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Passage through the rumen and the large intestine of sheep estimated from faecal marker excretion curves and slaughter trials

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External digesta markers (Yb-labelled diets and Co-EDTA) were given orally as a pulse dose to four pairs of Rasa Aragonesa twin ewe lambs, fed on either chopped or ground and pelleted lucerne hay, in order to estimate slow (k_1) and fast (k_2) rates of passage of liquid and solid phase from faecal marker excretion curves. After the faecal sampling period daily doses of the same markers were infused continuously for 5 d and the animals slaughtered. Concentrations of markers in the different compartments of the gut were determined and used to calculate mean retention times. The results showed that the rumen and the large intestine were the two main mixing compartments of the gut, accounting for more than 95 % of total mean retention time. Rates of passage estimated from faecal marker excretion did not accurately represent marker kinetics in the compartments of the gut derived from slaughter data. Accuracy in the estimation of fractional outflow rate from rumen (k_R) by k_1 was higher for low values of k_R whereas k_2 consistently overestimated large intestine outflow rate (k_{LI}), especially for high values of k_R. The relationship between outflow rates from the main two mixing compartments was important in influencing the accuracy of prediction of faecal estimates.

Rate of passage: Gastrointestinal tract: Sheep

Retention time of digesta in the different compartments of the ruminant gut, especially the ruminoreticulum, is one of the most important factors affecting the extent and site of digestion, and consequently the amounts and types of nutrients actually reaching the duodenum (Ellis, 1978). In addition, retention time is important when determining the physical regulation of voluntary intake (Ulyatt *et al*. 1986).

The direct measurement of digesta flow is laborious and generally involves the use of surgically modified animals; obtaining representative samples of rumen digesta is often difficult, especially from animals fed on forages in the long form (Mansbridge & Ørskov, 1980). The alternative approach, involving markers and mathematical models, has the potential for a more adequate description of the overall process and for delineating possible causal mechanisms; however, it also has the potential for errors in interpretation if the requirements of the approach are not met or the assumptions involved are not understood (Ellis *et al*. 1984). In this respect there is still a lack of agreement in the interpretation of kinetic parameters obtained from faecal marker excretion curves (Blaxter *et al*. 1956; Grovum & Williams, 1973; Ellis *et al*. 1979; Dhanoa *et al*. 1985).

In the present experiment, comparisons were made between outflow rates obtained from faecal marker excretion or from slaughter trials, and the reasons for discrepancies analysed. Preliminary results have already been presented as either posters or oral communications (Gasa *et al*. 1993; de Vega *et al*. 1994*a*,*b*).

Materials and methods

Animals and diets

Eight 11-month-old Rasa Aragonesa twin lamb ewes, averaging 33·6 (SE 0·75) kg live weight, were allocated to one of two diets of lucerne (*Medicago sativa*) hay either chopped (50 mm; diet C) or ground (2 mm) and pelleted (diet P). Intake was restricted to 90 % of previously established *ad libitum* values and diets were offered in twelve daily meals. Chemical composition of both diets is shown in Table 1.

Animals were kept in individual metabolic cages during the digestibility trial and the faecal sampling period, and in individual stands, bedded on sawdust, during the rest of the experiment. They had free access to water and mineral

Abbreviations: LP, lignin permanganate; TMRT, total mean retention time; TT, transit time.

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Table 1. DM (g/kg) and chemical composition (g/kg DM) of the diets

	Chopped lucerne hay	Pelleted lucerne hay
DM	847	877
Organic matter	857	832
Nitrogen	31·5	29·1
Ether extract	22	24
Neutral-detergent fibre	457	431
Acid-detergent fibre	315	312
Lignin permanganate	89	75

blocks. At 1 month before the experimental period the animals were treated orally against internal parasites with Thiabendazol.

Experimental procedure

The experiment lasted for 40 d of which the first 14 d were for adaptation and measurement of voluntary intake. The sheep were then placed in metabolic cages, fed at 90 % *ad libitum* and after 3 d, a 7 d digestibility trial was performed.

Once the animals were back in the standings, and after a 3 d resting period, they were given pulse doses of 10 g Yb-labelled diets and 50 ml of a solution containing 0·5 g of Co-EDTA. Labelled diets were orally dosed with the aid of a 50 ml plastic syringe barrel. Labelled diet C was previously chopped down with scissors to a size of about 5 mm. The Co-EDTA solution was orally introduced with the aid of a 10 ml plastic syringe. Bulk faecal samples were taken at 3, 4·5, 6, 7·5, 9, 12, 15, 18, 21, 24, 28, 33, 39, 48, 58, 72, 120 and 144 h post dose to construct the faecal marker excretion curves.

