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The physical digestion of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) in the foregut of sheep

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1. Sheep were fed once daily with 300 or 600 g dried perennial ryegrass (*Lolium perenne*) or white clover (*Trifolium repens*). Total rumen contents were sampled once daily at various intervals up to 24 h after feeding for 6 d.

2. Total weight of fresh rumen contents and dry matter (DM), organic matter and particulate matter concentrations were measured and the particle size distribution of rumen contents was analysed using a wet sieving technique.

3. There was a similar exponential loss of DM from the rumen with time after feeding for both perennial ryegrass and white clover. The rate of DM disappearance during the first 3 h after feeding was ten times greater for the higher intake and seven times greater for the lower intake than during the subsequent 21 h. The percentage of particulate material disappearing during the first 3 h was greater for white clover (57%) than for perennial ryegrass (49%).

4. Particle size distribution analysis of rumen contents showed that the most rapid rate of particle size reduction occurred during the first 3 h after feeding. Despite a rapid rate of breakdown there was no increase in the total weight of small particles in the rumen.

5. The rate of reduction in particle size during this period was greater for white clover than perennial ryegrass.

6. Microscopic examination of rumen particles showed a difference in particle geometry between white clover and perennial ryegrass which appeared to be related to anatomical features of the plant.

The anatomical and cell-wall characteristics of forage plants confer on them the necessary strength and rigidity to maintain their erectness. The same features also bear responsibility for the relative indigestibility of forages and cause them to be classified as bulky feeds of low nutrient density and high fibre content. These dietary limitations arise mainly from the physical properties of forages and in particular their resistance to breakdown.

The nutritive quality of the forage diet will, therefore, depend primarily on the efficiency of its initial processing, as it is eaten and passed through the reticulo-rumen, to a form which is more easily utilized by the digestive system. This initial and rate-limiting step in ruminant digestion is governed by the physical breakdown of feed particles, which occurs as a result of chewing during eating and rumination, microbial fermentation, and detrition by the muscular activity of rumen walls. Since these processes are responsible for controlling the physical breakdown of feed they also influence its suitability for passage from the rumen and, consequently, the voluntary intake of the animal (Balch & Campling, 1962). Thus, variation in physical breakdown may affect the extent, rate and nature of digestion within the reticulo-rumen and the ultimate supply of nutrients to the intestine.

Although the importance of these aspects of digestion has been discussed (e.g. Baldwin *et al.* 1977; Beever *et al.* 1980; Black *et al.* 1980), little is known of the relative contribution of the various processes to the breakdown of particulate matter, how this may change with the nature of the feed or of the consequences to subsequent digestion and utilization of feed components.

To define those factors which lead to variations in quality between forages it is necessary first to measure quantitatively the effects of breakdown processes in the ruminant foregut. The physical and anatomical characteristics of forage plants which contribute to their resistance to mechanical disruption could then be examined in relation to their breakdown,

Table 1. *Experimental design and dietary treatments*

	Dietary treatments					
Period no. Sheep no....	1	2	3	4	5	6
1	LG	HC	HG	LC	LG	HG
2	LC	HG	HC	LG	LC	HC
3	HC	LC	LC	HC	HG	LG
4	HG	LG	LG	HG	HC	LC

LG, low intake perennial ryegrass (*Lolium perenne*), 300 g dry matter (DM) intake/d; HG, high intake perennial ryegrass, 600 g DM intake/d; LC, low intake white clover (*Trifolium repens*), 300 g DM intake/d; HC, high intake white clover, 600 g DM intake/d.

digestion and passage. This would provide both a predictive indication of quality and valuable selection criteria for forage crop breeders.

The present work describes some of the physical differences between perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) during their initial digestion. These differences and related plant anatomical characteristics are examined in relation to the known contrast between the two species in chemical composition, digestion and animal performance (e.g. Rattray & Joyce, 1970; Ulyatt & MacRae, 1974).

MATERIALS AND METHODS

Feeds. Perennial ryegrass (var. S.23) and white clover (var. Sabeda) were harvested from pure sown 1-year-old leys. Both crops contained less than 10% weeds and unsown species. The fresh material was collected in hessian bags, frozen rapidly and stored at −20°. Preliminary trials had shown the thawed, wet material to be unpalatable and difficult to handle, consequently feeds were thawed overnight in a Unitherm forced-draught oven at 40° to approximately 850 g dry matter (DM)/kg and chopped to a staple length of 30 mm.

