.0 ($P<0.05$) and increased with increasing carbon-chain length. The faecal recovery of C_{31} alkane was higher than the mean of the values (0.843 ± 0.0242) reviewed by Dove and Mayes (1991), but our estimated recovery of C_{33} alkane was identical with the mean value of 0.872 ± 0.0125 reported by those workers. Faecal alkane concentrations were closely related to the proportion of luceme chaff in the diet, since the solvent-extracted SFM contributed almost no alkanes. However, faecal alkane recoveries were unaffected by the proportion of luceme in the diet.

Table 1. Faecal alkane recoveries in sheep consuming lucerne chaff supplemented with sunflower meal

	Alkane chain length[A]				
	C_{27}	C_{28}	C_{29}	C_{31}	C_{33}
Mean ± s.e.	0.630 ± 0.0125	0.699 ± 0.0104	0.765 ± 0.0108	0.877 ± 0.0136	0.872 ± 0.0179

[A] Insufficient dietary C_{30} or C_{32} to permit recovery estimate.

The mean *in vivo* digestibilities of diets A and B were 0.597 ± 0.0033 and 0.602 ± 0.0033 respectively. Despite the greater proportion of SFM in diet B, these were not significantly different from each other (s.e.d. 0.0085), but were significantly less ($P<0.05$) than the digestibilities of diet C (0.629 ± 0.0003) and diet D (0.611 ± 0.0112) which also differed significantly from each other. The response of diet digestibility to increasing SFM intake (diet A to diet D) was thus not linear.

When digestibilities were estimated using internal markers, there was no interaction between dietary treatment and method of estimation. We therefore present only means across diets A-D. The mean digestibility based on IADF (0.593 ± 0.0077) was less than but did not differ significantly from the overall mean *in vivo* digestibility of 0.610 ± 0.0052. By contrast, the mean digestibility estimated using lignin as an internal marker (0.642 ± 0.0039) was significantly greater ($P < 0.05$) than the mean *in vivo* digestibility. The digestibility calculated using C_{31} alkane as an internal marker (0.620 ± 0.0035) was slightly but not significantly higher than the mean *in vivo* digestibility, while the digestibility similarly estimated using C_{33} alkane (0.609 ± 0.0060) was almost identical with *in vivo* digestibility.

Estimates of luceme chaff intake were obtained by calculating total intake from faecal output and C_{33}- based digestibility, and subtracting from this the SFM intake computed from either Cr or TOHG.

Compared with the known mean lucerne chaff intake of 450 g/day (air-dry), that estimated by subtracting TOHG-based SFM intake from total intake was 436.5 ± 43.29 g/day. The equivalent value using Cr-based SFM intake was even closer, at 450.8 ± 39.70 g/day. Neither value was significantly different from known luceme intake. By contrast, the estimates of mean luceme intake calculated using lignin-based digestibilities for total intake were 509.2 ± 44.42 g/day (TOHG-based SFM intake) and 523.5 ± 40.07 g/day (Cr-based SFM intake). Both these values were significantly different from the known mean intake of 450 g/day *($P < 0.05$).*

DISCUSSION

Estimating supplement intake

Our results indicate that supplement intakes of 200–500 g/day can be estimated accurately either from faecal Cr excretion (Lobato *et al.* 1980) or from the accumulation of tritium in the body water pool (Dove 1984). Although there was no significant difference between the methods, the standard errors associated with the Cr-based method were smaller, and the indirect estimates of luceme chaff intake were closer to known intakes. Under field conditions, we have found either technique to be satisfactory (Dove 1984, Coombe *et al.* 1987), although we have some evidence that in the field, where supplement intake by some animals can be much lower, the TOHG-based method was the more accurate at low levels of intake (Coombe *et al.* 1987). Ultimately, the choice of method probably reflects the balance between the inconvenience of using a radioactive marker, versus that of using a stable marker which requires total faecal collection.

Estimating diet digestibility

In confirmation of earlier results (Penning and Johnson 1983) we found that IADF accurately estimated digestibility. In animals grazing cereal stubbles, we also found that IADF-based digestibilities gave better estimates of intake than those based on the *in vitro* digestibility of oesophageal extrusa (Coombe *et al.* 1987). Since the measured faecal recovery of C_{33} alkane proved to be identical with the recovery value assumed in the estimation of digestibility, the use of this alkane as an internal marker gave accurate estimates of digestibility and thus, after subtracting the known SFM intake, accurate estimates of luceme chaff intake. The faecal recovery of C_{31} alkane has been shown to be less consistent than C_{33} alkane (see Fig. *2a* of Dove and Mayes 1991), so the latter alkane would be the preferred one to use as an internal marker. By contrast with the above internal markers, diet digestibilities based on lignin were significantly greater than *in vivo* digestibilities, with consequent over-estimates of the intake of the herbage component of the whole diet.

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