

Composition, digestibility and particle-associated enzyme activities in rumen digesta as influenced by particle size and time after feeding

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ABSTRACT

Three bulls were given twice daily a diet of grass silage and barley (70:30) on a DM basis. Manual evacuation of rumen contents was made before feeding, 3, 6 and 9 h after feeding. The interval between two subsequent evacuations was 27 h. Particle size distribution in the digesta was determined by wet-sieving. The greatest diurnal variation in ruminal NDF pool occurred in the 3-mm fraction. Small particles (0.04–0.2 mm) contained less NDF and cellulose and more ADL compared with larger particles. Potential NDF digestibility determined by 288 h nylon bag incubation declined as particle size decreased except for the 0.04-mm fraction. NDF digestibility was positively correlated with the proportion of cellulose in NDF and negatively with that of ADL and hemicellulose. Chemical composition of rumen particulate DM was comparatively unaffected by the time after feeding but NDF digestibility decreased with time when averaged across particle sizes. Particle-associated CMCase and xylanase activities increased gradually as the particle size decreased. The activities were 3 to 9-fold higher in the 0.04-mm fraction than in the other four fractions. There was a close positive relationship between enzyme activities and content of neutral-detergent solubles in particles.

KEY WORDS: Rumen digesta, particle size, chemical composition, enzyme activity

INTRODUCTION

It is generally accepted that feed particles can only leave the rumen after reduction of particulate matter below a critical size (Poppi et al., 1980; Ulyatt et al., 1986). Feed is broken down physically by ingestive mastication and rumination. Particle size reduction by microbial fermentation alone is minor (Murphy and Nicoletti, 1984) but microbial digestion renders the plant material more sensitive to rumination. Rates of particle size reduction, fermentation and

passage are the major constraints describing digesta kinetics in the rumen. These processes affect rumen fill, feed intake and fermentation end-products.

Microbial attachment to fibrous substrates is an important prerequisite of the degradation of these substrates in the rumen (Cheng et al., 1984). The adherent microbial population may comprise 70–80% of the microbial organic matter (Craig et al., 1987), and there is a close relationship between the rate of digestion of fibre and quantity of adherent bacteria (Gerson et al., 1988) or particle-associated enzyme activities (Silva et al., 1987; Huhtanen and Khalili, 1992). Particle-associated microbial population is dependent on available attachment sites, and is affected by rumen environment (Forsberg and Lam, 1977; Silva et al., 1987; Huhtanen and Khalili, 1992). However, there is little information on distribution of the adherent population relative to particle size or particle composition. Gerson et al. (1988) reported a greater microbial density in small particles than in large particles of meadow hay.

The objectives of the present study was to determine the quantity and distribution of NDF among different particle size fractions, and also between digestible and indigestible fractions. The chemical composition, digestibility and particle-associated enzyme activities as influenced by particle size and time after feeding were determined with a view toward improving the understanding of their possible significance with respect to rumen function dynamics.

MATERIAL AND METHODS

Animals and diets

Three Friesian bulls (mean live weight 520 kg), each fitted with a rumen cannula, were used. The experimental diet consisted of grass silage made from timothy (*Phleum pratense*) and barley in the ratio of 70:30 on a DM basis. A daily feed allowance of 7 kg DM day⁻¹ was provided in two equal meals at 7 a.m. and 7 p.m. The animals were kept in individual standings and had free access to water. A commercial mineral mixture (100 g day⁻¹) was given to meet the animals' requirements.

Experimental procedure

The experimental measurements were carried out after an adaptation period of 21 d. Rumen pool size of total digesta was measured by manually removing all the rumen contents. Measurements were made at 7, 10, 13 and 16 h on four consecutive days. The time interval between each emptying was therefore 27 h. In later studies (unpublished) this procedure had no effect on rumen microbial activity as indicated by a similar degradation of hay DM in 24 h before and

during the 3-d rumen evacuation period. Rumen contents was weighed, thoroughly mixed, sampled and returned to the rumen. Each sample was divided into two subsamples of which one was used for determination of DM content and subsequently for NDF, ADF and ADL analyses. The other subsample was used for particle size analyses and enzyme assays.