After a 2 d rest period, and for five consecutive days, daily doses of 6 g Yb-labelled diets and 60 ml of a solution containing 0·2 g of Co-EDTA were administered in twelve equal portions at feeding time to mimic a continuous infusion. Lignin was also used as internal marker. Three faecal samples were taken at 3, 9 and 21 h on the fifth day of marker administration to check for steady-state conditions of external markers, and the following morning animals were slaughtered, after administration of a sedative-analgesic. The gut was removed and separated into ruminoreticulum, omasum, abomasum, small intestine, caecum, ascending colon and a fraction including transverse colon, descending colon and rectum. Digesta contents were weighed and sampled for chemical composition, particle size and marker analysis. As marker concentration did not differ among the different fractions of the hindgut it was considered as a single mixing compartment, hereafter referred to as the large intestine.

Marker techniques

Co-EDTA was prepared according to the technique developed by Udèn *et al*. (1980), and the Yb-labelled diets by soaking the forages in a buffer of acetate solution (0·1 M-acetic acid adjusted to pH 6·0 with NH_4OH) for 3 h and then overnight in the same solution containing 17 mg of ytterbium acetate/kg DM. The labelled material was washed several times with distilled water and allowed to dry.

Digesta and faecal samples were dried at 105° for 24 h, ground to pass through a 1 mm screen and analysed for marker concentrations by adding 15 ml 1·5 M-HNO_3 + 0·027 M-KCl and 5 ml 7 M-$HClO_4$ to the ashed (550° for 8 h) material. After boiling gently for 3 min, samples were filtered and marker concentrations determined by atomic emission spectrometry using a Perkin-Elmer P-40 spectrophotometer (Perkin Elmer, Uberlingen, Germany).

Faecal marker excretion curves were fitted to the multi-compartmental model developed by Dhanoa *et al*. (1985), which was restricted to two compartments as the rumen and the caecum are assumed to be the main functional mixing pools of the gut. This model consists of a multiplicative equation which includes single and double exponential terms:

$$y = Ae^{-c_1 t}\exp[-Be^{-c_2 t}],$$

where A, B, c_1 (k_1) and c_2 ($k_2 - k_1$) are parameters estimated by iterative procedures. In the model k_1 and k_2 are estimates of the outflow rate of digesta through the rumen and the large intestine, although not necessarily in that order. Estimates of transit time (TT) and total mean retention time (TMRT) were also calculated as

$$TT = \sum_{i=3}^{N-1} \frac{1}{k_2 + (i-2)(k_2 - k_1)},$$

and

$$TMRT = 1/k_1 + 1/k_2 + \sum_{i=3}^{N-1} \frac{1}{k_2 + (i-2)(k_2 - k_1)}, \quad k_2 > k_1.$$

Model fitting was performed using a DEC VAX/750 computer and the MLP statistical package (Ross, 1987).

Outflow rates from the different compartments of the digestive tract in the slaughter trial were estimated according to the procedures described by Faichney (1975), using the equation

$$K = F/Q,$$

where K represents the fractional outflow rate (/h) from the compartment, F the rate of administration of markers (mg/h) and Q the amount of marker (mg) actually present in the compartment, calculated as

$$Q = C \times A,$$

where C represents the concentration of markers in the digesta at steady-state conditions (mg/g DM) and A the amount of DM (g) in the pool considered.

Chemical analysis

The DM content of each feed and digesta sample was determined by oven drying at 105° for 24 h, and organic matter in the diets by ashing at 550° for 8 h. Total N was determined by the Kjeldahl method. Neutral-detergent fibre, acid-detergent fibre and lignin permanganate (LP) were measured on dried samples according to the method of Goering & Van Soest (1970). Ether extract was determined by the method of the Association of Official Agricultural Chemists (1985).

Particle size analysis

The particle size distribution of the contents of the different compartments of the gastrointestinal tract (rumen, reticulum, omasum, abomasum, small intestine and large intestine) was determined using a wet-sieving apparatus as described by Evans *et al*. (1973). Sieve sizes were chosen in a geometric progression (Kennedy, 1984), being of 0·15, 0·30, 0·60, 1·20 and 2·40 mm (length of side of square hole). Between 15 and 65 g digesta (about 5 g DM) was thoroughly dispersed in about 200 ml water and washed onto the top sieve of the apparatus. Water flow was set at 4·85 litres/min and the apparatus was run for 5 min. The sieves were then inspected so that any clumps of particles could be dispersed and the apparatus was run for another 5 min. Particles retained on each sieve were collected onto tared filter papers and dried at 70° for 48 h. The dry weights were expressed as a percentage of the total DM in the sieved sample.

