

## A quantitative model of reticulo-rumen particle degradation and passage

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Labelled particles were prepared by mordanting low concentrations (0.1 or 5 g/kg dry matter) of chromium to neutral-detergent-extracted stems (1–2 mm or 10 mm in length) of bromegrass (*Bromus inermis*). These were used in the study of reticulo-rumen particle kinetics of four steers given bromegrass hay and from the results a quantitative model of particle digestion and passage was developed. At the 0.1 g Cr/kg concentration there was minimal interference with digestibility of the feedstuff. The ratio, dry weight of the reticulo-rumen large-particle pool (> 3.35 mm):small-particle pool (< 3.35 mm) was 2:1. It was derived from the model that volatile fatty acids (VFA) and carbon dioxide in the rumen were produced mainly from large particles, and that between 500 and 700 g/kg hay dry matter was digested in the reticulo-rumen. It was also derived from the model that a major portion, 200 (SE 110) g/kg, of the hay dry matter was rapidly solubilized and that the material leaving the reticulo-rumen was composed of small particles (500–840 g/kg), large particles (100–160 g/kg) and an unknown portion of soluble dry matter of hay (0–400 g/kg). Disappearance from the large-particle pool in the model involving the lowest Cr level was directed to formation of VFA and CO<sub>2</sub> (0.68 (SE 0.04) of total flow) to the small-particle pool (0.25 (SE 0.06) of total flow) and direct passage from the reticulo-rumen (0.07 (SE 0.002) of total flow). The disappearance from the small-particle pool was to VFA and CO<sub>2</sub> production and to the omasum accounting for 0.14 (SE 0.18) and 0.86 (SE 0.24) respectively, of the total flow. It was concluded that the low-level-mordanting technique in combination with appropriate sampling yielded a realistic quantitative description of forage breakdown and movement processes in the digestive tract of cattle.

### Particulate kinetics: Reticulo-rumen model

Clearance of feed residues from the reticulo-rumen has long been recognized as a major process determining and, therefore, controlling intake and digestion of forages and, as a result, productive performance of the ruminant (Faichney, 1986). Clearance depends on two interacting processes: the first being breakdown through the physical reduction in particle size plus microbial digestion, and the second being physical passage from the reticulo-rumen. Although the qualitative aspects of clearance processes are well understood, increased use of mathematical models to examine control processes and increasing interest in manipulating rumen dynamics to improve efficiency depends on understanding the quantitative importance of these processes (Ulyatt *et al.* 1986).

Until now the best estimates of the rates of clearance of particles from the rumen have been made using indigestible or undigested material. However, use of such material does not yield a measure of the outflow of digestible nutrients, which probably would be intermediate between the flow pattern of fluids and solids (Church, 1979).

Chromium mordanting fulfils most of the desirable criteria for particulate marking. It yields a stable marker of solids forming hexacoordinate ligands with hydroxyl groups that are very difficult to hydrolyse (Uden *et al.* 1980). Cr mordanted to fibre is the most tenaciously bound of the particulate markers which have been examined (Ellis *et al.* 1980).

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Concentrations of Cr in excess of 80 mg/g dry matter (DM) render plant cell walls essentially indigestible (Colucci, 1979); as the content of Cr on the fibre decreases, the digestibility of fibre increases. Moreover, Cr bound to feedstuffs at high levels has been found to affect the rate of passage, probably due to altered specific gravity (Ehle, 1984).

The objective of the present work was to evaluate low-Cr-concentration mordanting of neutral-detergent-extracted forage particles as a means to attain marked particles that will yield biologically realistic measurements of particle kinetics in the rumen.

#### MATERIALS AND METHODS

##### *Animals, diets and management*

Four 15-month old Hereford steers weighing approximately 400 kg were prepared with rumen fistulas of 100 mm diameter. Each animal was maintained in an individual metabolism crate with continuous lighting at ambient temperatures of 20–22° for 2 weeks before and during the experiment. Bromegrass hay (*Bromus inermis*) harvested at mid- to late-bloom, was chopped through a 76 mm screen and offered at 2 h intervals using an automatic feeding device. Body-weight was maintained by providing 450 g hay DM each 2 h. Composition (/kg DM) of the diet was 13.9 g nitrogen, 672 g cell-wall constituents (neutral-detergent fibre; NDF) and 349 g acid-detergent fibre (ADF) with > 900 g large particles (LP) (those retained on screens of aperture 3.35 mm and larger after wet-sieving (Dixon & Milligan, 1985)). Cobalt mineralized salt blocks (Canadian Salt Ltd, Montreal) and water were available *ad lib*.

##### *Preparation of the Cr mordanted particles*

The leafy material was first separated from the stems by hand. Half the stems were ground in a Wiley Mill grinder with a screen aperture of 2 mm (small particles; SP), and the other half were cut to 10 mm lengths (LP). The ground stems were then sieved by a wet-sieving procedure (Dixon & Milligan, 1985) that allowed the particles to pass through a 2 mm screen but not through a 1 mm screen. Those passing were discarded. The particles retained by the 1 mm screen were kept and immersed in acetone for 24 h, in methanol for 24 h to dissolve the cuticle, and then washed in boiling neutral detergent (Goering & Van Soest, 1970). The stems were finally rinsed with distilled water.

The neutral-detergent-extracted bromegrass stems were mordanted with <sup>51</sup>Cr-containing sodium dichromate by a modified technique (Uden *et al.* 1980) using 0.1 or 5 g Cr/kg fibre DM. The quantities of <sup>51</sup>Cr used were 10.9 mCi/kg fibre in the sodium dichromate solution for the LP and 41.7 mCi/kg fibre in the binding solution for the SP. The weight of sodium dichromate solution was four times that of the plant fibre and the heating time was 3 h. The dry, mordanted stems were finally washed in boiling neutral detergent for 1 h and then washed thoroughly with tap-water until the solution was colourless. All the particles were then dried at 65° for 24 h, weighed, and the radioactivity/g DM was determined for each fraction using a gamma spectrometer (Model 8000; Beckman Instruments, Fullerton, California).