Rumen digesta was fractioned into various particle size groups using a wet-sieving technique. The sample (60 g wet weight) was first sieved using screens (diameter 20 cm) with mesh sizes 3.0, 1.0 and 0.5 mm. The slurry passed through the screens was then filtered through two nylon bags (pore sizes 200 and 40 μm). The bag of larger pore size (external dimensions 6 x 12 cm) was placed within the bag of smaller pore size (external dimensions 8 x 16 cm). The tops of the bags were tied with a tube of running tap water. During rinsing the bags were occasionally squeezed. All particle size distribution measurements were made in triplicate.

Immediately after rumen evacuation one sample of rumen contents was sieved, using a similar procedure as described above, in order to measure enzyme-activities of microbes firmly attached to rumen particles. After sieving, the material was transferred quantitatively into nylon bags (pore size 40 μm), washed under running tap water and squeezed thoroughly by hand. For determination of particle-associated carboxymethylcellulase (CMCase) and xylanase activities, a sample of 1.0 g of each particle size fraction was used. The details of enzyme extraction and enzyme assays are described by Silva et al. (1987) and Huhtanen and Khalili (1992). The DM content of the particles was determined in duplicate. Enzyme activities were expressed as μmol reducing sugars produced per minute per g DM under the conditions used. The activities were corrected for the substrate breakdown during the extraction procedure.

Rumen particulate fractions of different size (> 3, 1-3, 0.5-1, 0.2-0.5 and 0.04-0.2 mm) were analyzed for NDF, ADF and ADL according to Goering and Van Soest (1970). Cellulose (C) was calculated as ADF-ADL, and hemicellulose (HC) as NDF-ADF. Potential digestibility of DM, NDF (NDFD) and ADF (ADFD) of the rumen contents and each particle size group was determined by 288-h ruminal incubation in nylon bags (pore size 40 μm). The residues were analyzed for NDF and ADF. The undegraded residues were considered to be indigestible NDF (INDF). Digestible NDF (DNDF) was calculated as total NDF-INDF.

Statistical analyses

The model used to analyze rumen pool size data was: $A_i + H_j + e_{ij}$, where A and H are the effects of animal and evacuation time. For some parameters, the effect of time was further separated into single degree comparisons using

polynomial contrasts. The model used to analyze composition, degradability and enzyme activities of rumen particles was: $A_i + H_j + e_{ij} + S_k + (AS)_{ik} + (HS)_{jk} + e_{ijk}$, where A, H and S are the effects of animal, time and particle size, and e_{ij} and e_{ijk} are main plot (6 df) and sub-plot (24 df) errors. Correlation coefficients between chemical composition, NDFD and enzyme activities were calculated.

RESULTS

The silage used contained 207 g DM kg⁻¹ and 24.6 g N, 520 g NDF and 281 g ADF kg⁻¹ DM. The corresponding values for barley were 871, 21.1, 187 and 46, respectively. The silage was of high quality in terms of low pH (3.73) and low concentrations of fermentation acids and ammonia N.

The amount of rumen digesta decreased from 55.2 kg at 3 h to 39.0 kg at 12 h after feeding (linear trend $P < 0.001$) (Table 1). As diurnal variation in digesta composition was small, differences in the pool size of DM and cell wall constituents were similar to those in the total digesta. The reduction in the total weight of DM occurred in two phases with an initial rapid loss during the first

TABLE 1

The effect of time after feeding on the pool size of rumen digesta, dry matter (DM), distribution of DM in various particle groups and pool size of cell wall constituents