Mean particle size was estimated following the procedures described by Pond *et al*. (1984) where the calculated value indicates the size of the theoretical sieve which retains 50 % of the particles.

Statistical analysis

Intake and digestibilities, and whole digesta, DM, organic matter, acid-detergent fibre, and LP contents in the different compartments of the gut were analysed by means of one-way ANOVA following the methods proposed by Steel & Torrie (1980). Differences in digesta weights between diets were analysed separately for each compartment of the gut.

Mean particle size of the digesta contents of the different compartments was analysed as a split-plot, with animals as the main plot and gut compartment the subplot.

Faecal marker excretion data and outflow rates through each compartment of the gut were analysed as a split-plot design, animal being the main plot and marker the subplot.

The intake of one of the animals on diet C during the continuous marker infusion period was significantly lower than during the previous period, hence it was removed from the analysis and treated as a missing value.

Significant differences between treatment means were compared by the least significant difference test.

Faecal marker parameters k_1 and k_2 were compared with slaughter-derived passage rates through the rumen and large intestine respectively, by means of paired *t* tests.

Results

Mean daily intakes and digestibility coefficients are shown in Table 2. Average DM intake was not significantly affected by the physical form of the forage, although intakes of diet P tended to be larger. During continuous administration of markers, intake was slightly lower than planned (65·5 and 88·3 g DM/kg metabolic weight ($W^{0·75}$) for diets C and P respectively), probably due to the stress caused to the animals. On the other hand digestibility coefficients were significantly larger on diet C for crude protein ($P < 0·001$), DM ($P < 0·01$) and organic matter ($P < 0·05$) but not for neutral-detergent fibre ($P > 0·05$).

Table 2. DM intake (g/kg live weight$^{0·75}$), and DM, organic matter (OM), crude protein (CP), and neutral-detergent fibre (NDF) digestibility coefficients (%) of lucerne hay which was chopped (C) or ground and pelleted (P), during the digestibility trials

(Mean values for four sheep)

	C	P	SE (6 df)	*F*	*P*
DM intake	71·8	94·3	8·58	3·44	0·1130
Digestibility					
DM	53·5	47·1	1·22	14·03	0·0096
OM	54·0	48·7	1·41	6·95	0·0387
CP	68·8	58·7	0·67	114·75	0·0001
NDF	32·8	32·3	3·45	0·01	0·9175

Table 3. Weights (g) of whole digesta (WD), DM, organic matter (OM), acid-detergent fibre (ADF) and lignin permanganate (LP) in the different compartments of the digestive tract of animals consuming lucerne hay which was chopped (C) or ground and pelleted (P)

	C	P	Residual SD*
Rumen			
WD	4190	3529	842·6
DM	748	616	200·5
OM	668	547	181·9
ADF	341	271	77·4
LP	131	106	30·6
Omasum			
WD	117	106	42·0
DM	25	23	9·8
OM	22	24	6·6
ADF	11	12	3·7
LP	4	4	2·0
Abomasum			
WD	185	192	85·0
DM	20	19	9·5
OM	18	19	6·8
ADF	9	8	3·9
LP	4	3	1·5
Large intestine			
WD	765	916	311·3
DM	130	207	50·1
OM	110	168	42·2
ADF	55	80	22·3
LP	20	31	8·9

* There was one missing value.

Table 4. Mean particle size (mm) of digesta in the different compartments of the digestive tract of animals consuming lucerne hay which was chopped (C) or ground and pelleted (P)

	C	P	Mean	Residual SD (21 df)*
Reticulum	0·81^{a}	0·45^{a}	0·63	
Rumen	0·86^{a}	0·45^{a}	0·65	
Omasum	0·29^{b}	0·29^{c}	0·29	0·031†
Abomasum	0·30^{b}	0·36^{b}	0·33	
Small intestine	0·27^{b}	0·29^{c}	0·28	
Large intestine	0·25^{b}	0·26^{c}	0·25	
Mean	0·46	0·35		
Residual SD (5 df)*	0·083‡			

$^{a, b, c}$ Values in the same column with unlike superscript letters were significantly different ($P < 0·05$). For differences between diets within each compartment see pp. 381–383.

* There were ten missing values.

† For comparisons between gut compartments, between diets within each gut compartment and between gut compartments within each diet.