Animals. Six 2-year-old Clun Forest wether sheep, between 50 and 54 kg body-weight, were prepared with large removable rumen cannulas according to Moseley & Jones (1979*a*). The animals were housed indoors in individual pens over slatted floors and, between experiments, maintained on dried grass with free access to water. All animals were previously treated for intestinal parasites with thibenzole (2-(thiazol-4-yl)benzimidazole; Merck, Sharp and Dohme Ltd, Moddeston, Herts).

Experimental design. The six sheep were randomly allocated to four dietary treatments in a split-plot design over four feeding periods as shown in Table 1. Diets were given once daily at 09.00 hours and each treatment period of 15 d comprised 9 d adaptation and 6 d during which rumen samples were obtained. Representative samples of whole rumen contents were collected once daily from each animal at 3, 6, 9, 12, 18 and 24 h after feeding. Sampling sequences were organized to allow a minimum of 18 h between subsequent samples and no animal was sampled more than once between feeds.

Rumen sampling. Total rumen contents were removed, weighed and subsampled after mixing by the method outlined by Moseley & Jones (1979*a*). Two 200 g samples of whole rumen contents were obtained by plunging a vessel into the mixed rumen contents and were immediately stored at −20° for subsequent analysis. Two further 100 g samples were obtained for DM and organic matter (OM) analyses.

Analyses. DM analysis on whole rumen contents was carried out on duplicate 100 g samples by drying to constant weight at 100° in a forced-draught oven. OM was determined by subtraction after ashing overnight at 450° in a muffle furnace and particulate material

Table 2. *Sieve sizes used in particle size separation*

Sieve no.	1	2	3	4	5	6	7	8	9
Aperture (mm)	4·00	2·00	1·18	0·60	0·42	0·30	0·15	0·10	0·05

Table 3. *Dry matter (DM) digestibility (in vitro), nitrogen and cell-wall content of white clover* (Trifolium repens) *and perennial ryegrass* (Lolium perenne)

	Clover	Grass	SE (df 11)
DM digestibility	0·732	0·746	0·005
N (mg/g DM)	43·3	28·1	1·03
Cell-wall content (mg/g DM)	333	476	9·9

was measured as that proportion of the total DM retained above the 0·05 mm sieve. The particle-size spectrum of whole rumen contents was measured on duplicate 100 g samples of whole rumen contents by the wet sieving technique of Jones & Moseley (1977) using the range of sieve sizes shown in Table 2. The two smallest fractions were separated by washing on individual sieves using the effluent from the initial sieving procedure. Cell wall content of the feeds was estimated from the neutral detergent residue by the method of Van Soest (1963*a*, *b*) and nitrogen by the conventional macro-kjeldhal procedure. In vitro digestibility was estimated by the procedure of Tilley & Terry (1963). The results were subjected to analysis of variance for a split-plot design with the four feeding treatments as main plots and the six sampling periods as sub-plots. The results are tabulated as the mean values of six sheep for each sampling period and as the grand means for each feed treatment with their standard errors.

Representative samples of bulked particulate material from each sieve fraction at each sampling time were prepared on permanent slides mounted in Euparal and examined microscopically.

RESULTS

In vitro digestibility for both grass and clover was similar but the grass contained more cell-wall components and less N than the clover (Table 3). At the higher intake level grass-fed sheep had a significantly higher ($P < 0·1$) mean weight of total rumen contents than clover-fed sheep (Table 4) but the weight of DM in the rumen was similar.

This resulted in a significantly higher mean DM concentration in rumen contents for clover diets (75·5 and 68·6 g/kg at the higher and lower intakes respectively) compared with the grass diets (68·6 and 54·2 g/kg, SE 3·19). The concentration of OM in DM, although lower for clover diets (832 and 809 g/kg) compared with grass diets (855 and 827 g/kg, SE 17·0), was not significantly different.

The reduction in the total weight of DM, OM and particulate matter in the rumen with the time after feeding occurred in two distinct phases with an initial rapid loss followed by a more gradual decline. During the first 3 h after feeding the losses of DM and OM were similar for both grass and clover feeds with approximately 58 and 40% of the intake disappearing for the higher and lower intakes respectively. At the higher intake there was a significant difference ($P < 0·01$) between the species in the percentage of particulate material lost from the rumen during this period, with 57% of clover and 49% of the grass

Table 4. *Total weight of wet contents, dry matter, organic matter and particulate matter in the rumen at various times after feeding high* (600 *g/d*) *and low* (300 *g/d*) *levels of perennial ryegrass* (Lolium perenne) *and white clover* (Trifolium repens)

(Mean values for six sheep)