	Time after feeding (h)				SEM	Significance of effect		
	3	6	9	12		Linear	Quadr.	Cubic
Total digesta (kg)	55.2	50.4	44.8	39.0	1.41	***	NS	NS
Total DM (g)	5819	5245	4784	3950	163	***	NS	NS
Distribution of DM (g)								
> 3.0 mm	1853	1665	1313	787	70.5	***	†	NS
1.0 – 3.0 mm	475	424	315	280	44.6	*	NS	NS
0.5 – 1.0 mm	301	245	246	255	20.9	NS	NS	NS
0.2 – 0.5 mm	487	518	433	612	40.1	NS	NS	†
0.04 – 0.2 mm	585	457	411	383	25.7	**	†	NS
< 0.04 mm	2117	1936	2066	1632	60.5	**	†	*
Cell wall components (g)								
NDF	3155	2708	2476	2072	134.4	**	NS	NS
ADF	1506	1291	1131	920	75.2	***	NS	NS
ADL	153	142	135	122	6.4	**	NS	NS
Cellulose	1352	1149	996	798	70.7	***	NS	NS
Hemicellulose	1649	1416	1344	1152	69.0	**	NS	NS

SEM = standard error of means

Significance: † ($P < 0.10$); * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$)

3 h after feeding followed by a more gradual decline between 3–12 h after feeding. The rate at which DM left the rumen was 548 g h^{-1} between 0–3 h, and 208 g h^{-1} between 3 and 12 h. The amount of DM leaving the rumen over the first 3 h after feeding was calculated as follows: the amount of DM present in the rumen at 3 h was subtracted from the sum of DM present before feeding and that ingested. The greatest decrease in rumen DM pool occurred in the 3-mm fraction accounting for 57% of the total amount of DM disappeared between 3–12 h after feeding.

The changes in the particle size distribution with time after feeding is shown in Fig. 1. There was a reduction in the 3-mm fraction and generally an increase in the 0.2- and <0.04 -mm fractions with time but very little change occurred in other fractions.

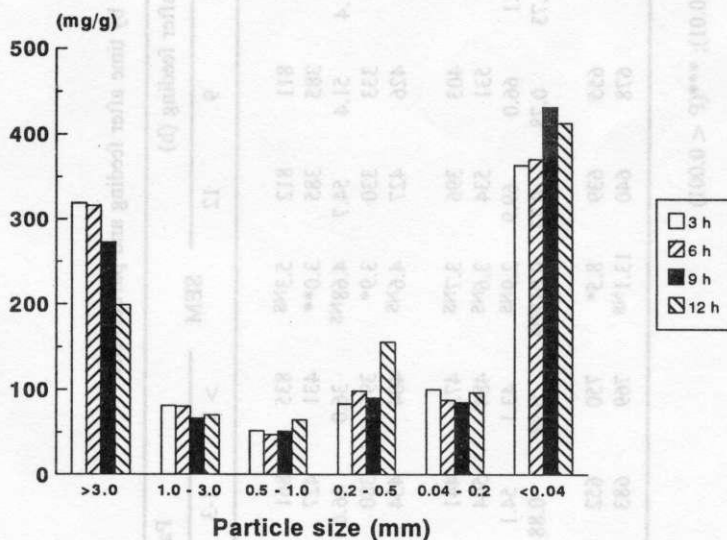


Figure 1. Change in particle size distribution of rumen contents with time after feeding.

Postprandial changes in the composition of rumen particulate matter were fairly small (Table 2). The only significant ($P < 0.05$) differences were in ADF and C content which both increased with time. The NDFD decreased ($P < 0.05$) with time, and a similar trend was also observed for ADFD. On the other hand, chemical composition, NDFD and ADFD varied significantly ($P < 0.001$) with particle size. NDF and ADF contents were much lower and ADL content higher in the 0.04-mm fraction than in the other fractions. The composition of NDF was also affected by the particle size; the proportion of C decreased and that of HC and ADL increased gradually as the particle size decreased. The difference was

TABLE 2

Chemical composition of feed particles affected by time after feeding and particle size