‡ For comparisons between diets.

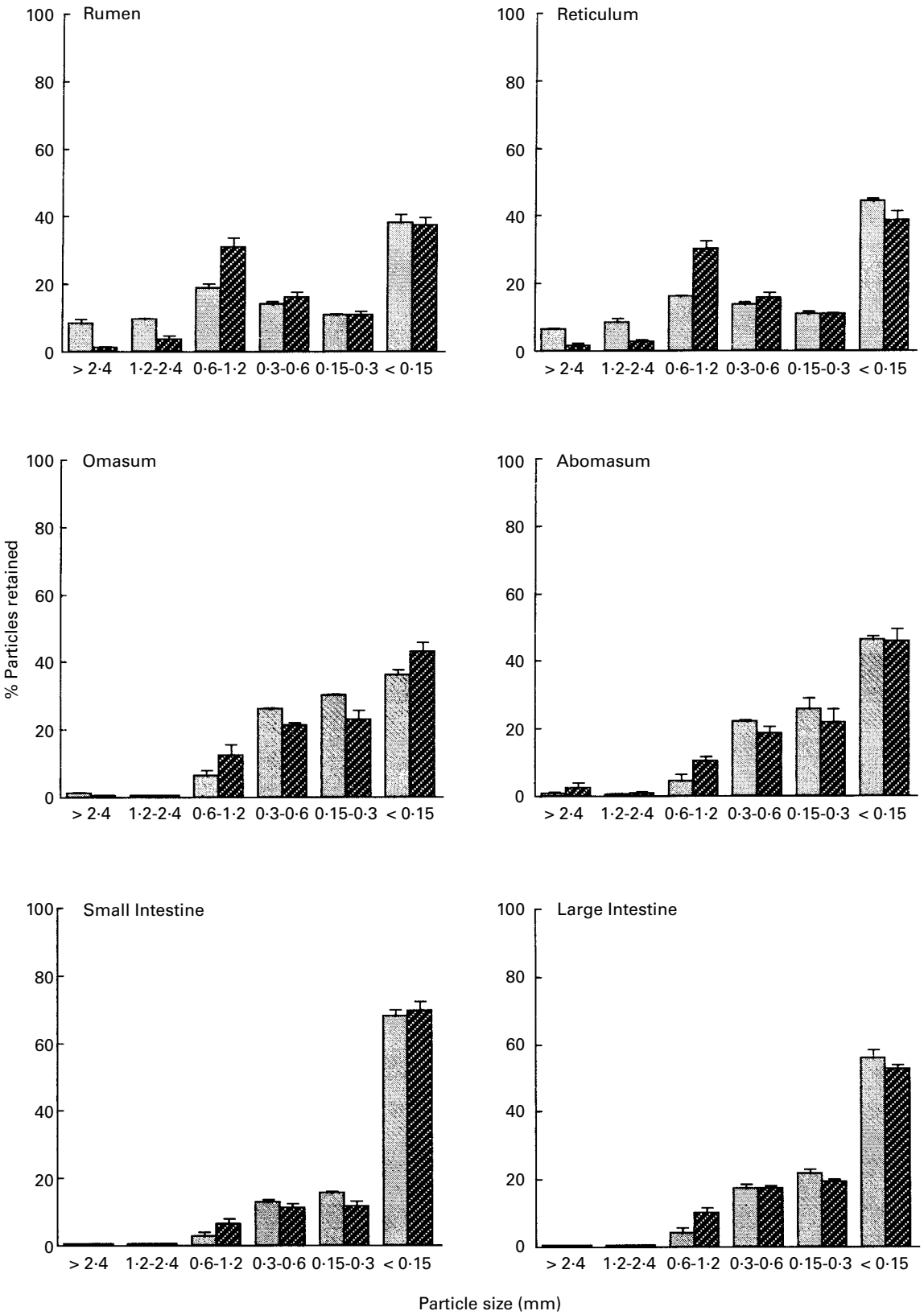

Fig. 1. Particle size distribution within the different compartments of the gut from sheep fed on lucerne hay which was chopped (▦) or ground and pelleted (▨).

