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Estimating digestibility and faecal output in lambs using internal and external markers

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SUMMARY

Twenty fine-wool, ruminally cannulated lambs (average weight 45·9 kg) were used in a completely random design to evaluate the ability of three internal markers to predict dry matter digestibility and two external markers to estimate faecal output. Lambs were allotted randomly to one of four diets: 100% prairie hay (PH), 100% lucerne hay (LH), 50% prairie hay: 50% sorghum grain (PS) and 50% lucerne hay: 50% sorghum grain (LS). The trial consisted of a 14-day adaptation period followed by a 7-day total faecal collection period. Feed and faecal samples were subjected to 96 h ruminal fluid and 48 h acid–pepsin digestions, followed by extraction with acid detergent (IVADF) or neutral detergent (IVNDF) solution. Dry matter digestibility (DMD) calculated from feed:faeces ratios of IVADF, IVNDF and acid detergent lignin (ADL) was compared with *in vivo* apparent digestibility. Ytterbium-labelled forage (YLF) and dysprosium-labelled faeces (DLF) were pulse-dosed via ruminal cannulae, and faecal Yb and Dy excretion curves were fitted to a one-compartment, age-dependent model for estimation of faecal output, particulate passage rate (PPR) and mean gastro-intestinal retention time. *In vivo* DMD in lambs fed PH was greater ($P < 0·05$) than DMD calculated from IVNDF, IVADF and ADL. In lambs fed LH and LS, *in vivo* DMD did not differ ($P > 0·05$) from marker estimates. *In vivo* DMD for lambs fed PS did not differ from IVNDF or IVADF estimates but was greater than ($P < 0·05$) the ADL estimate. No differences ($P > 0·05$) were observed in recovery among the three internal markers for any of the diets. Faecal output for lambs fed PH did not differ ($P > 0·05$) from marker estimates but was overestimated by 15 to 20% by YLF and DLF. Faecal output for lambs fed LH was similar to the estimate from YLF, but less than ($P < 0·05$) the estimate with DLF. For lambs fed PS, faecal output did not differ from marker estimates, but YLF and DLF values were 16% lower and 17% higher, respectively. No significant differences were observed in actual and estimated faecal output for lambs fed the LS diet. Estimates of PPR with DLF were numerically greater than YLF estimates for all diets except LS. Correspondingly, mean gastro-intestinal retention time was less ($P < 0·05$) for DLF compared with YLF for all diets except LS.

INTRODUCTION

Assessment of faecal output and total tract digestibility is important in studies evaluating the nutritional status of ruminants. In grazing ruminants, voluntary intake estimates are derived by measuring faecal output and diet indigestibility. Several direct and indirect methods are available to determine faecal output (total faecal collection, chromic oxide, rare earth markers). Direct measurements of grazed forage digestibility are not possible, however, and estimates are usually obtained from *in vitro*, *in situ* or internal marker approaches.

Total faecal collection has been used by a numbe of workers (Handl & Rittenhouse, 1972; Cordova Wallace & Pieper, 1978), but the technique is generall regarded as expensive and labour intensive, and ma alter normal grazing behaviour (Brisson, 1960 Corbett, 1960; Cordova *et al.* 1978). Althoug chromic oxide has been used extensively in pastur nutrition studies, variation in marker recovery diurnal variation in marker excretion and analytica difficulties have been noted as problems with th technique (Raleigh, Kartchner & Rittenhouse, 1980 Prigge *et al.* 1981). Recently, rare earth elements hav received considerable attention as markers for deter

mination of particulate passage rate (Ellis *et al.* 1982). Compartmental modelling techniques (Ellis, Matis & Lascano, 1979) applied to faecal marker excretion curves from a pulse dose of rare earth markers can be used to determine faecal output as well as particulate passage rate (Krysl, McCollum & Galyean, 1985); however, limited data are available regarding the validity of faecal output estimates determined with this technique.