	Time after feeding (h)				SEM	Particle size (mm)					SEM	
	3	6	9	12		> 3	1-3	0.5-1	0.2-0.5	0.04-0.2		
In DM (mg/g)												
NDF	796	786	811	812	5.3NS	835	861	858	836	615	5.2****	
ADF	374	362	385	385	3.0**	431	427	426	404	194	3.6****	
ADL	52.8	51.4	51.4	54.7	4.68NS	36.0	46.6	51.0	62.8	66.4	3.6****	
Cellulose (C)	321	310	333	330	3.9*	395	380	375	342	128	3.0****	
Hemicellulose (HC)	422	424	426	427	4.6NS	404	434	433	432	421	4.7****	
In NDF (mg/g)												
C	390	383	403	396	3.7NS	473	441	436	408	206	2.9****	
HC	542	549	531	534	3.6NS	484	504	504	517	686	4.2****	
ADL	68.1	67.1	66.0	69.9	2.9NS	43.1	54.1	59.5	75.2	107.9	2.4****	
C/HC-ratio	0.77	0.73	0.78	0.77	0.012NS	0.98	0.88	0.87	0.79	0.31	0.009****	
Degradability (mg/g)												
NDF	681	678	655	639	8.5*	750	652	599	567	750	6.5****	
ADF	692	682	678	640	13.1NS	769	683	636	596	682	13.0****	

SEM = standard error of means
 Significance: + (P < 0.10); *(P < 0.05); **(P < 0.01); ****(P < 0.001)

especially distinct between the 0.02- and 0.04-mm fractions. The NDFD decreased from 750 mg g⁻¹ in the 3-mm fraction to 567 mg g⁻¹ in the 0.2-mm fraction. In the smallest fraction (0.04 mm) NDFD was similar to that in the 3-mm fraction but ADFD was lower.

There was a significant ($P < 0.001$) time x particle size interaction in NDFD (Fig. 2). Among the four largest fractions, the smaller the particle size the later the maximum in NDFD was reached. Among the four largest particle fractions, the NDFD and composition were highly correlated (ADL: -0.847; C: 0.623; HC: -0.667; ADL/NDF: -0.825; C/NDF: 0.799; HC/NDF: -0.590).

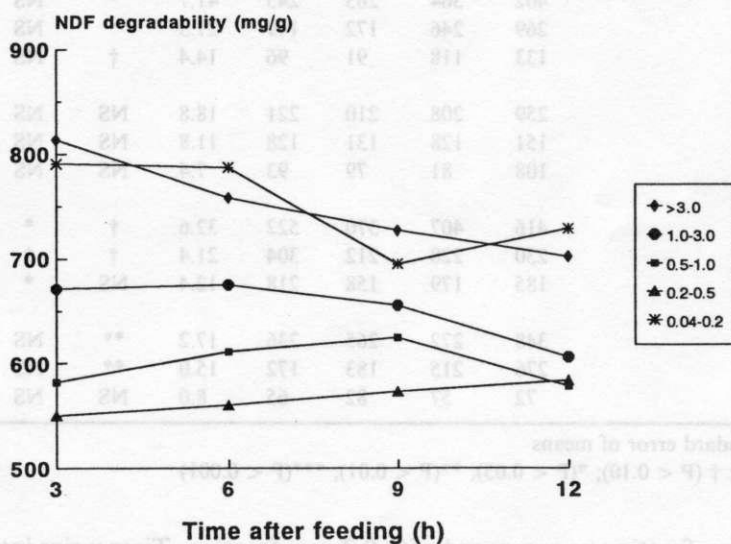


Figure 2. Changes in potential NDF digestibility with particle size and time after feeding.