Table 5. Slow (k_1) and fast (k_2) fractional outflow rates (/h), transit time (TT; h) and total mean retention time (TMRT; h) of cobalt-EDTA (Co) and ytterbium-labelled diets (Yb) in animals consuming lucerne hay which was chopped (C) or ground and pelleted (P)

(Mean values for four sheep)

	C	P	Mean	SE (6 df)*	*P*
k_1					
Co	0·054	0·080	0·067[a]	0·0029	0·0102
Yb	0·045	0·059	0·052[b]		
Mean	0·050	0·069			
SE (6 df)†	0·0070				
P	0·0901				
k_2					
Co	0·475	0·648	0·562	0·0517	0·0568
Yb	0·301	0·479	0·390		
Mean	0·388	0·564			
SE (6 df)†	0·0776				
P	0·1608				
TT					
Co	6·4	6·5	6·5[a]	0·38	0·0027
Yb	10·2	8·0	9·1[b]		
Mean	8·3	7·3			
SE (6 df)†	0·94				
P	0·4498				
TMRT					
Co	29·0	21·3	25·2[a]	1·31	0·0045
Yb	39·4	27·4	33·4[b]		
Mean	34·2	24·3			
SE (6 df)†	4·68				
P	0·1863				

[a, b] Mean values within a category with unlike superscript letters were significantly different ($P < 0·05$).
* For comparisons between markers.
† For comparisons between diets.

The weights of the rumen, omasum, abomasum and large intestine contents were not significantly affected by diet type (Table 3), whereas mean particle size was significantly affected by the interaction diet × gut compartment (Table 4). Particle size of digesta was larger in animals fed on diet C for the reticulum and the rumen ($P < 0·001$), with no statistically significant differences between diets for the other compartments. For diet C there were no differences in mean particle size from omasum onwards, whereas for diet P abomasum showed values lower than for rumino-reticulum but higher than for omasum, small intestine and large intestine, with no differences between the latter compartments. The model used for statistical analysis of particle size data proved satisfactory ($P = 0·0001$). The particle size distribution within each compartment of the gut is shown in Fig. 1.

Fractional outflow rates (k_1, k_2), TT and TMRT of Co-EDTA and Yb-labelled diets, estimated from faecal marker excretion curves, are presented in Table 5. Grinding and pelleting tended to increase rate of passage of both markers although differences were not significant. Estimates of TT and TMRT were not affected by diet although TMRT tended to be higher on diet C. Co-EDTA showed faster rates of passage and shorter TT, and consequently shorter TMRT than Yb-labelled diets ($P < 0·01$). The split-plot model explained most of the variability in TMRT ($P = 0·0022$), TT ($P = 0·0113$) and k_1 ($P = 0·0088$).

The concentration of markers in the three faecal samples taken the day before slaughter did not change with time as shown by the regression coefficient values which were not

Table 6. Fractional outflow rates (/h) from the ruminoreticulum (k_R), omasum (k_O), abomasum (k_A) and large intestine (k_{LI}) of cobalt-EDTA (Co), ytterbium-labelled diets (Yb) and lignin permanganate (LP) in sheep consuming lucerne hay which was chopped (C) or ground and pelleted (P)

	C	P	Mean	Residual SD
k_R				
Co	0·073	0·162	0·117	
Yb	0·041	0·068	0·054	0·0117 (10 df)*
LP	0·024	0·045	0·035	
Mean	0·046	0·092		
Residual SD (5 df)†	0·0359			
k_O				
Co	1·964	5·666	3·815	
Yb	1·168	1·864	1·516	2·0293 (9 df)‡
LP	0·934	3·072	2·003	
Mean	1·355	3·534		
Residual SD (5 df)†	4·2631			
k_A				
Co	3·297	6·075	4·686	
Yb	2·617	2·675	2·646	1·7738 (9 df)‡
LP	1·318	3·404	2·361	
Mean	2·411	4·051		
Residual SD (5 df)†	4·7639			
k_{LI}				
Co	0·142	0·148	0·145	
Yb	0·166	0·138	0·152	0·0221 (10 df)‡
LP	0·180	0·149	0·165	
Mean	0·163	0·145		
Residual SD (5 df)†	0·0846			

* For comparisons between markers, between diets within each marker and between markers within each diet.
† For comparisons between diets.
‡ For comparisons between markers.

different from zero ($P > 0{\cdot}1$). From these results steady-state conditions were assumed.