Dietary treatment	Period after feeding (h) 0	3	6	9	12	18	24	Treatment mean
				Wet contents				
Clover								
High		4950	5092	4950	4583	4758	3975	4718
Low		4650	4458	4675	4208	4317	3683	4332
Grass								
High		5508	5675	5050	5167	4833	4342	5096
Low		4608	4592	4350	4450	4225	4117	4390
				SE 258				199
				(df 100)				(df 15)
				Dry matter (g)				
Clover								
High	739	436	433	398	336	304	215	409
Low	467	361	332	315	291	250	204	317
Grass								
High	746	433	432	412	344	276	216	409
Low	459	340	296	259	249	226	194	289
				SE 20·5				14·6
				(df 100)				(df 15)
				Organic matter (g)				
Clover								
High	646	367	367	340	280	244	166	344
Low	397	303	272	252	233	196	157	259
Grass								
High	665	373	373	361	292	230	175	353
Low	394	293	254	216	201	182	149	241
				SE 18·3				13·2
				(df 100)				(df 15)
				Particulate matter (g)				
Clover								
High	419	228	211	177	132	118	84	158
Low	246	189	144	126	105	83	78	121
Grass								
High	399	237	216	185	152	107	67	161
Low	221	165	127	103	95	80	55	104
				SE 13·0				8·4
				(df 100)				(df 15)

particulate matter intake disappearing. There was no difference at the lower intake however, and both clover- and grass-fed sheep lost approximately 34% of the particulate matter intake in the first 3 h.

Fig. 1 shows the percentage changes in the mean particle-size distribution of rumen contents with time after feeding. The distributions are expressed as the percentage of the total weight of particulate material collected on each of the nine sieve fractions described in Table 2. For each of the feeds there was a reduction in the 4 mm fraction and an increase in the percentage of the fraction collected on the 0·15 mm sieve with time but very little change in the percentage of other fractions.

The rate of change in the distribution of particle sizes was greatest during the first 3 h

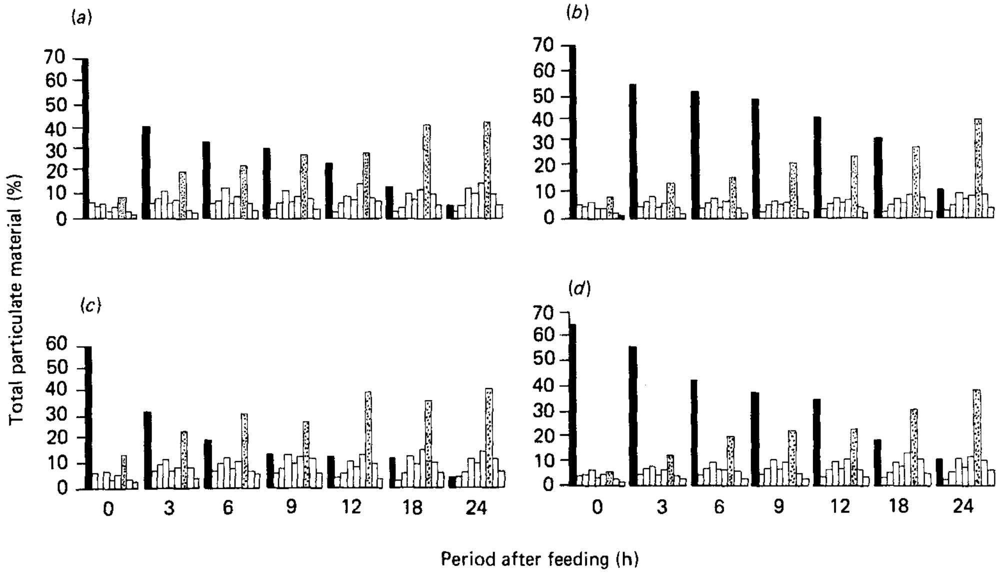

Fig. 1. Change in the particle size distribution of rumen contents with time after feeding in sheep receiving white clover (*Trifolium repens*) (*a*, *c*) and perennial ryegrass (*Lolium perenne*) (*b*, *d*) at either 600 g/d (*a*, *b*) or 300 g/d (*c*, *d*) dry matter intake. Each block diagram is composed of the nine sieve fractions described in Table 2. (■), Particles > 4·0 mm; (▧), particles > 0·15 mm.

after feeding for all treatments. However, the percentage loss of 4 mm clover particles was significantly greater ($P < 0{\cdot}01$) for both high- and low-intake treatments than that of 4 mm grass particles in the corresponding treatments during this period.