In vitro fermentation methods like those developed by Tilley & Terry (1963) have been used extensively to estimate forage digestibility. Addition of concentrate supplements to roughage-based diets may present problems with *in vitro* methods because of associative effects (Henning *et al.* 1980; Mehrez *et al.* 1983). Such interactions between feeds are difficult to mimic with *in vitro* systems (Van Soest, 1982). Feed to faeces ratios of internal and external markers have been used to estimate digestibility, and marker-based methods should be more sensitive to associative effects than *in vitro* approaches. However, most internal markers predict *in vivo* digestibility with varying degrees of accuracy (Streeter, 1969; Raleigh *et al.* 1980; Muntifering, 1982; Fahey & Jung, 1983; Penning & Johnson, 1983; Hunt *et al.* 1984). Recently, residues from *in vitro* fermentation subjected to acid (IVADF) or neutral (IVNDF) detergent extraction have been proposed as internal markers (Berger, Klopfenstein & Britton, 1979; Waller *et al.* 1980; Cochran *et al.* 1986). Information on reliability of these markers for estimation of apparent digestibility is limited. This study evaluated the use of rare-earth markers to estimate faecal output and IVADF, IVNDF and acid detergent lignin (ADL) to estimate *in vivo* digestibility in lambs fed diets of varying roughage to concentrate ratios.

MATERIALS AND METHODS

Experimental details. Twenty fine-wool, castrated male lambs (average weight 45·9 kg) were used in a completely random design. Lambs were fitted with permanent ruminal cannulae, housed individually in 1·5 × 3·0 m pens and had access to fresh water and trace mineral salt. The trial consisted of a 14-day adaptation period and a 7-day collection period. Lambs were allotted randomly to one of four diets: 100% prairie hay (PH), 100% lucerne hay (LH), 50% prairie hay:50% sorghum grain (PS) and 50% lucerne hay:50% sorghum grain (LS). Chemical composition of the two hays and sorghum grain is shown in Table 1. Hay was chopped to pass a 2·54 cm screen and sorghum grain was finely ground to pass a 0·5 cm screen. To ensure complete feed consumption, lambs were fed at 1·8% of body weight.

During the 7-day collection period, total dry matter intake and faecal output (via faecal collection bags) were recorded, and a 10% aliquot of faeces was frozen. Subsequently, faeces were composited by lamb over the 7-day period. At the start of the 7-day collection period, each lamb was dosed via ruminal cannula with gelatin capsules containing ytterbium-labelled forage (YLF) and dysprosium-labelled faeces (DLF). Lambs fed LH or LS were dosed with 13·9 g of lucerne hay (dry matter) containing 144·7 mg Yb, while those fed PH or PS received a dose of 14·0 g of prairie hay (dry matter) containing 137·8 mg Yb. Lambs were dosed with 14·7 g of faecal dry matter containing 195·4, 121·4, 105·2 and 50·6 mg Dy for LH, LS, PH and PS, respectively. Hays and faeces (obtained from a composite faecal sample of each treatment) were labelled (Teeter, Owens & Mader, 1984; McCollum & Galyean, 1985) by soaking 50 g of dry matter per litre of a solution containing 2·5 g of $YbCl_3.xH_2O$ for 24 h. After soaking, excess fluid was decanted and remaining hay and faeces were washed with deionized water every hour for a 6 h period and dried at 50 °C for 48 h in a forced-air oven. Rectal grab samples of faeces were obtained from lambs at 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 42, 48, 54, 60, 72, 84, 96, 108 and 120 h after dosing. Total collection values used to obtain digestibility estimates were adjusted for the weight of faeces removed by grab sampling.

Table 1. *Chemical composition (%) of experimental diet components**

Item	Diet component		
	Prairie hay	Lucerne hay	Sorghum grain
Dry matter	93·3	92·8	90·2
Ash	9·1	9·2	2·1
Crude protein	10·2	18·4	11·3
Neutral detergent fibre	64·5	39·4	21·5
Acid detergent fibre	39·3	29·5	4·3
Acid detergent lignin	6·1	7·5	1·4

* Dry matter basis.

Analytical methods. Composited faecal material from the 7-day collection period was divided into four subsamples, two of which were freeze-dried and two dried at 50 °C in a forced-air oven. Faeces were then ground to pass a 2 mm screen in a Wiley mill and dry matter (D.M.) was determined (Association of Official Analytical Chemists, 1984). Feed samples taken daily throughout the collection period were composited and ground in a Wiley mill to pass a 2 mm screen and analysed for dry matter, ash and Kjeldahl nitrogen by standard procedures (Association of Official Analytical Chemists, 1984). Neutral detergent fibre, acid detergent fibre and acid detergent lignin were determined according to Goering & Van Soest (1970). Feed and faecal samples were subjected to a 96 h *in vitro* fermentation and 48 h acid–pepsin digestion

(Tilley & Terry, 1963), followed by acid detergent or neutral detergent extraction. Procedures followed those of Waller *et al.* (1980) and Ellis, Bailey & Taylor (1984) for IVADF and IVNDF, respectively. Acid detergent lignin was determined on faeces following procedures of Goering & Van Soest (1970).