The distribution of NDF in various particle pools is shown in Table 3. The greatest reduction with time after feeding occurred in the total NDF and DNDF retained by the 3.0 mm sieve (linear trend $P < 0.001$). The amount of NDF in the 0.04-mm fraction also decreased linearly with time. As a result of decreasing NDFD with time, relative diurnal changes in the amount of rumen NDF retained by 3.0 and 1.0-mm sieves were greater for digestible than for indigestible fraction. The average rate of disappearance of particles was 96.7 and 17.4 g h⁻¹ for NDF retained by the 3.0 and 1.0-mm sieves.

The effects of time and particle size on particle-associated enzyme activities are shown in Table 4. Both CMCcase and xylanase activities increased with time (linear effect $P < 0.05$). The enzyme activities increased gradually as the particle size decreased from > 3.0 mm to 0.2–0.5 mm, and increased to 3.0–4.5-fold in

TABLE 3

The effect of time after feeding on distribution of NDF in various fractions

Particle size (mm)	Time after feeding (h)				SEM	Significance of effect		
	3	6	9	12		Linear	Quadr.	Cubic
> 3.0								
Total	1534	1391	1089	664	66.4	***	†	NS
DNDF	1248	1058	787	469	50.2	***	NS	NS
INDF	287	333	302	197	21.8	*	*	NS
1.0 – 3.0								
Total	402	364	263	245	41.7	*	NS	NS
DNDF	269	246	172	149	27.5	*	NS	NS
INDF	133	118	91	96	14.4	†	NS	NS
0.5 – 1.0								
Total	259	208	210	221	18.8	NS	NS	NS
DNDF	151	128	131	128	11.8	NS	NS	NS
INDF	108	81	79	93	7.4	NS	NS	NS
0.2 – 0.5								
Total	416	407	370	522	32.6	†	*	NS
DNDF	230	228	212	304	21.4	†	†	NS
INDF	185	179	158	218	12.4	NS	*	NS
0.04 – 0.2								
Total	348	272	265	236	17.2	**	NS	NS
DNDF	276	215	183	172	15.0	**	NS	NS
INDF	72	57	82	65	8.0	NS	NS	†

SEM = standard error of means

Significance: † ($P < 0.10$); * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$)

the 0.04-mm fraction as compared with 0.2-mm fraction. Time x size interaction both in CMCase and xylanase activity was significant ($P < 0.01$). Xylanase increased from 20.3 to 44.7 between 3 – 12 h post-feed in the 3-mm fraction while the variation in smaller particles was much less; e.g. 32.4 to 46.8 in the 0.5-mm fraction and 40.0 to 47.7 in the 0.2-mm fraction. The content of neutral-detergent (ND) solubles of particulate DM was highly correlated with CMCase ($r = 0.953$) and xylanase ($r = 0.899$).

DISCUSSION

The feeding level used ($65 \text{ g DM (kg LW)}^{-1}$) was restricted below to appetite to ensure that the meals were eaten in a short period. Rapid loss of DM from the rumen during the 3 h after feeding agrees with the observations of Moseley and Jones (1984) and Aitchison et al. (1986). Since the frequency of reticulum contractions increases during eating (Balch, 1971), greater DM loss during this

TABLE 4

Particle-associated enzyme activities ($\mu\text{mol/g DM/min}$) in various particle size groups

	Time after feeding (h)				Particle size (mm)					SEM	
	3	6	9	12	SEM	> 3	1-3	0.5-1	0.2-0.5		0.04-0.2
Reducing sugars produced without substrate ($\mu\text{mol/g DM}$)	1.96	2.47	2.82	2.23	0.224NS	1.04	1.10	0.57	0.98	8.12	0.223***
CMCase	23.0	22.8	22.6	28.4	0.88*	9.2	8.7	10.0	16.9	76.3	0.95***
Xylanase	44.7	50.0	61.1	65.9	3.05*	31.9	34.3	40.1	41.8	129.0	1.57***

SEM = standard error of means

Significance: † ($P < 0.10$); * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$)

period may be due to increased passage rate (Moseley and Jones, 1984). Rapid fermentation of forage cell solubles (Van Soest, 1982) and barley starch may also explain the higher removal rate of DM during the initial period after feeding in the present study. The high content of NDF in rumen particulate matter at 3h after feeding also reflects a rapid loss of non-cell wall material either due to solubilization or fermentation.