Plate 1 shows particulate material collected on the 1·18 mm mesh sieve from rumen contents sampled at 6 and 18 h after feeding. These are typical of the contrasting appearance of particles collected from clover- and grass-fed animals throughout the range of sizes and sampling times, with the exception of the largest-particle fraction, which contained a percentage of relatively unchanged plant fragments. The major feature of note was the difference in particle shape; the breakdown of grass produced long thin or thread-like structures while the clover produced more blockish, irregular shapes. Closer examination showed that the grass particles consisted of thread-like strands of vascular tissue and epidermal sheets where the fracture lines run longitudinally along the length of the leaves and stems. The clover particles consisted largely of epidermal tissue with little evidence of vascular structures, while the fracture lines occurred in all planes with equal frequency, giving rise to irregular shaped particles with no dominant axis.

DISCUSSION

The intake levels used in the present study were restricted to well below *ad lib* levels to ensure that meals were eaten completely in a relatively short period. The period of time spent eating was usually between 20 and 40 min and the maximum time taken was approximately 50 min. Under this regimen the loss of total DM from the rumen showed a similar pattern with time after feeding for grass and clover within the same intake level. The main feature was the very rapid rate of loss of DM from the rumen during the first 3 h followed by a reduced rate which did not change significantly over the subsequent 21 h.

Table 5 shows that the rate of DM disappearance of both grass and clover at the lower

Table 5. *Disappearance of dry matter and of particulate matter from the rumen of sheep fed on perennial ryegrass* (Lolium perenne) *or white clover* (Trifolium repens)

(Mean values for six sheep)

	Period 0–3 h after feeding			Period 3–24 h after feeding		
	Total DM loss (g)	Rate of loss (g/h)	Proportion of rumen pool lost	Total DM lost (g)	Rate of loss (g/h)	Proportion of rumen pool lost
Dry matter						
Clover						
High	303·6	101·2	0·74	221·0	10·5	0·54
Low	105·2	35·1	0·33	157·1	7·5	0·50
Grass						
High	308·7	102·7	0·76	221·3	10·4	0·55
Low	117·8	39·2	0·41	147·2	7·0	0·51
SE (df 20)	23·6	7·9	0·07	23·5	1·1	0·06
Particulate matter						
Clover						
High	197·2	65·8	0·46	141·3	6·7	0·62
Low	50·5	16·2	0·20	118·2	5·6	0·60
Grass						
High	160·0	52·7	0·40	171·8	8·2	0·71
Low	55·5	18·3	0·25	110·5	5·3	0·67
SE (df 20)	20·9	7·0	0·09	20·8	0·99	0·05

intake was approximately five times greater during the first 3 h after feeding than during the subsequent 21 h, while at the higher intake this differential increased to a factor of ten. These differentials between the feeding and the post-feeding periods, although somewhat higher, compare with published information. Reid *et al.* (1979) showed a threefold increase in the rate of DM disappearance from the rumen, during the first 5 h after feeding compared with the subsequent 19 h, for sheep given lucerne (*Medicago sativa*) chaff, while Balch (1958) showed a two- to threefold increase in cattle for a range of diets containing hay and concentrates. During a once daily feeding regimen the rapid reticular and omasal activity which coincides with eating is normally followed by a period of relatively lax gastric activity. This generally lasts until rumination activity increases approximately 9–12 h after feeding (Pearce, 1965*a*, *b*; Reid *et al.* 1979). Should a longer period before sampling be allowed after feeding, then a greater proportion of lax gastric activity would be included in the calculation, thus reducing the hourly rate. The difference in time allowed between feeding and sampling in the present work (3 h) and that allowed in previous studies (5–6 h) may, therefore, be sufficient to account for the discrepancies.

This increased rate of DM loss was sustained in the initial period after feeding in which chewing during eating was a major activity. The act of eating has been shown to be accompanied by a marked increase in reticular and omasal contractions which, in turn, lead to an elevated rate of flow of digesta from the rumen (Balch 1958; Freer *et al.* 1962; Reid, 1963). It is, therefore, likely that a significant proportion of the DM loss from the rumen during this period was due to an increased rate of flow to the hind-gut

The results (Table 5) also show an increased rate of loss of DM during the first 3 h at the higher intake for both clover and grass. This effect has been well documented for forages given at varying levels of intake (see Balch & Campling, 1962) and has also been observed between forages of contrasting voluntary intake characteristics when fed *ad lib.* (McRae & Ulyatt, 1974; Moseley & Jones, 1979*b*). These authors have all shown that this effect was accompanied by an elevated rate of DM flow from the rumen to the hind-gut. Although there was little difference between grass and clover in the loss of total DM during the first 3 h, the results (Table 5) showed there was a greater proportion of particulate material lost with the clover feed compared with the grass at the higher intake. This difference could only occur by a more rapid reduction in particle size for the clover feed and subsequently a loss of the smaller particles either by fermentation and absorption of products or by passage to the hind-gut.