##### *Experimental design and schedule*

The experimental design was a 4 × 4 Latin square. The four treatments entailed 92 g LP (10 mm)/animal or 24 g SP (1–2 mm)/animal mordanted with either 5 or 0.1 g Cr/kg DM. Equal quantities of particles were placed in six different locations: top and bottom of the reticulum, rumen ventral sac, rumen dorsal sac, and top and bottom of the caudal sac. The quantities added to the reticulo-rumen were 2.02 × 10<sup>-3</sup> g Cr for low-level SP; 7.75 × 10<sup>-3</sup> g Cr for low-level LP; 8.21 × 10<sup>-2</sup> g Cr for high-level SP; 3.14 × 10<sup>-1</sup> g Cr for high-level

LP. The radioactivity of the material as put into the reticulo-rumen was  $18\text{--}36 \times 10^6$  counts/minute (cpm) depending on binding efficiency. After addition the reticulo-rumen contents were mixed by hand. The radioactive material was introduced into the reticulo-rumen at the beginning of each week for 4 weeks, and the sampling by hand from the same locations as particle additions commenced 2 h after each dosing. The first two were separated from the last two sampling weeks by 1 week to allow the radioactivity to decrease and rest the animals. Sampling was done at 2 and 4 h, and then every 3 h for the following 57 h, and every 6 h for the last 72 h. Faeces were sampled from the rectum at the same times.

#### *Stability of Cr binding and digestibility of Cr-bound particles*

Efficiency of  $^{51}\text{Cr}$  binding was determined by measuring the proportion of total radioactivity associated with the stems after being exposed in the reaction mixture for various times. Stability of binding was estimated from the proportion of radioactivity lost from the stems in boiling neutral-detergent solution after different periods of heating. Digestibility of the mordanted particles was measured by the nylon-bag suspension in the rumen for 72 h (McQueen *et al.* 1980).

#### *Particle size determination*

The reticulo-rumen samples from the steers receiving the mordanted LP were sieved by the screen-by-screen wet-sieving method described by Dixon & Milligan (1985) with the exception that the up and down cycle was repeated ten times before repeating the process for the next smaller screen. Screens of 7.74, 4.0 and 3.35 mm apertures were used to separate LP ( $> 3.35$  mm) from SP ( $< 3.35$  mm including microbial DM). The rumen samples from the animals receiving the mordanted SP were sieved, but the faecal samples were not. All samples were dried at  $65^\circ$  for at least 24 h before transfer to plastic vials for triplicate counting (Model 8000, gamma spectrometer; Beckman Instruments).

Eating boluses were collected from the four steers by way of the rumen cannulas to verify by sieving the bolus LP and SP flows to the LP pool and SP pool respectively. The reticulo-rumen was emptied of its contents and the digesta were mixed and partly sieved to find the SP pool for the Grovum & Williams' (1973) procedure.

#### *Calculations and statistical analysis*

Radioactivity (cpm) was converted to a per g DM basis and then transformed to natural logarithmic values. These values were plotted against time after dosing with  $^{51}\text{Cr}$ , and linear regression equations were calculated using SPSSX programs (Nie & Hull, 1983). Univariate equations of type:  $Y = \text{intercept} + \text{slope } X$ , were used to calculate the values for turnover time (TT), pool size ( $A$ ) and flow ( $a$ ) as described by Shipley & Clark (1972) for a first-order kinetic pool. The analyses of variance for TT,  $A$ , and  $a$  were conducted using an SPSSX program (Nie & Hull, 1983). Subsequently, a quantitative reticulo-rumen model was developed for forage particles. The model values were determined as follows (Fig. 1).

- (1) Feed intake: weight of feed DM intake;
- (2) flow from the eating bolus to the LP pool: equal to flow  $a$  for the LP (verified by sieving the bolus);
- (3) flow from the eating bolus to the SP pool: (disappearance from SP pool) – (flow toward SP pool from LP pool), (verified by sieving the bolus);
- (4) soluble component: (feed) – (flow to LP pool + flow to SP pool);
- (5) LP pool size:  $A$  for LP;
- (6) SP pool size:  $A^1$  for SP;
- (7) disappearance from LP pool:  $a$  of LP;