The rate of removal of NDF and DNDF from the rumen appeared to be a more exponential process. The following relations between rumen pool size of NDF and DNDF (> 0.04 mm) and time after feeding were calculated: $\text{NDF} = 8.191 \times e^{-0.0450t}$, $r = 0.993$; $\text{DNDF} = 7.898 \times e^{-0.654t}$, $r = 0.996$. Using the value of 0.010–0.015 for the fractional passage rate of DNDF from the rumen (Huhtanen and Khalili, 1991; Huhtanen and Jaakkola, 1992) the fractional rate of digestion of DNDF would be 0.050–0.055.

The large proportion (above 60%) of DM in the rumen was below the size (< 1.0 mm) which is considered to be the threshold size to particle passage (Poppi et al., 1980). Therefore, the reduction in particle size by comminution during eating or rumination does not appear to be the main rate limiting factor in clearing digesta from the reticulo-rumen (Ulyatt et al., 1986; Kennedy and Murphy, 1988).

The changes in chemical composition of particulate DM with size were broadly similar to those reported by Waghorn et al. (1986) and Jung et al. (1990) for lucerne diets. The difference in NDF content between the smallest particle size group and the other groups was, however, greater in our study. This may be due the smaller mesh size used in our study (0.04 mm) than in the studies of Jung et al. (1990) (0.15 mm) and Waghorn et al. (1986) (0.25 mm). Lower NDF content in the small particles may partly be related to a greater attachment of bacteria to small as compared with large particles (Legay-Carmier and Bauchart, 1989). An increasing N content in rumen particles with decreasing size (Waghorn et al., 1986) supports this. It is also possible that some rumen protozoa was included in the 0.04-mm fraction thereby decreasing NDF content. Rumen protozoa contains fairly large amounts of storage polysaccharides (Czerkawski, 1986) which may not be completely soluble in NDF detergent but soluble in ADF. The different composition of the 0.04-mm fraction may also be associated with the smaller particle size and lower C/HC-ratio in barley than in silage.

The effect of time after feeding on the composition of particulate DM and NDF was small when averaged across particle sizes, in agreement with Waghorn et al. (1986). However, the differences were greater when calculated from rumen NDF pool, e.g. C/NDF decreased from 429 to 385 mg g⁻¹ and HC/NDF increased from 523 to 556 mg g⁻¹ at 3 and 12 h post-feeding.

The NDFD of digesta declined as the particle size decreased from > 3.0 mm to 0.2–0.5 mm. Similarly, Jung et al. (1990) reported a lower *in vitro* DM

digestibility for small than for large particles. This relationship may reflect the fermentation history of the particles. Higher NDFD of the 0.04-mm fraction in the present study may be related to a greater particle loss from the bags during the 12-d ruminal incubation. Although chewing during ingestion and rumination are of major importance in particle size reduction, particle size has also decreased during *in situ* incubation (Nocek and Kohn, 1988). Among the particle size groups ≥ 0.2 mm, NDFD was influenced by the composition of NDF with ADL being the major determinant of NDFD. The C/NDF-ratio was positively and that of HC/NDF negatively correlated with NDFD. This is consistent with the greater rate of removal of C than that of HC from the rumen (0.058 vs. 0.038h⁻¹). These observations agree with higher ruminal and total digestibility of C than that of HC in cattle given similar diets to that used in the present study (Jaakkola et al., 1991; Khalili and Huhtanen, 1991).

Significant time x particle size interaction in NDFD may be related to the changes in the age and composition of particles with time. The pool size of > 3.0 mm particles increases during eating relatively more than that of other fractions, and consequently NDFD increases more due to higher NDFD of feed than ruminal digesta (Jung et al., 1990). On the other hand, later particle size reduction by rumination provides recently ingested feed particles from the 3-mm fraction to the other fractions thereby increasing their NDFD.