It has been shown that very little material greater than 1 mm in length may be found in the abomasum or omasum (e.g. Troelson & Campbell, 1968; Grenet, 1970; Reid *et al.* 1977) and most estimates place the value at less than 1% of the total DM passing. This has led to the concept of a critical particle size where the reticulo-rumen allows the selective passage of particles which are smaller than 1 mm and retains those which are greater. If this concept of a critical particle size is applied to the values shown in Fig. 1 then the change in the percentages of particles < 1 mm and > 1 mm with time after feeding may be plotted (Fig. 2). This shows very clearly the reduction in the percentage of large particles and the reciprocal increase in the percentage of small particles for both feeds. For both levels of feeding, the clover showed a more rapid rate of change compared with the grass. The reduction in the percentage of large particles (> 1 mm) in the rumen during the eating period was 32 and 18 respectively for clover and grass at the higher intake and 33 and 9 at the lower intake. The extent of the difference between the feeds in the rate of breakdown may be gauged from the time taken to reach the equivalence point at which there were equal percentages of large (> 1 mm) and small (< 1 mm) particles in the rumen. The clover feed required only 3·5 h to reach this point, whereas the grass required 12 h.

Perhaps a more realistic picture of what happens may be seen in Fig. 3, which shows

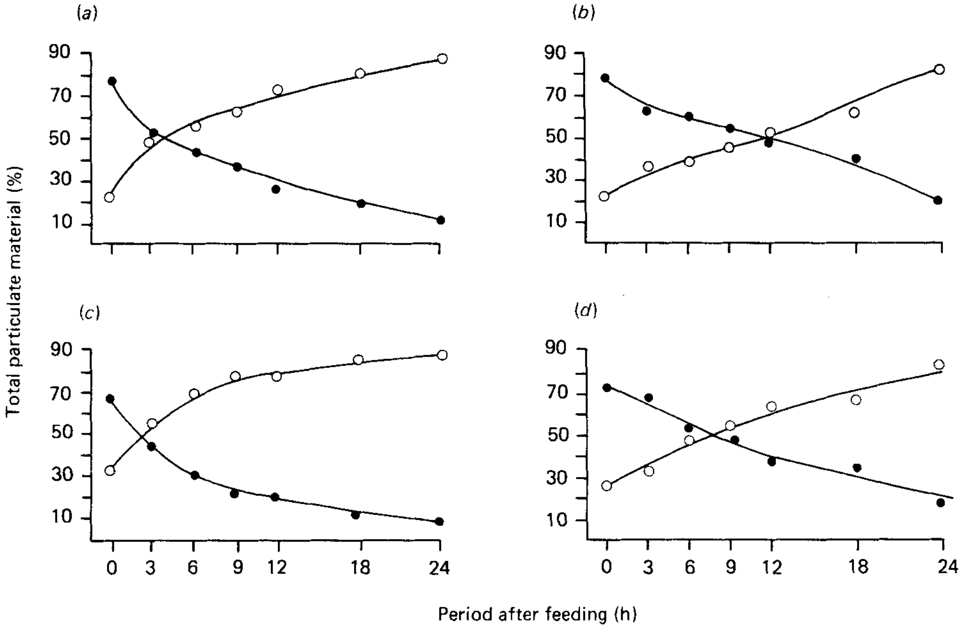

Fig. 2. Change in the percentage of particles greater and smaller than 1 mm in the rumen contents of sheep with time after feeding white clover (*Trifolium repens*) (*a*, *c*) and perennial ryegrass (*Lolium perenne*) (*b*, *d*) at 600 g/d (*a*, *b*) and 300 g/d (*c*, *d*) dry matter intake. Mean values for six sheep.