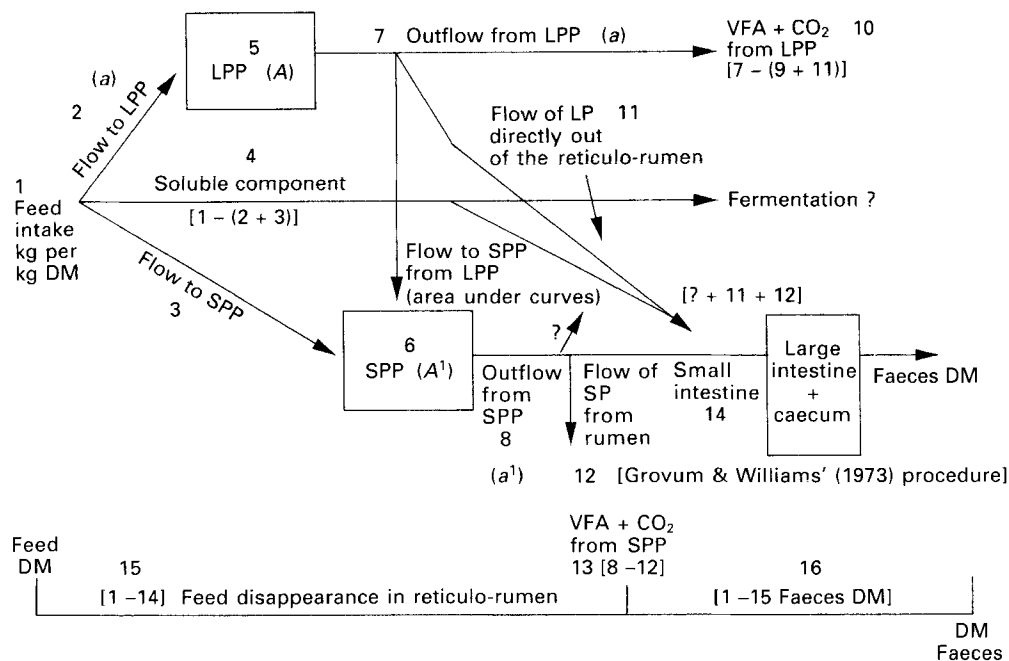


Fig. 1. Derivation of quantitative kinetic model of ruminant digestion of hay using <sup>51</sup>Cr-mordanted bromegrass (*Bromus inermis*) stems. See p. 467 and below for key to nos. 1-18.

LPP, large particle (LP) pool; SPP, small particle (SP) pool; A, LPP size, VFA, volatile fatty acids; A<sup>1</sup>, SPP size; a, slope originating from LP added; a<sup>1</sup>, slope originating from SP added;  $Y = \ln \alpha_0 - ax$ ;

$$\alpha_0 = \frac{A^*}{A} = \frac{\text{Total radioactivity (counts/min) given}}{\text{pool size}}$$

(8) disappearance from SP pool: a<sup>1</sup> of SP;

(9) flow toward SP pool from LP pool: obtained by comparing the area under the curve of the SP originating from LP to the area under the curve of LP disappearance;

(10) volatile fatty acids (VFA)+CO<sub>2</sub> and methane from the LP pool: disappearance from LP pool - (flow toward SP pool from LP pool + flow of LP directly out of the reticulo-rumen);

(11) flow of LP which go directly out of the reticulo-rumen: percentage of the total faecal DM weight formed by LP DM weight (obtained by wet sieving the faeces);

(12) outflow of the reticulo-rumen from the SP pool: obtained by Grovm & Williams' (1973) procedure in animals dosed with SP;

(13) VFA + CO<sub>2</sub> and CH<sub>4</sub> from the SP pool: disappearance of SP pool - outflow of reticulo-rumen from the SP pool;

(14) small intestine flow: (soluble component escaping rumen fermentation unknown) + (flow of LP which goes directly out of the reticulo-rumen) + (outflow of reticulo-rumen from SP pool);

(15) digestibility of DM in the reticulo-rumen: DM - flow into small intestine (unknown);

(16) post-rumen digestibility: DM - (digestibility in rumen (unknown) and residual in faeces);

(17) total DM digestibility: DM feed - DM faeces;

(18) post-rumen TT: obtained by the procedure of Grovm & Williams (1973).

### *Analytical technique*

Cell-wall contents were determined by the procedure of Goering & Van Soest (1970) and N was determined by the Kjeldahl method (Association of Official Analytical Chemists, 1975).

## RESULTS AND DISCUSSION

### *Particle sieving technique*

Several authors have proposed that the particulate DM present in the rumen can be kinetically represented by two pools: an LP pool and an SP pool (Hungate, 1966; Poppi *et al.* 1981). Although Dixon & Milligan (1985) demonstrated that there was no clearly discernible size below which all particles were equally eligible to leave the rumen, a two-pool model involving an SP and LP pool is likely to be a useful simplification in describing the kinetics of particulate matter in the rumen. Dixon & Milligan (1985) concluded that for cattle, using the current sieving techniques, the DM retained by a 3.2 mm screen provided an appropriate minimum definition of the LP pool because fewer than 150 g faecal particles/kg were of this size category.

The method of wet sieving used in the present study was described by Dixon & Milligan (1985). The mechanical sieving techniques used in other studies where the orientation of particles and screens may be different (Jones & Moseley, 1977; Poppi *et al.* 1980) could give different results. Consequently, differences in particle size distribution measured using different sieving techniques may, to a large extent, reflect differences in the basis of selectivity of the techniques, rather than actual particle size difference between experiments. For this reason comparisons of particle size distribution with other studies using different sieving techniques should be made with caution (Dixon & Milligan, 1985).

### *Stability test and digestibility*

The efficiency of binding Cr was inversely proportional to the concentration of Cr in the bathing solution (Table 1). This indicates that a limited number of sites are available for binding, or at least access to additional sites was increasingly difficult. The percentage of associated Cr subsequently lost from the stems in boiling neutral-detergent solution was also inversely proportional to the Cr concentration of the mordanting solution used (Table 1). With increased time-period of heating during mordanting there was increased extent and tenacity of binding (Table 1). This longer heating time-period might favour Cr binding to hydroxyl groups of components more resistant to degradation.

The digestibility of the LP and SP of NDF-extracted bromegrass stems was practically unaffected by mordanting with 0.1 g Cr/kg DM (Table 2). The fibres mordanted with 5 g Cr/kg DM had their digestibility substantially reduced. This is in agreement with Uden *et al.* (1980), who stated that when the concentration of Cr on mordanted particles was increased (from 0 to 80 mg/g DM) the *in vitro* cell wall digestibility decreased drastically.