Although ingested plant material is rapidly colonized in the rumen (Cheng et al., 1984), particle-associated enzyme activities increased between 3–12 h after feeding, in agreement with Williams et al. (1989). They observed that the number of microorganisms attached to digesta particles were similar at 2 and 20 h after feeding, and the increases in enzyme activity did not occur as a result of increased population but were due to increased activities in an attached population. Greater diurnal variation in enzyme activities, NDFD and also NDF pool size in the 3-mm fraction than in the 0.5- and 0.2-mm fractions indicates that most of ingested forage enters this fraction, and that particle breakdown proceeds rapidly. On the other hand, the amounts of NDF entering the small particle pools from large particles by comminution during rumination, and the amounts leaving by digestion and passage were almost equal even although the animals were fed twice daily.

The higher enzyme activities in the 0.04-mm fraction than in the other fractions may relate to the increased surface area. Bacterial attachment is proportional to the surface area exposed to attack (King, 1966). It is also possible that rumen protozoa included in this fraction had cellulolytic activity (Coleman, 1985). The positive correlation between the enzyme activities and the content of ND solubles support the conclusion of Kennedy and Milligan (1988) that the latter in small, water-extracted particles might approximate DM of adherent microbes. Except for the 0.04-mm fraction, the content of ND solubles in

particulate DM was lower than the values reported for microbial DM in particles by Craig et al. (1987). They used ^{15}N as a microbial marker to estimate microbial contamination.

CONCLUSIONS

The data presented here indicated that ND solubles disappeared rapidly from the particulate matter in the rumen. Chemical composition of particulate DM varied with particle size. These changes were related to the availability of the fibre in rumen fermentation. The changes in the composition of NDF suggested that the rates of digestion of fibre components were different. The gradual increase in particle-associated enzyme activities with time after feeding and decreasing particle size suggested that a relatively long period is needed to establish maximum activity of the adherent population.

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STRESZCZENIE

Skład, strawność i aktywność enzymów związanych z cząstkami paszy w treści żwacza w zależności od wielkości cząstek i czasu po karmieniu

Doświadczenie przeprowadzono na bykach karmionych dwa razy dziennie dawką składającą się z kiszonki z trawy i sruły jęczmiennej (70:30). Całą treść żwacza wybierano ręcznie przez przetokę żwacza przed karmieniem oraz w 3, 6 i 9 godzin po karmieniu. Przerwa między kolejnym wybieraniem treści wynosiła 27 godz. Próby treści żwacza rozdzielono „na mokro” na sitach na frakcje zawierające cząstki o różnej wielkości. Największe dobowe zmiany w ilości NDF stwierdzono we frakcji cząstek większych niż 3 mm. Małe cząstki (0,04–0,2 mm) zawierały, w porównaniu z cząstkami dużymi, mniej NDF i celulozy a więcej ADL. Ilość potencjalnie strawnego NDF, oznaczonego podczas 288 godzinnej inkubacji woreczków w żwaczu, zmniejszał się wraz ze zmniejszaniem się wielkości cząstek, z wyjątkiem frakcji 0,04 mm. Strawność NDF była dodatnio skorelowana z udziałem w niej celulozy a ujemnie z udziałem ADL i hemicelulozy. Stwierdzono nieznaczny wpływ czasu po karmieniu na skład chemiczny SM treści żwacza, natomiast strawność NDF malała wraz z wielkością cząstek. Aktywność karboksymetylcelulozy i ksylanazy zwiększała się wraz ze zmniejszaniem się wielkości cząstek. Aktywność ta była 3–9 razy większa dla frakcji cząstek 0,04 mm niż dla pozostałych. Stwierdzono dodatnią zależność pomiędzy aktywnością enzymów i zawartością związków rozpuszczalnych w obojętnym detergencie.