The outflow rates from ruminoreticulum (k_R), omasum (k_O), abomasum (k_A) and large intestine (k_{LI}) of Co-EDTA, Yb-labelled diets and LP estimated at slaughter are presented in Table 6. The ruminoreticulum and the large intestine were shown to be the main mixing compartments, the cumulative retention time in these two pools ($1/k_R + 1/k_{LI}$) accounting for more than 95% of the mean retention time in the four mixing compartments of the gut ($1/k_R + 1/k_O + 1/k_A + 1/k_{LI}$). Rumen outflow rates of all markers were significantly increased by grinding and pelleting, but the highest values were recorded with Co-EDTA. Differences between Yb-labelled particles and LP were significant only for diet P. Large intestine outflow rate was independent of both marker and diet type and, except for Co-EDTA on diet P ($P > 0{\cdot}05$), was always faster than rumen outflow rate ($P < 0{\cdot}05$). The model used for statistical analysis of the data was adequate for all the parameters considered ($P = 0{\cdot}0001$ for k_R, $P = 0{\cdot}0321$ for k_O, $P = 0{\cdot}0096$ for k_A and $P = 0{\cdot}0013$ for k_{LI}).

As shown in Fig. 2, mean retention time in the two main mixing compartments of the gut ($1/k_R + 1/k_{LI}$) was not accurately represented by faecal marker excretion parameters ($1/k_1 + 1/k_2$), low mean retention time values being generally overestimated and high values generally underestimated by faecal outflow rates. The regression coefficient of $1/k_1 + 1/k_2$ *v.* $1/k_R + 1/k_{LI}$ was 0·99 ($y = -0{\cdot}29 + 0{\cdot}99x$; r^2 0·62; $P < 0{\cdot}001$), although when data from the ewe which presented the higest values of $1/k_1 + 1/k_2$ were removed the coefficient became 0·38 ($y = 10{\cdot}40 + 0{\cdot}38x$; r^2 0·71; $P < 0{\cdot}001$). As shown in Fig. 3 increasing values of k_R were reflected in increasing values of k_1 although generally to a lesser extent. There was no significant difference

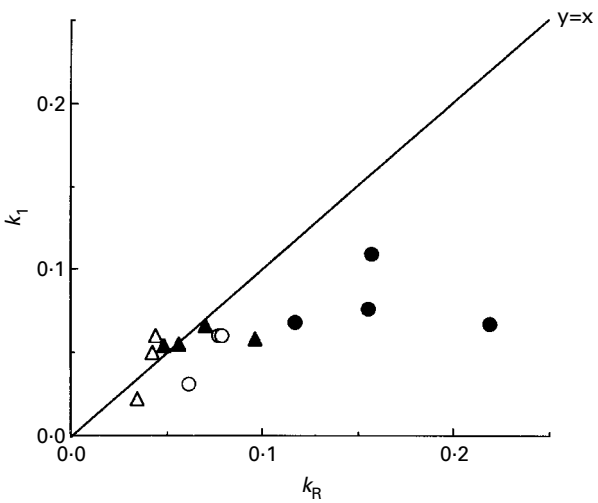

Fig. 3. Relationship between fractional outflow rate from the rumen estimated from slaughter samples (k_R) and from faecal marker excretion curves (k_1), as affected by diet (lucerne hay which was chopped (○, △) or ground and pelleted (●, ▲)) and indicated by marker (cobalt-EDTA (○, ●) or ytterbium-labelled diets (△, ▲)).

(*t* 0·27; 10 df) between the regression coefficients for Yb-labelled diets ($k_1 = 0{\cdot}03 + 0{\cdot}38k_R$; r^2 0·31; $P = 0{\cdot}1919$) and Co-EDTA ($k_1 = 0{\cdot}04 + 0{\cdot}24k_R$; r^2 0·33; $P = 0{\cdot}1795$) in the relationship between k_R and k_1. The values of k_R were significantly higher than k_1 for Co-EDTA ($P < 0{\cdot}05$ for both diets C and P) but not for Yb-labelled particles ($P > 0{\cdot}1$). Differences were larger for diet P as a result of the higher outflow rates recorded with this diet. By contrast, and as shown in Fig. 4, k_{LI} values were always higher than k_2, for both Co-EDTA ($P < 0{\cdot}05$ for both diets C and P) and