The loss of  $^{51}\text{Cr}$  during rumen incubation was nearly identical to the loss of DM (Table 1). Thus, the label appears to be associated with components of the extracted particles that are removed during digestion. Nevertheless, the fate of Cr liberated by digestion is unknown. One might expect that the Cr would remain bound to very small particles because of the strong hexacoordinate ligands formed with hydroxyl groups during mordanting (Uden *et al.* 1980). Ellis *et al.* (1980) stated that feedstuffs mordanted with Cr constitute labelled material in which there is little doubt as to marker location because migration within and between particles is low.

Table 1. *Binding of chromium to neutral-detergent-extracted bromegrass (Bromus inermis) stems as influenced by Cr concentration during mordanting\**  
(Mean values with their standard errors)

	Cr concentration for mordanting (g Cr/kg stems)	Period of heating (h)					
		1		3		24	
		Mean	SE	Mean	SE	Mean	SE
Proportion of added $^{51}\text{Cr}$ bound to stems ( $\times 100$ )	100	17.8	2.7	35.5	5.8	70.4	6.9
	20	27.8	4.4	47.1	7.0	72.6	10.8
	15	27.0	4.3	48.3	7.2	82.8	7.9
	10	29.9	5.9	53.9	8.6	88.9	13.3
	5.0	36.8	7.4	68.4	12.3	90.1	12.4
	0.1	51.2	9.5	84.2	15.8	91.0	14.0
Proportion of stem $^{51}\text{Cr}$ removed by boiling neutral detergent ( $\times 100$ )	100	14.1	1.3	8.4	0.9	6.8	0.5
	20	26.8	1.9	14.2	1.1	12.5	1.3
	15	24.8	2.6	14.1	1.3	11.5	0.5
	10	26.8	2.2	14.6	1.7	10.6	0.9
	5.0	38.5	3.1	24.4	2.1	16.9	1.4
	0.1	37.1	3.4	28.6	2.6	29.9	2.3

\* For details of procedures, see pp. 466–467.

Table 2. *In situ rumen digestibility (72 h) of non-mordanted and chromium-mordanted particles of neutral-detergent-extracted bromegrass (Bromus inermis) stems\**  
(Mean values with their standard errors)

Size of particles (mm)	Cr level (g/kg DM)	DM digestibility		Loss of $^{51}\text{Cr}$	
		Mean	SE	Mean	SE
10	0	0.31	0.02		
	0.1	0.24	0.02	0.34	0.06
	5.0	0.20	0.08	0.22	0.07
1–2	0	0.50	0.03		
	0.1	0.48	0.08	0.50	0.06
	5.0	0.32	0.06	0.33	0.08

\* For details of procedures, see pp. 466–467.

#### *Kinetic regression equations based on rumen sampling*

The high  $R^2$  values (Table 3) indicate that LP and SP pools in the rumen were reasonably represented as homogenous first-order kinetic pools. This is in agreement with experiments (Dixon & Milligan, 1985) in which disappearance from the rumen of fluid, particulate, and microbial markers, occurs according to first-order kinetics. The better fit of SP than LP probably reflects the fact that more particles were introduced into the rumen in the small form and, therefore, sampling would entail less statistical variation. Also, mixing of SP is likely to occur more rapidly than for LP. The marker disappearance slopes and turnover times were not significantly different ( $P > 0.05$ ) between SP and LP, whether mordanted with 5 or 0.1 g Cr/kg DM, and animal differences explained the majority of the variations

Table 3. Analysis of variance and orthogonal contrasts on pool kinetic values based on rumen sampling  
(Mean values with their standard errors)

Mordanting level...	5 g Cr/kg DM				0.1 g Cr/kg DM				Main effects		Source of variance:		
	10 mm		1-2 mm		10 mm		1-2 mm		P	Treatment	Week	Animal	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE					
Slope ( $\times 10^{-2}$ )	3.69	0.51	3.63	0.72	4.05	0.76	3.98	1.40	0.145	0.308	0.753	0.038	
TT (h)	27.13	2.78	28.20	4.49	24.91	2.52	25.27	2.46	0.164	0.250	0.802	0.051	
$\lambda$ (g)	3724	762.00	1929	309	3815	703	1632	580	0.009	0.061	0.132	0.416	
$a$ (g/h)	135	19.00	69.0	15.0	154	30.0	65.0	26.0	0.030	0.004	0.231	0.987	
$R^2$	0.91	0.04	0.98	0.01	0.89	0.02	0.99	0.01	—	—	—	—	
Orthogonal contrasts*													
	Slope				TT				$\lambda$				$a$
5 g v. 0.1 g Cr/kg	0.083				0.067				0.698				0.566
Small v. long particles	0.716				0.609				0.000				0.001

DM, dry matters; TT, turnover time;  $\lambda$ , size of pool;  $a$ , flow.  
\* Significance of orthogonal contrast which compared both Cr levels and both sizes of particles.

Table 4. Analysis of variance and orthogonal contrasts on pool kinetic values based on faecal sampling  
(Mean values with their standard errors)