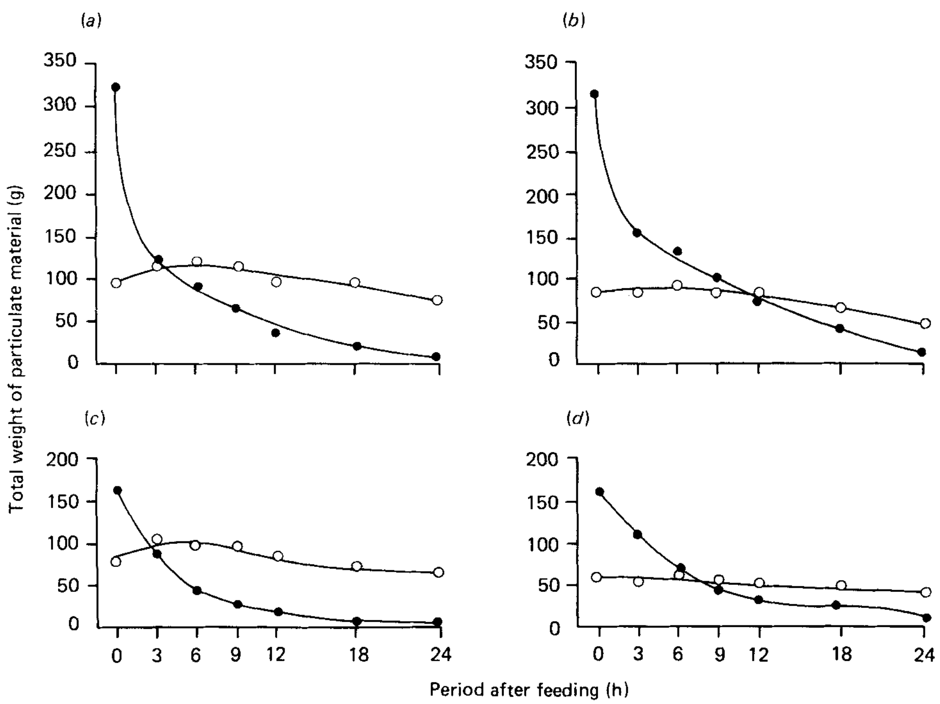

Fig. 3. Change in the total weight of particles greater and smaller than 1 mm in the rumen contents of sheep with time after feeding white clover (*Trifolium repens*) (*a*, *c*) and perennial ryegrass (*Lolium perenne*) (*b*, *d*) at 600 g/d (*a*, *b*) and 300 g/d (*c*, *d*) dry matter intake. Mean values for six sheep.

the changes in the total weight of particles > 1 mm and < 1 mm in the rumen with time. The loss of particles > 1 mm during the first 3 h appears greater than suggested by the percentage change since it represents the net effect of both size reduction and loss of particles from the rumen. With the clover feed the percentage of the total weight of large particles lost increased from 46 to 61 with intake while the corresponding losses for the grass were 32 and 52. Fig. 3 also shows that there was no significant change in the total weight of the 1 mm particle fraction throughout the 24 h period, confirming the rapid loss of particulate material from the rumen following its breakdown. The fate of the DM lost from the rumen was not determined directly, but the rapidity with which particulate matter disappeared during the eating period suggests that a large percentage must have been lost by passage to the hind-gut.

The prime importance of the initial digestion processes may also be gauged from the findings of Reid *et al.* (1979) who showed that in sheep 50% of the large particle fraction (> 1 mm) of a dried-lucerne feed was broken down by chewing during eating, while Poppi *et al.* (1981) reported that the large particles of leaf and stem fractions of tropical grasses were broken down by 46 and 31% respectively during chewing by sheep. Reid *et al.* (1979) also showed that during the first 5 h after feeding less than 4% of the total rumen DM was ruminated, and this was consistent with the findings of Pearce (1965*a*, *b*) who concluded that during once daily feeding, rumination did not occur to any great extent during the first 9–12 h. Furthermore, Prins & Clark (1980) described the fermentation of carbohydrates in meal-fed ruminants as a successive process in which cell-wall polysaccharides did not reach their maximum rate of breakdown until late in the digestion cycle. According to Bailey (1967), in cows fed once daily, there was little or no disappearance of cell-wall constituents from the freshly ingested forage in the rumen up to 6 h after feeding.

Published evidence therefore shows clearly that during the early stages of digestion neither rumination nor microbial fermentation play a significant part in the physical breakdown of particulate material. Consequently, the high degree of particle detrition observed during the first 3 h after feeding must have been due mainly to chewing during eating.

The present findings stress the importance of physical breakdown to both voluntary intake and subsequent digestion. Variation in the resistance of forage plants to the physical forces employed during digestion will affect these determinants of forage nutritive quality and must arise from differences in specific plant characteristics. The microscopic examination of particulate material collected from the rumen during the present work thus provided an opportunity to identify some of these characteristics.