In vitro fermentations were conducted with 0·5 g substrate in 120 × 26 mm plastic tubes. Four tubes were inoculated for each feed for each analysis (IVNDF and IVADF). Two tubes were inoculated for each of the four faecal composites from each lamb for each analysis (IVNDF and IVADF). Composited ruminal fluid inoculum was collected via ruminal cannulae from two mature beef steers maintained on a chopped hay ration of approximately 50% lucerne hay and 50% prairie hay. Inoculum and McDougall's artificial saliva were mixed in a 1:4 ratio with a final volume of 35 ml/tube, flushed with CO_2, capped and placed in a 39 °C water bath. After 96 h, tubes were centrifuged at 1000 *g*, supernatant fluid was discarded, 35 ml of acid–pepsin solution were added (Tilley & Terry, 1963) and tubes were returned to the water bath. Tubes were shaken three times daily throughout the 96 h *in vitro* fermentation and 48 h acid–pepsin digestion. Following incubation, tubes were stored at 0 °C before detergent extraction. Tubes were thawed and contents transferred to 600 ml Berzelius beakers. For IVNDF and IVADF isolation, 100 ml of the appropriate detergent solution were added (decalin excluded) to beaker contents and refluxed for 1 h (Goering & Van Soest, 1970). Following refluxing, samples were filtered through pre-weighed Whatman No. 541 filter paper, rinsed, oven-dried (100 °C) and reweighed.

Individual faecal grab samples were dried (100 °C) and ashed (500 °C), after which ash was solubilized with 3·1 N HCl. Ytterbium and Dy were determined by atomic absorption spectrophotometry using an acetylene, nitrous oxide flame (McCollum & Galyean, 1985).

Calculations. Dry matter digestibility was determined for each lamb from feed to faeces ratios of IVADF, IVNDF and ADL. Faecal recovery of the three internal markers was calculated by multiplying marker concentration in faecal composites by actual faecal output and dividing by marker intake. Faecal Yb and Dy excretion curves were fitted to a one-compartment model (Ellis, Matis, Pond, Lascano & Telford, 1984) using the non-linear regression option (Marquardt method) of SAS (1984). The equation for the one-compartment model is as follows:

$$Y = k_0.(t-\tau).(k_1)^2.e^{-k_1(t-\tau)}$$

where Y = expected concentration in the faeces (μg/g faecal D.M.) sampled at time t; k_0 = scaling factor with units μg/g × h such that when $t = 0$, $k_0 \times k_1 = C_0$, where C_0 is the initial concentration of marker within the age-dependent compartment with units of μg/g of digesta dry matter; k_1 = the age-dependent rate parameter for a γ_2 distribution of passage rates which increase with age, t, in the age-dependent passage compartment (this asymptotic passage rate is used to estimate $\bar{k}_1$; Matis 1984, 1987) and τ = time from dose until first appearance of marker in faeces.

Parameters estimated by fitting the above model to faecal marker concentration were then used to calculate the following: $\bar{k}_1$, the mean passage rate (per h) averaged over all past residence times, which for the γ_2 distribution of passage rates = $k_1 \times 0{\cdot}59635$ (Ellis, Matis, Pond, Lascano & Telford, 1984); faecal output (g/h) = dose of marker (μg)/k_0 and mean gastro-intestinal retention time = $2/k_1 + \tau$, where $2/k_1$ equals the mean compartmental retention time where a γ_2 distribution of rates was assumed in the model (Matis, 1984). A time dimension for faecal output calculated as shown above is derived from k_0 because it involves both concentration at $t = 0$ and the rate parameter k_1.

Statistical analyses. During the experiment, a lamb fed the LS diet experienced digestive disturbances and was removed from the study. Thus the number of lambs per treatment was five for all diets except LS, where data for only four lambs were used. Estimates of digestibility were analysed by analysis of variance as a split-split-plot design with effects of diet, lamb within diet, marker, marker × diet, marker × lamb within diet, faeces drying method, faeces drying method × diet and faeces drying method × marker × diet included in the model. Diet, marker and drying method were main plot, subplot and sub-subplot, respectively. Estimates of faecal output and passage rate parameters were analysed by analysis of variance as a split-plot design with effects of diet, lamb within diet, marker and marker × diet. Because of marker × diet interactions ($P < 0{\cdot}05$), comparisons among markers were made within diets using a model including effects for marker, lamb within diet and lamb within diet × marker. Lamb within diet and lamb within diet × marker were pooled and used as the error term with 16 degrees of freedom (D.F.) for digestibility data, 12 D.F. for marker recovery and faecal output data and 8 D.F. for passage rate data. For the LS diet, error D.F. were reduced to 12, 9 and 6, respectively. If the F-test for marker effects was significant ($P < 0{\cdot}05$), differences among means were determined by the least significant difference method (Snedecor & Cochran, 1980).