Mordanting level...	5 g Cr/kg DM						0.1 g Cr/kg DM						Source of variance:					
	10 mm		1-2 mm		10 mm		1-2 mm		10 mm		1-2 mm		Main effects		Treatment	Week	Animal	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P					
TT <sub>1</sub> (h)	38.28	12.57	23.61	5.21	31.04	5.05	22.50	4.55	0.004	0.004	0.021	0.726	0.045					
TT <sub>2</sub> (h)	22.67	2.82	15.96	1.04	24.32	2.34	14.11	1.09	0.002	0.002	0.001	0.377	0.093					
Transit (h)	10.68	2.50	24.30	3.84	10.39	1.30	14.69	2.35	0.001	0.001	0.001	0.019	0.008					
D <sub>50</sub> (h)	50.08	10.11	32.89	5.26	46.05	5.98	30.40	4.86	0.004	0.004	0.001	0.560	0.056					
TMRT (h)	71.62	16.13	58.70	9.39	65.51	8.52	51.43	8.23	0.011	0.011	0.002	0.819	0.034					
R <sup>2</sup>	0.88	0.03	0.72	0.04	0.91	0.02	0.86	0.03	—	—	—	—	—					
	Orthogonal contrasts*																	
	TT <sub>1</sub>	TT <sub>2</sub>	Transit						D <sub>50</sub>	TMRT								
5 g v. 0.1 g Cr/kg	0.179	0.901	0.883						0.117	0.115								
Small v. long particles	0.005	0.000	0.000						0.000	0.000								

DM, dry matter; TT<sub>1</sub>, turnover times for label in reticulo-rumen; TT<sub>2</sub>, turnover time for label in post-rumen tract; transit, time elapsed from introduction of Cr-mordanted particles to the appearance of the marker in the faeces; D<sub>50</sub>, time for 50% excretion of the marker; TMRT, total mean retention time.

\* Significance of orthogonal contrast which compared both Cr levels and both sizes of particles.



between these treatments ( $P < 0.05$ ). The LP pool size ( $A$ ) was significantly greater than the SP pool size ( $A^1$ ,  $P < 0.001$ ). The disappearances from each pool ( $a$  and  $a^1$ ) were different ( $P < 0.01$ ), with the LP pool flow being greater ( $P < 0.05$ ) than the SP pool flow.

Particle sizes and Cr concentrations were used in the treatments, consequently orthogonal contrasts (Table 3) were necessary to separate the effects of these two factors on the pool kinetic variables. The TT differences tended to depend on Cr concentrations ( $P < 0.10$ ), while pool sizes and flows were related to particle size ( $P < 0.001$ ). The shorter TT of the particles treated with 0.1 g Cr/kg DM than those treated with 5 g Cr/kg DM may reflect the greater digestibility of the former preparation. The size of, and flow of DM through, the kinetic pool of LP were roughly twice as great as for SP. Therefore, the values for TT of SP and LP pools were nearly equal. These observations are consistent with results of Dixon & Milligan (1985), who found that the LP pool was about 600 (SE 70) g/kg and the SP pool was 400 (SE 50) g/kg of the total rumen particle pool for steers given long hay.

The fractional turnover rate of the  $^{51}\text{Cr}$ -labelled material in the LP pool itself would be equal to the sum of the rate-constants of the digestion of LP DM and the mechanical breakdown of the LP to SP, plus the small proportion of LP which passes directly out of the reticulo-rumen. These combined processes for our cattle given a diet of chopped hay sum to a daily turnover rate of 0.88 (SE 0.09) for high Cr particles and 0.96 (SE 0.1) for low Cr particles. These values agree with the observations of Dixon & Milligan (1985) who measured a rumen LP pool fractional turnover rate of 0.82/d for steers given chopped hay.

The daily turnover rate of the SP pool was 0.85 (SE 0.16)/d for the high-Cr particles and 0.95 (SE 0.1)/d for the low-Cr particles. These results are consistent with those of Dixon & Milligan (1985) who observed that the fractional outflow rate (FOR) of lignin in the rumen SP pool was 1.07/d. This was similar to the mean FOR (1.17/d) of the 2.0–3.2, 1.0–2.0 and 0.25–0.5 mm mesh particle groups (Dixon & Milligan, 1985).

*Rate constants derived from the changes in concentrations of marker in the faeces*

TT for label in the reticulo-rumen ( $\text{TT}_1$ ) and the post-rumen tract ( $\text{TT}_2$ ), time-interval for 500 g/kg excretion of the marker ( $D_{50}$ ) and total mean retention time (TMRT) were all significantly affected ( $P < 0.05$ ) by particle size, but not by level of Cr treatment (Table 4).

$\text{TT}_1$  of SP was 23.6 (SE 4.4) h and 22.5 (SE 2.5) h for high-Cr and low-Cr markers respectively. However,  $\text{TT}_1$  of LP as determined from faecal sampling (38.3 (SE 12.6) h high-Cr, 31.0 (SE 5.1) h low-Cr) was greater ( $P < 0.05$ ) than the TT of LP calculated from rumen sampling (27.1 (SE 2.78) h high Cr, 24.9 (SE 2.5) h low-Cr). The faecal sampling technique, however, yields the TT of overall LP flow, including both the reticulo-rumen breakdown and digestion of LP, plus passage of the resulting SP. A rate-limiting step when clearing dietary residues from the reticulo-rumen is passage through the reticulo-omasal orifice (Ulyatt *et al.* 1986). Since particle size reduction is virtually a prerequisite for passage through this orifice (Evans *et al.* 1973; Ulyatt *et al.* 1976; Welch & Smith, 1978; Poppi *et al.* 1981), it is important to realize that the undigested component of the LP pool has a TT for the total reticulo-rumen, determined by the time-interval for breakdown of the LP pool plus time for passage through the SP pool.

Calculation of TT from the descending phase of label in faecal SP following rumen administration of labelled LP yielded an average value of 47 (SE 8) h. Addition of the rumen TT of LP plus the rumen TT of SP yielded an average of 51 (SE 5) h. Across individual trials, the descending phase TT and sum of rumen turnover were correlated, with a regression coefficient of 0.82. Thus, calculation of TT from the SP in the descending phase appears to relate more closely to digestive events than did calculation of TT of the LP from rumen sampling (31–38 h).

The direct passage of LP from the rumen would speed up the reticulo-rumen TT of LP

determined by rumen sampling. It would also speed up reticulo-rumen TT determined from faecal sampling.