The differences observed in the shape of particles produced by the breakdown of grasses compared with legumes (Plate 1) was also recorded by Troelson & Campbell (1968) in the omasal contents of sheep given alfalfa (*Medicago sativa*) and a range of grass species. These authors contended that grass particles, by virtue of their long thread-like shape, would suffer greater restriction in their movement in the rumen compared with the compact blockish structures of the legume, thus giving rise to a longer retention time and lower voluntary intake. Evans *et al.* (1973) showed that in the rumen contents of cows given hay the density of smaller particles was greater than that of coarser particles. They also observed that a greater proportion of the smaller, denser particles occurred in the ventral regions of the rumen and the reticulum, and suggested that there was a cyclic movement of digesta in the rumen which carried these particles towards the reticulum. This published and present evidence would suggest that there is a sorting process in the rumen, in which particles are selected on the basis of their size, density and shape and presented at the reticulum for passage to the hind-gut.

The size and shape of particulate material formed during breakdown would seem to depend on the anatomical and cellular features of the plants. Microscopic examination of rumen contents showed the vascular and epidermal tissues to be the most prevalent and persistent. Furthermore, differences between the forages in the rate of size reduction, orientation of tissue fracture and persistency within the rumen appeared to be related to differences between the plants in orientation of vascular tissue, thickening and shape of epidermal cells, cuticular thickness and waxiness of leaves.

These features endow the forage plants with a degree of toughness and elasticity which directly influence their ability to resist the physical forces employed during chewing and, therefore, their nutritive value. The considerable variation which has been observed among grasses and clovers in vascular tissue anatomy (Sant & Rhodes, 1970) epidermal cell thickness and shape (Lees *et al.* 1982), cuticular thickness and waxiness of leaves (Daly, 1964; Martin & Juniper, 1970; Moseley, 1983) may, therefore, offer a basis for the selection of forages of improved nutritive value.

Further studies with a range of forages are being carried out to establish these relationships and will provide information on the comparative digestion of these forages, especially with regard to their physical breakdown and passage of particulate material.

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EXPLANATION OF PLATE

Particles from the 1·18 mm sieve fraction of rumen contents from sheep given perennial ryegrass (*Lolium perenne*) after 6 h (*a*) and after 18 h (*b*) or white clover (*Trifolium repens*) after 6 h (*c*) and after 18 h (*d*).

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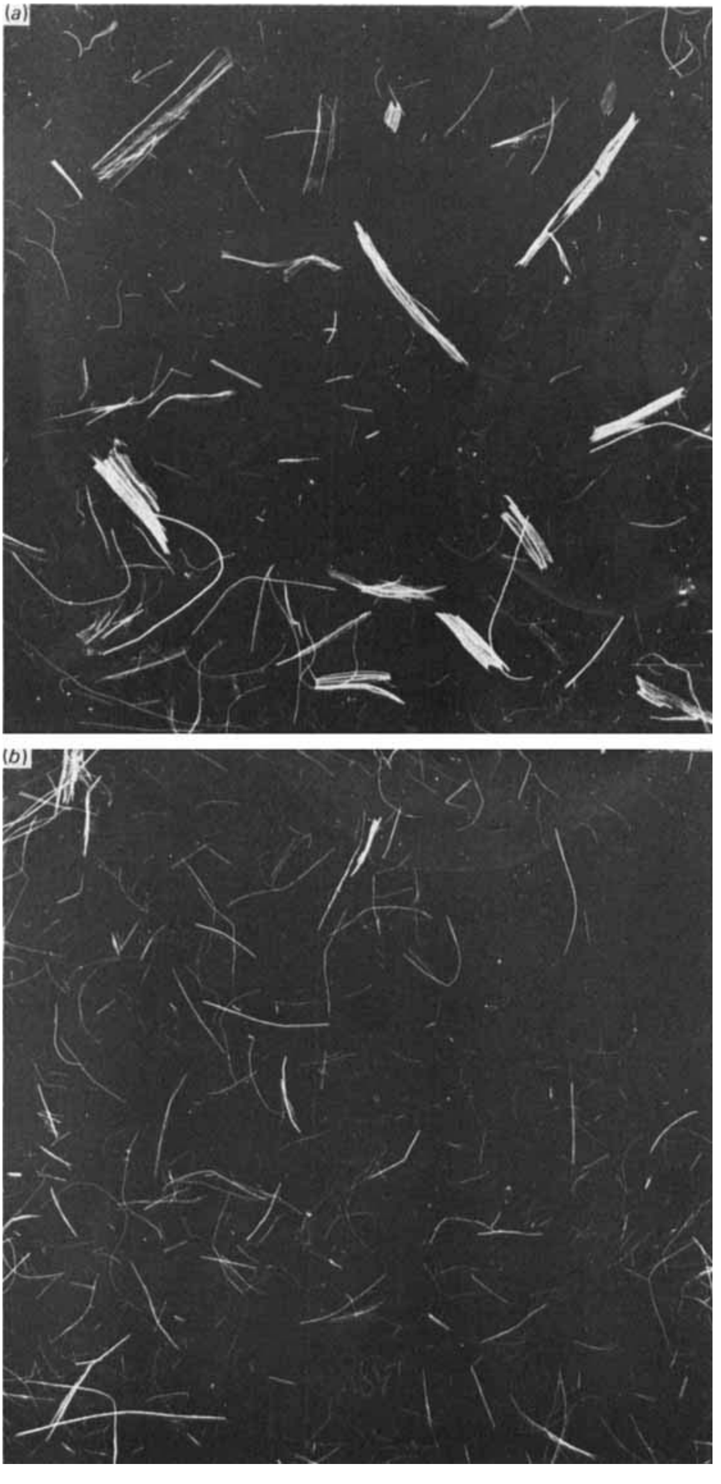