RESULTS AND DISCUSSION

Digestibility. Method of drying faeces did not alter dry matter digestibility (DMD) estimates; thus, values were averaged over the two drying methods. These results are in agreement with Van Soest (1982),

Table 2. *Apparent dry matter digestibility coefficients (%) in lambs fed hay and hay:grain diets*

Diet	Method of determination*				
	In vivo	IVNDF ratio	IVADF ratio	ADL ratio	S.E.
Prairie hay	61·6	53·2	50·6	50·3	1·7
Lucerne hay	64·0	59·8	58·8	61·4	1·8
50% Prairie hay: 50% sorghum grain	71·6	68·6	66·4	62·0	2·5
50% Lucerne hay: 50% sorghum grain	75·4	76·0	71·9	70·2	2·7

* IVNDF = indigestible neutral detergent fibre, IVADF = indigestible acid detergent fibre, ADL = acid detergent lignin.

Table 3. *Marker recovery (%) in lambs fed hay and hay:grain diets*

Diet	Method of determination*			
	IVNDF ratio	IVADF ratio	ADL ratio	S.E.
Prairie hay	82·0	77·3	77·6	5·4
Lucerne hay	90·2	87·7	93·8	8·2
50% Prairie hay: 50% sorghum grain	92·6	85·7	75·7	11·8
50% Lucerne hay: 50% sorghum grain	101·7	87·4	82·2	13·5

* IVNDF = indigestible neutral deterent fibre, IVADF = indigestible acid detergent fibre, ADL = acid detergent lignin.

who reported that faeces is not as susceptible to the formation of artifact lignin as is feed.

Apparent DMD of PH estimated from IVNDF, IVADF and ADL was lower ($P < 0·05$) than *in vivo* DMD (Table 2). Faecal recoveries of IVNDF, IVADF and ADL were low (82·0, 77·3 and 77·6%, respectively; Table 3), indicating that extensive disappearance of marker may have occurred during gastro-intestinal transit. Cochran *et al.* (1986) and Hunt *et al.* (1984) reported similar results with IVADF in ruminants consuming prairie hay and orchard-grass hay, respectively. Likewise, the IVNDF ratio underestimated *in vivo* DMD in ruminants consuming prairie hay (Cochran *et al.* 1986), buffel-grass and coastal bermuda-grass (Lippke, Ellis & Jacobs, 1986). In contrast to present results, Hunt *et al.* (1984) reported DMD estimates using lignin ratios that were similar to *in vivo* values for cattle consuming orchard-grass hay.

In vivo DMD for LH (Table 2) did not differ ($P > 0·05$) from estimates obtained with any of the markers; however, the IVADF value tended ($P < 0·10$) to be lower than the *in vivo* value. Faecal recoveries (Table 3) were considerably greater than those obtained with PH. Cochran *et al.* (1986) reported *in vivo* DMD of steers fed cubed lucerne could be predicted accurately by either IVNDF or IVADF, with faecal recoveries of 101·2% and 99·1% for IVNDF or IVADF, respectively. Thus, feed processing method might have an effect on the predictive ability of these markers, or obtaining representative samples of diet for analysis may be more easily accomplished with cubed *v.* coarsely ground hay.

In vivo DMD for PS (Table 2) did not differ from DMD estimates from IVNDF or IVADF. The ADL estimate, however, was lower ($P < 0·05$) than the *in vivo* value. No differences were noted among the three markers. Faecal recoveries reflected errors in digestibility estimates but were generally greater than those obtained with PH alone.

In vivo DMD for LS (Table 2) did not differ ($P > 0·05$) from marker estimates. Faecal recoveries were variable and greater than those noted for lambs fed the PS diet (Table 3).

In conclusion, *in vivo* DMD of limit-fed (1·8% of body weight) lambs was consistently higher than DMD estimated by IVNDF, IVADF or ADL ratio, reflecting low marker recovery in the faeces. Because digestibility estimates are generally coupled with faecal output estimates to determine forage intake of free-grazing ruminants, the use of these markers to provide quantitative estimates of DMD may be questionable.