The size of the reticulo-rumen particles influenced ( $P < 0.01$ ) the estimate of  $TT_2$  (Table 4). Label from the LP passed more slowly through the hind-gut. This may have been due to LP which passed directly out of the rumen.

The concentration of Cr, and consequently a change in the digestibility of the particles, did not appear to have any significant effect on the TT,  $D_{50}$  or TMRT (Table 4). On the other hand, the latter three values were greater ( $P < 0.01$ ) for LP than for SP. This is in agreement with Van Soest (1982), who stated that particle size *per se* tends to have its own effect on passage, with SP passing faster than LP.

There is relatively no breakdown of LP in the post-ruminal tract. The breakdown of LP is dependent on four main factors: chewing during eating, chewing during rumination, microbial degradation and detrition. The most important factors are the first two, the last two factors having relatively very little influence (Ulyatt *et al.* 1986). Some authors such as Grenet (1970), Poppi *et al.* (1980), Uden & Van Soest (1982) have stated that there is very limited breakdown of LP in the post-ruminal tract despite the fact that there is considerable fermentation in the caecum and proximal colon (Ulyatt *et al.* 1975).

There were important variations between animals which gave rise to large standard errors. While the disappearance TT for SP was apparently smaller than TT for the outflow of residual SP, they were not significantly different (Tables 3 and 4). The SP pool was calculated twice, once for TT disappearance (first-order kinetics) where the SP pool was  $A^1$ , and a second time where the rumen content was emptied and partly sieved to find the SP pool with the Grovum & Williams' (1973) procedure. This last SP pool was slightly smaller than the first one, but not significantly different. This SP pool was used to calculate outflow from the SP pool in the model. This is why TT of the SP pool in the first-order kinetic calculations is larger than  $TT^1$  in the Grovum & Williams' (1973) procedure and does not implicate a larger outflow of residual SP than disappearance of SP from the SP pool.

#### *Quantitative model (0.1 g Cr/kg DM)*

A kinetic model using values derived from the particles subjected to low-level Cr mordanting is presented in Fig. 2. Some 20% of the DM of the eating bolus was soluble. DM flow to the LP pool accounted for 680 (SE 150) g/kg total DM or 850 (SE 190) g/kg particulate DM of the eating bolus. The DM flow directly to the SP pool was 120 (SE 30) g/kg total DM or 150 (SE 60) g/kg bolus particulate DM. The finding that DM of the eating bolus was composed (/kg) of 800 g in particles and 200 g in soluble form when the hay contained more than 900 g/kg DM in LP, is in agreement with Ulyatt *et al.* (1986) who stated that approximately 350 g/kg DM of fresh forage are solubilized by chewing, while only 200–300 g/kg are solubilized from dried diets. There are large quantitative differences in the results of experiments measuring the particle sizes of eating boluses in the literature; many of these can be explained in terms of the sieving techniques used (Ulyatt *et al.* 1986). Nevertheless, chewing during eating is efficient in releasing the soluble components. First-order kinetic calculations indicated that the LP pool was 3.82 (SE 0.47) kg and the SP pool was 1.63 (SE 0.18) kg, or 0.70 and 0.11 of the particulate DM in the rumen. When samples of rumen contents were wet-sieved, 650 (SE 50) g/kg and 330 (SE 30) g/kg particulate DM were measured to be in LP and SP respectively. Thus, the calculated values were in general overall agreement with the sieving-technique results.

The breakdown of large particles to small particles depends on four main factors: chewing during eating, chewing during rumination, microbial degradation and detrition. The two first factors are the most important (Ulyatt *et al.* 1986). The bolus sieving in the present paper concerns only one of these factors which is chewing during eating. Three

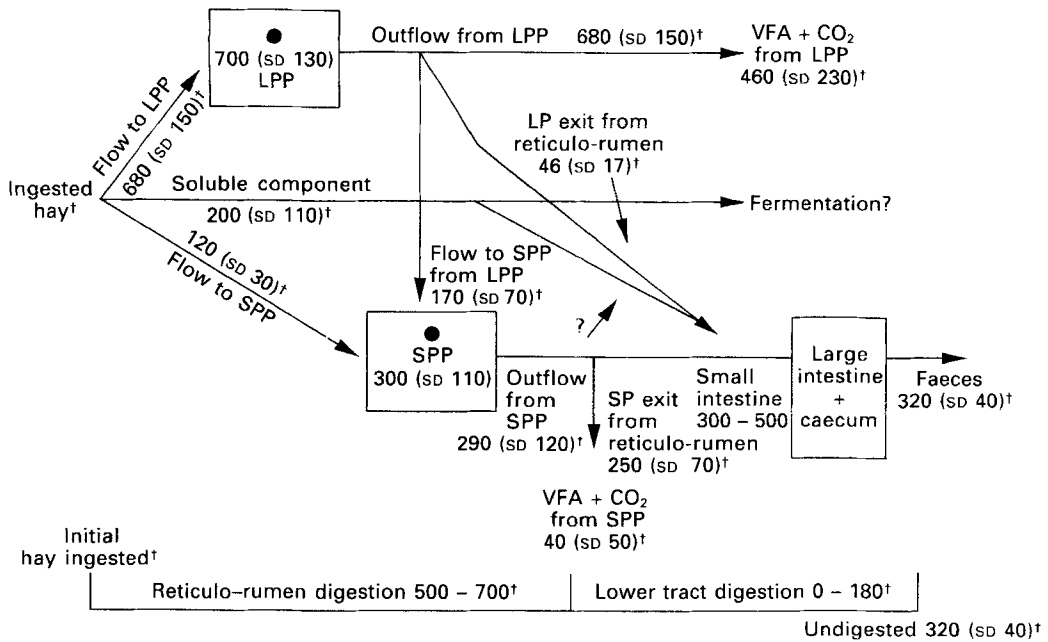


Fig. 2. Quantitative kinetic model of ruminant digestion of hay using bromegrass (*Bromus inermis*) stems mordanted with a low level of  $^{51}\text{Cr}$  (0.1 g Cr/kg fibre dry matter (DM)). Proportion of total particulate DM in the reticulo-rumen (●). Large particle (LP) pool (LPP); small particle (SP) pool (SPP); VFA, volatile fatty acids. † Values are g/kg hay DM ingested.