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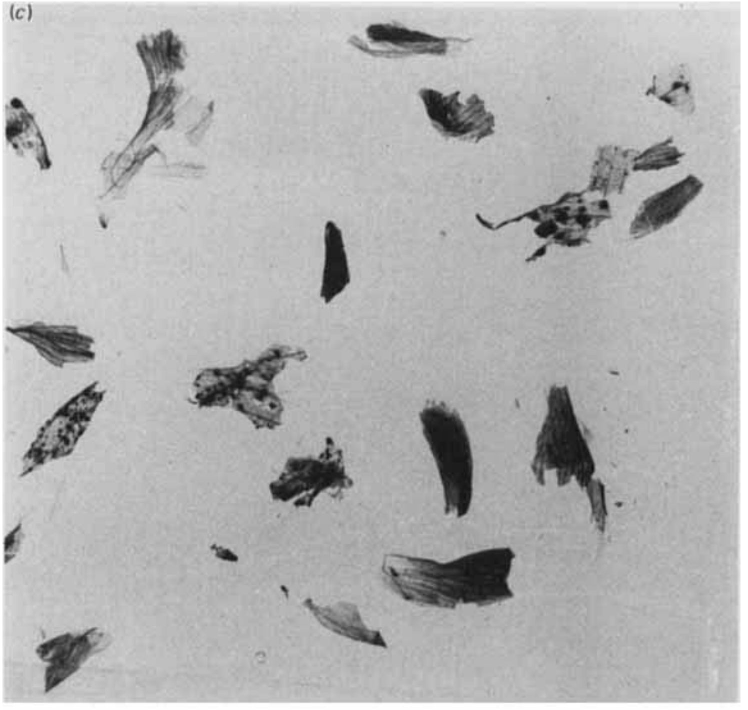
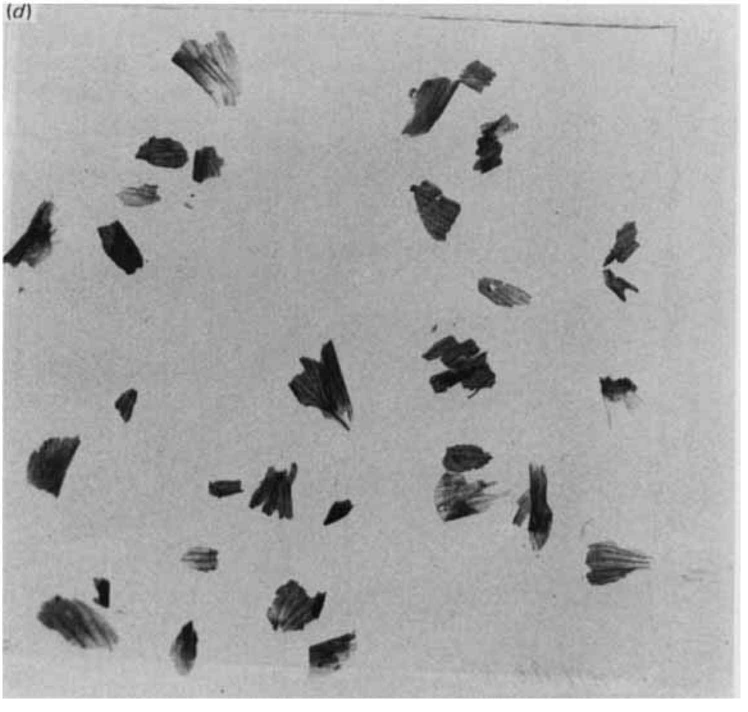
(*Facing p.* 390)





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