Faecal output. Faecal output (Table 4) in lambs consuming the PH diet did not differ significantly from, but was numerically less than, estimates derived

Table 4. *Faecal output estimates (g/day) in lambs fed hay and hay:grain diets*

Diet	Method of determination			S.E.
	Total faecal collection	Ytterbium-labelled hay	Dysprosium-labelled faeces	
Prairie hay	276·5	332·0	318·0	34·3
Lucerne hay	260·6	267·2	392·0	21·0
50% Prairie hay: 50% sorghum grain	204·4	172·5	240·1	23·9
50% Lucerne hay: 50% sorghum grain	166·4	155·5	139·2	17·3

Table 5. *Particulate passage rate, tau and mean gastrointestinal time in lambs fed hay and hay:grain diets*

Diet	Method of determination		S.E.
	Ytterbium-labelled hay	Dysprosium-labelled faeces	
Particulate passage rate (per h)			
Prairie hay	0·033	0·045	0·003
Lucerne hay	0·040	0·048	0·005
50% prairie hay: 50% sorghum grain	0·028	0·044	0·004
50% lucerne hay: 50% sorghum grain	0·030	0·030	0·004
τ (h)			
Prairie hay	21·5	17·3	1·4
Lucerne hay	16·9	13·5	1·5
50% prairie hay: 50% sorghum grain	23·1	20·7	1·1
50% lucerne hay: 50% sorghum grain	22·0	14·1	1·0
Mean gastro-intestinal retention time (h)			
Prairie hay	57·9	44·6	2·1
Lucerne hay	48·0	40·1	1·9
50% prairie hay: 50% sorghum grain	67·9	49·0	4·6
50% lucerne hay: 50% sorghum grain	64·0	55·3	4·6

from YLF and DLF. These values represent 20% and 15% overestimations in faecal output for YLF and DLF, respectively. In contrast, Krysl *et al.* (1985) reported a pulse dose of Yb-labelled forage overestimated faecal output by only 3% in lambs consuming prairie hay *ad libitum*.

Faecal output in lambs consuming the LH diet was similar to estimates derived from YLF, but DLF overestimated faecal output by 50% ($P < 0·05$, Table 4). The 2·5% overestimation for YLF is similar to results of Krysl *et al.* (1985) for lambs fed lucerne hay *ad libitum*.

In lambs fed the PS diet, faecal output was numerically greater but not significantly different from the estimate derived from YLF (16% underestimation). Although not significant, DLF again overestimated faecal output by 17%. No significant differences between actual faecal output and YLF (6·6% underestimation) or DLF (16% underestimation) estimates (Table 4) were noted in lambs fed the LS diet.

Estimates using YLF did not differ statistically ($P > 0·05$) from total faecal collection for lambs consuming any of the diets; however, substantial over- and underestimations were noted. Estimates with DLF did not differ ($P > 0·05$) from total faecal collection for lambs consuming PH, PS or LS diets, although substantial errors were also apparent. Whether these techniques offer any advantages over more widely used methods (e.g. chromic oxide) is questionable.

Passage rate and mean gastro-intestinal retention time data from the one-compartment model are shown in Table 5. Considerable differences were noted between YLF and DLF in estimates of passage rate constants. Particulate passage rate estimates were faster ($P < 0·05$) with DLF than YLF with PH and PS diets but not different ($P > 0·05$) for LH and LS diets. Calculated first appearance of marker (τ) was less ($P < 0·05$) with DLF than YLF only in lambs fed LS; however, τ was numerically less for DLF than YLF with all diets. Mean gastro-intestinal retention

time was greater ($P < 0.05$) when estimated using YLF than when using DLF for PH, LH and PS diets.

Because faecal particulate matter would be of a size capable of passing the reticulo-omasal orifice (Ulyatt *et al.* 1986), one might expect faster passage rate constants with labelled faeces than would be obtained using labelled feeds. It is unlikely that differences between the rare earth elements *per se* affected results, because Goetsch & Galyean (1983) reported no differences in passage rate estimates between Yb- and Dy-labelled lucerne hay in beef steers.

Recently, Erdman & Smith (1985) reported that Yb selectively labels the small particle fraction of feedingstuffs when used in an immersion labelling approach. Present data suggests Yb-labelled forage does not completely mimic the flow of small particles because passage rate constants were generally less than those obtained with DLF. In experimental situations where both estimates of passage rate and faecal output are desired, pulse doses of rare earth-labelled feeds may be a useful technique; however, for prediction of faecal output alone, the use of rare earth-labelled feeds, in conjunction with a one-compartment, age-dependent model (Ellis, Matis, Pond, Lascano & Telford, 1984), does not appear to offer distinct advantages over more commonly used methods.

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