results are seen from chewing during eating: long forages are reduced to a size that can be incorporated into a bolus and swallowed; soluble nutrients, especially from fresh forages, are released for fermentation; and the inner structures of forage material are exposed to enable effective invasion of the ruminal microbes. Rumination serves two purposes: it damages regurgitated digesta to expose internal plant structures further for microbial attack, and it reduces the particle size of refractory material (Ulyatt *et al.* 1986). In this model, which is a simplification of the numerous digestion processes, only the original flow from feed (eating bolus) to the two pools (LP and SP) is considered. Rumination will influence the model by affecting the marker kinetics from the pools ( $a$ ,  $a^1$ ).

Disappearance of DM from the LP pool consisted of production of VFA, CO<sub>2</sub> and CH<sub>4</sub>, (460 (SE 230) g/kg original feed DM), LP which go directly out of the reticulo-rumen (46 (SE 17) g/kg), and LP which go to the SP pool (170 (SE 70) g/kg). Thus, fermentation products accounted for 0.68 DM disappearance from the LP pool. Flow from the LP pool constituted 0.59 of flow into the SP pool. Flow of large particles directly out of the reticulo-rumen was relatively small (0.07 of the DM flow out of the LP pool), which is in agreement with Dixon & Milligan (1985). These authors noted that only 0.11-0.15 of the faecal particulate DM was retained by 3.2 mm mesh and larger screens. This was in agreement with our observation that 0.16 (SE 0.06) of the particulate DM in the faeces was in LP (> 3.2 mm).

The present values do not yield an estimate of VFA, CO<sub>2</sub> and CH<sub>4</sub> produced by the fermentation of the soluble components. However, one might expect that some part of the soluble components originating from the eating bolus may pass readily out of the reticulo-rumen and be absorbed in the lower digestive tract. Van Soest (1982) stated that fine, soluble and liquid matter escape the rumen more rapidly than coarse, light, solid matter,

although Dixon & Milligan (1985) noted that the FOR of even the smaller rumen particle groups is considerably less than that of water. However, for materials that are passed rapidly, time of fermentation may limit the extent of digestion in the rumen (Owen & Goetsch, 1986). Some of the soluble fraction would also be assimilated by rumen microbes and pass through the reticulo-omasal orifice in this form.

There is a significant difference between the model where 60–70% of the disappearance of LP from the LP pool was by digestion and the much smaller losses occurring during the 72 h incubation in nylon bags. Breakdown and digestion of particles are influenced by many factors: breakdown by chewing eating, chewing rumination, microbial degradation, and detrition. In nylon bags only the two last factors have an influence. Breakdown by chewing in both eating and rumination is a very important factor for microbial digestion by facilitating access of bacteria to internal structures and dissolving the DM. Microbes cannot easily penetrate the undamaged epidermis of plant material except via stomata (Cheng *et al.* 1980). The overall effect of chewing during eating and rumination on digestion has been studied by comparing the digestion of a chewed diet with that of the unchewed material. Bailey & Balch (1961) avoided chewing during eating by feeding cows via a rumen fistula: rumination time increased from 430 to 630 min/d. Bailey (1962) also found a greater rate of digestion of DM, fibre, protein and ash in chewed grass than in unchewed grass when they were digested in the rumen in nylon bags. Similarly, Poppi *et al.* (1981) showed not only that the rate of digestion of tropical grasses in nylon bags was faster for chewed (0.022/h) than unchewed (0.016/h) leaf and stem fractions, but also that lag time before significant digestion occurred was considerably greater in unchewed (15.5 h) than in chewed material (3.1 h). A. John (unpublished results) found a similar effect using fresh ryegrass and fescue; the initial rate of DM digestion in nylon bags was 105% greater in chewed grass than in the same material unchewed. In this model technique the mordanted particles were not in nylon bags. Thus the three last factors influencing breakdown of particles were acting on the LP and the SP. The relatively small amount of mordanted particles added to the rumen were available for chewing during rumination and so had their epidermis damaged during this chewing. Also, to some extent, to put NDF particles bound with Cr directly into the rumen mimics the entry of natural particles into the reticulo-rumen after the washing process of chewing during eating where considerable soluble materials are washed into the saliva.

The DM disappearance from the SP pool included formation of VFA, CO<sub>2</sub> and CH<sub>4</sub> (40 (SE 50) g/kg original feed DM or 140 (SE 170) g/kg total DM from this pool) and outflow from the rumen (250 (SE 70) g/kg original feed or 860 (SE 240) g/kg total DM of this pool). The quantities of VFA plus CO<sub>2</sub> produced in this way are relatively small, but this is logical because about 0.60 of the SP pool originates from the LP pool, and consequently has already been subjected to fermentation. On the other hand, it could be expected that the intensity of microbial attack on the small particles would be greater due to a larger surface per unit weight. Nevertheless, Pond *et al.* (1984) stated that simple reduction in particle size does not mean the material will be more digestible, or that the rate of digestion will be greater. Akin & Burdick (1981) found that many small forage particles were deeply stained with acid phloroglucinol, indicating a high content of lignin and low digestibility, in which case no increase in digestibility would be predicted from further particle size reduction or increased surface area exposure.