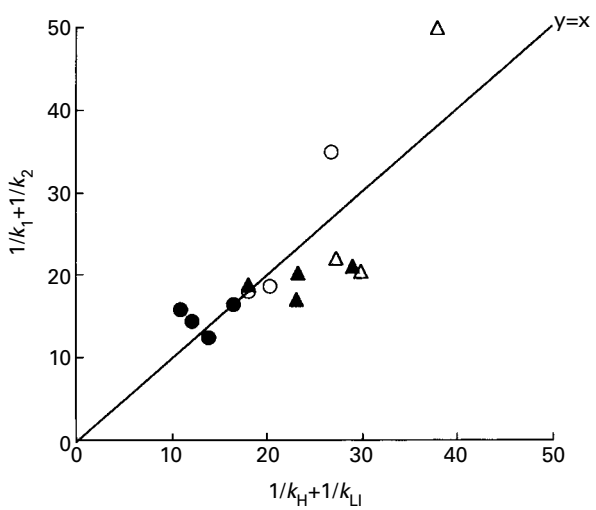

Fig. 2. Relationship between retention time in rumen and large intestine estimated from slaughter data ($1/k_R + 1/k_{LI}$) and faecal marker excretion curves ($1/k_1 + 1/k_2$), as affected by diet (lucerne hay which was chopped (○, △) or ground and pelleted (●, ▲)) and indicated by marker (cobalt-EDTA (○, ●) or ytterbium-labelled diets (△, ▲)). For details of regression analysis, see p. 386.

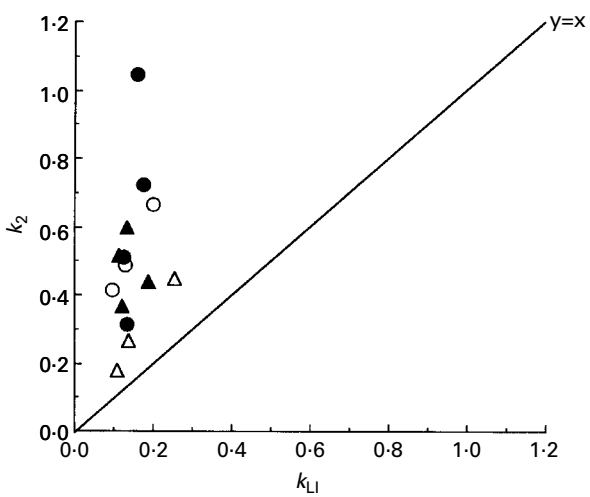

Fig. 4. Relationship between fractional outflow rate from the large intestine estimated from slaughter samples (k_{LI}) and from faecal marker excretion curves (k_2), as affected by diet (lucerne hay which was chopped (○, △) or ground and pelleted (●, ▲)) and indicated by marker (cobalt-EDTA (○, ●) or ytterbium-labelled diets (△, ▲)).

Yb-labelled particles ($P < 0{\cdot}1$ and $P < 0{\cdot}01$ for diets C and P respectively).

Discussion

Diet processing resulted in slight differences in chemical composition, which may be easily explained by selective losses during the grinding and pelleting of the lucerne. A larger DM intake and lower digestibility coefficients, associated with higher rumen outflow rate values were also recorded, as previously shown by Shaver *et al*. (1986), Woodford & Murphy (1988) and others.

There was a good relationship between Yb k_R and DM intake: Yb k_R (%/h) = $-0{\cdot}48 + 0{\cdot}078$ DM intake (g/kg $W^{0{\cdot}75}$ per d); r^2 0·64; $P = 0{\cdot}0304$, which was not accompanied by differences between diets in rumen load (Table 3). This linear relationship would probably have resulted from diet-derived differences in the proportion of particles in the rumen of a size capable of passage through the reticulo-omasal orifice (Table 4 and Fig. 1), together with a higher rate and extent of degradation of diet P, and differences in water turnover (Table 6).

As shown in Table 6, the contribution of the large intestine to total tract (excluding the small intestine) mean retention time was higher on diet P (51, 32 and 23 % for Co-EDTA, Yb-labelled diets and LP respectively) than C (33, 19 and 11 % respectively). The highest values were always recorded with Co-EDTA irrespective of diet. Differences between markers were mainly due to the different values of rumen outflow rate since liquids and solids do not behave independently in the large intestine as shown by Faichney & Boston (1983) and the present results. Moreover, retention time in both omasum ($1/k_O$) and abomasum ($1/k_A$) was quantitatively of minor importance (< 3 %).

Differences observed between average k_R (Table 6) and average k_1 (Table 5) were larger than those previously reported by Mira & MacRae (1982) or Mudgal *et al*. (1982) but similar to those recorded by Cruickshank *et al*. (1989) with young lambs fed *ad libitum* on high-quality forages. These differences were due to the kinetic behaviour of the liquid marker, as Yb-labelled particles showed similar average k_R and k_1 ($P > 0{\cdot}05$) for both C and P diets.

Similarly, k_{LI} (Table 6) was largely overestimated by k_2 (Table 5), this overestimation being greater than that found by Dixon *et al*. (1982) and Faichney & Boston (1983). However, in this earlier work k_2 was estimated by either applying first-order kinetics to caecal samples (Dixon *et al*. 1982) or by mathematical simulation (Faichney & Boston, 1983), while in our current experiment k_2 was derived from the multicompartmental model developed by Dhanoa *et al*. (1985). This model is known to produce higher k_2 estimates than other models (Gasa & Sutton, 1991). Moreover, our k_{LI} values were estimated with reference to the entire large intestine, and hence may have been underestimated with respect to the values obtained from caecal and proximal colon by the workers previously mentioned.

In a simulation study, Cruickshank *et al*. (1989) elegantly theorized that the ratio caecal : rumen outflow rates rather than the outflow rate values *per se* would influence the faecal excretion curve, and would dictate how accurately the excretion curve from the rumen is replicated in faeces. In the present experiment, the relationship k_{LI}/k_R was also observed to affect the accuracy of k_1 as an estimate of k_R. Cruickshank *et al*. (1989) estimated rumen outflow rate from duodenal or abomasal marker excretion curves, and caecal outflow rate from simulation, using the model of Grovum & Williams (1973), and assuming that the parameter was adequately represented by faecal k_2. The authors themselves pointed out that estimations of rumen outflow rate could be slightly erroneous as a result of the diurnal variation in rumen digesta content observed in grazing sheep and the associated fluctuations in marker concentration. On the other hand, the assumption that caecal outflow rate is adequately described by faecal k_2 may lead to large errors in its estimation (Ellis *et al*. 1979 and the present results). Slaughter experiments give more confidence to data used to estimate actual outflow rates, and our results support, experimentally, the suggestion that the caecum–large intestine can modify the faecal marker excretion curve. This leads to biased estimates of rumen outflow rate from faecal k_1. At high rumen outflow rates, analysis of the descending portion of the faecal marker excretion curve is likely to result in an erroneous estimate as shown to happen with Co-EDTA, especially on diet P, where the k_{LI}/k_R was lower, resulting in severe underestimates of k_R from k_1.

Cruickshank *et al*. (1989) suggested that multicompartmental models (Dhanoa *et al*. 1985) would minimize the bias from the estimate of k_R from k_1 as these models estimate simultaneously, and not sequentially, the rumen and large intestine outflow rates. However, in our present experiment the multicompartmental model of Dhanoa *et al*. (1985) did not provide reliable digesta kinetic estimates, and discrepancies between outflow rates estimated from slaughter or faecal marker excretion curves were large for both rumen and large intestine. Moreover, the model was originally developed for values of $k_2 > k_1$, which was not always the case in our experiment. Faichney & Boston (1983) and Cruickshank *et al*. (1989), among others, have already reported that the ruminoreticulum may not always have the longest mean retention time, and Dhanoa *et al*. (1985), describing the multicompartmental model, have stated that 'it fulfills the requirement of providing two rate constants which in theory relate to the two compartments having the longest mean retention time, although it has yet to be demonstrated clearly that they can be identified as the rumen and the caecum'. In our study the largest underestimates of k_R from k_1 were coincident with the largest overestimates of k_{LI} from k_2, as a result of the joint estimation of k_1 and k_2 by the model.

The TT calculated from this model (Table 5) also failed to represent retention time in tubular sections of the gut and mixing compartments other than the rumen and the large intestine (Table 6). This suggests that the faecal variable TT may also include events produced in either the ruminoreticulum or the large intestine. In addition, in the model of Dhanoa *et al*. (1985) TT is obtained from an expression including both k_1 and k_2, so misleading estimations of any parameter will give erroneous TT values.

At high outflow rates single-dose markers given orally may well result in a significant proportion of marker reaching the abomasum more rapidly, in turn modifying the faecal marker excretion curve. This is especially so with

liquid markers as shown by Cafe & Poppi (1994) who demonstrated that a significant proportion of imbibed water bypasses the rumen to go directly to the abomasum. As a result, multicompartmental models are likely to produce erroneous estimates of transit kinetics of markers through the rumen when their outflow rate is high, although they prove satisfactory for low outflow rates. Events which occurred in the large intestine were not represented at all by faecal k_2, and the discrepancies were larger also at higher rumen outflow rates. This fact precludes the use of multicompartmental models to estimate k_{LI}, even for low outflow rates. Further research is needed to develop a model to analyse faecal concentration curves accurately. Recent attempts have used multi-compartmental, double-marker models (Aharoni *et al*. 1994) but their accuracy in estimating rumen and large intestine outflow rates has yet to be checked experimentally for a wide range of diets and animals.

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