According to the kinetic calculation of the present study, material leaving the reticulo-rumen consisted of LP, SP and soluble components that were equivalent to 0.07, 0.25 and 0.20 of original feed DM. These results are consistent with the idea that the relative resistance of DM to passage is related to size (Poppi *et al.* 1980; Weston & Cantle, 1984). Clearance rate within diets approaches zero for the largest particle fraction and progressively increases as the size of particles decreases (Weston & Kennedy, 1984).

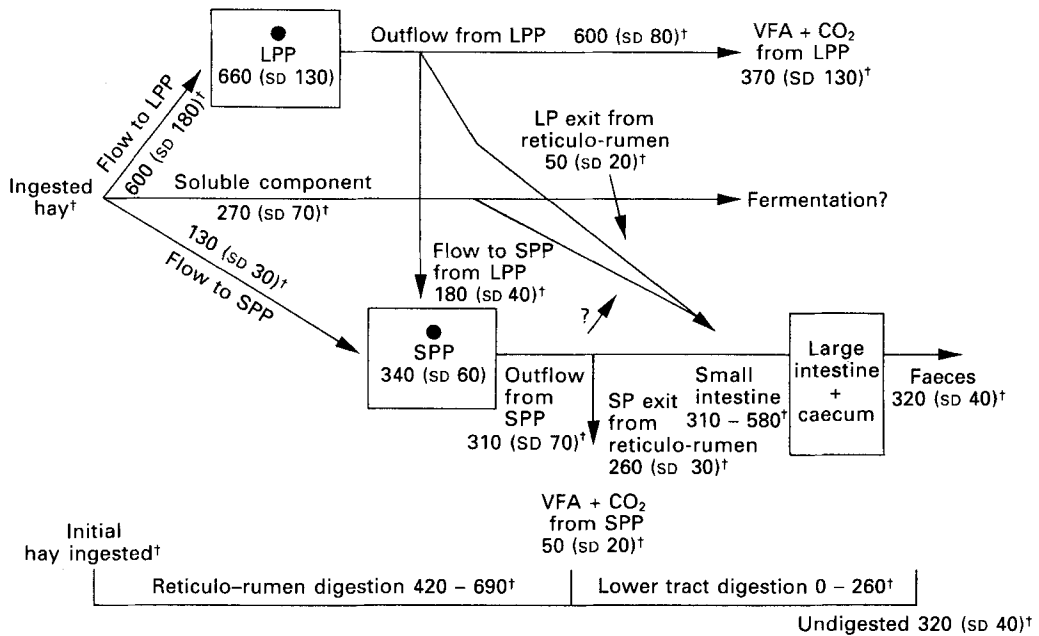


Fig. 3. Quantitative kinetic model of ruminant digestion of hay using bromegrass (*Bromus inermis*) stems mordanted with 5 g <sup>51</sup>Cr/kg fibre dry matter (DM). Proportion of total particle DM in the reticulo-rumen (●). Large particle (LP) pool (LPP); small particle (SP) pool (SPP); VFA, volatile fatty acids. † Values are g/kg hay DM ingested.

The apparent DM digestibility of the hay was 684 (SE 38) g/kg, of which 0.74–1.00 (depending on the site of digestion of soluble components) occurred in the reticulo-rumen. Previously, the proportion of the total digestible organic matter digested in the rumen in cattle given forage diets has been estimated as 0.71–0.82 for oaten chaff (Redman *et al.* 1980), 0.79 for wheaten straw (Sriskandarajah *et al.* 1982), 0.82–0.89 for spear grass hay (Hunter & Sieberg, 1980) and 0.80–0.93 for mixed grass and legume hay (Kennedy, 1982).

#### Comparison between models using results from low-Cr and high-Cr markers

Comparison of kinetic models (Figs. 2 and 3) for the two concentrations reveals that there was a greater proportion of soluble components, less LP, and the same proportion of SP in the high-Cr model compared with the low-Cr model. On the other hand, the SP pool was larger. Less VFA plus CO<sub>2</sub> and CH<sub>4</sub> were produced from the LP pool. The LP which go directly out of the reticulo-rumen and the flow toward the SP pool from the LP pool were both increased in the high-Cr model. Disappearance from the SP pool, and outflow of the rumen from the SP pool were similar between the two concentrations as well as the VFA plus CO<sub>2</sub> and CH<sub>4</sub> production from the SP pool. The VFA and CO<sub>2</sub> and CH<sub>4</sub> produced from the LP pool were less for the 5 g Cr/kg DM model. The main differences between the two models appeared to be a consequence of lower digestibility of particles containing the higher level of Cr, and underscore marker effects on values obtained. The other kinetic values seem to be relatively similar within the relatively large standard deviations.

Large standard deviations indicate differences between animals in particle size reduction by chewing during eating. This was also found by Ulyatt *et al.* (1986) who stated that there are consistent differences between animals in the effectiveness of chewing.

Formation of fermentation products (VFA, CO<sub>2</sub>, CH<sub>4</sub>) in the reticulo-rumen accounted for 0.72 (low-Cr markers) to 0.61 (high-Cr markers) of the total feed DM digested. This is

supported by Sutton (1972) who stated that production of VFA in the rumen fermentation provides between 500 and 700 g/kg of the total digestible energy.

This model is a simplification of the multiple digestive processes acting in the ruminant animal. The technique yields quantitative understanding of different digestive functions. Nevertheless, more work remains to be done to improve the actual descriptive field which was limited to NDF extracted bromegrass stems.

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