Enzyme activities of rumen particles and feed samples incubated *in situ* with differing types of cloth

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Three ruminally cannulated non-lactating dairy cows were used to investigate the effects of six different bag cloth types with pore size (μm) : free surface area (%) ratios of 200 : 45, 41 : 33, 16 : 5, 10 : 2, 6 : 5 and 1 : 2 respectively on the disappearance of grass silage DM and neutral-detergent fibre (NDF), and on particle-associated carboxymethylcellulase (*EC* 3.2.1.4; CMCase) and xylanase (*EC* 3.2.1.8) activities extracted from feed residues. Another objective was to compare microbial activity inside the bags and in rumen ingesta. Rumen incubation periods were 3, 6, 12, 24, 48 and 96 h. DM and NDF disappearance and particle-associated enzyme activities were greatly reduced with the smaller pore size and/or open surface area. Re-analysing some of the data as a 2 × 2 factorial (pore size × free surface area) indicated that, generally, free surface area rather than pore size affected the disappearance of feed components and particle-associated enzyme activities. Enzyme activities were highly correlated with NDF disappearance at 6–48 h of incubation. Cumulative area under CMCase and xylanase activity curves explained 0·79 and 0·88 of the variation in NDF disappearance when different cloth type and 6–48 h incubation data were combined. Weighted mean enzyme activities inside the bags were less than 0·35 those in rumen ingesta. The highest activity values inside the bags (24 or 48 h) were less than 0·50 those found in rumen ingesta. The lower microbial activity inside the bags explains the slower rates of NDF digestion reported with *in situ* techniques than with rumen evacuation techniques. The general assumption of similar microbial activity inside the bags and in rumen ingesta is not justified by the present results, and caution must be taken in interpreting *in situ* results quantitatively for feed evaluation systems.

Neutral-detergent fibre: Rumen: Enzyme activity

Kinetic parameters describing ruminal protein degradation and neutral-detergent fibre (NDF) digestion are typically assessed by the *in situ* technique. Despite the popularity of the technique, the details of the method (e.g. bag pore size, sample : surface area of bags ratio, incubation periods, diet of the host animals, particle size of the sample, washing procedures, microbial contamination and particle loss) further discussed in the reviews by Nocek (1988) and Michalet-Doreau & Ould-Bah (1992) have not been standardized. In addition, several techniques (e.g. markers, sampling sites, models) are used to estimate parameters of passage kinetics, with different models for estimating ruminal protein degradability or NDF digestibility. Although many variables of the procedures have been studied in detail, the most critical assumption of the method, i.e. that the microbial activity inside the bag equals or at least resembles that in rumen ingesta, has not been verified. The studies of Meyer & Mackie (1986) indicated that microbial numbers inside the bags were much lower than those in the surrounding ingesta. Huhtanen & Khalili (1992) and Nozière & Michalet-Doreau (1996) reported markedly lower particle-associated carboxymethylcellulase (*EC* 3.2.1.4; CMCase) and xylanase (*EC* 3.2.1.8) activities inside the bags than in rumen ingesta. Lower microbial activity inside the bags may explain the underestimation of *in vivo* rates of NDF digestion when determined by the *in situ* technique (Tamminga *et al.* 1989; Huhtanen *et al.* 1995).

Abbreviations: AUC, area under curve; CMCase, carboxymethylcellulase; NDF, neutral-detergent fibre.

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The present study was designed to assess the effects of bag cloth type on the disappearance of feed components in the rumen and on particle-associated enzyme activities extracted from undegraded feed residues. To compare microbial activity inside the bags and in the surrounding ingesta, enzymes were also extracted from rumen particulate matter.

Experimental methods

Animals and feeding

Three non-lactating Finnish Ayrshire cows, each fitted with a permanent rumen cannula, were used. The cows received an all-grass silage diet meeting maintenance requirements. In addition to silage, cows received a commercial mineral–vitamin supplement. An adaptation period of 3 weeks was allowed before measurements were made.

Nylon bag incubations

To avoid any confounding effects of dietary components, the same silage was given to the cows and used for *in situ* incubations. The silage contained 228 g DM/kg and in DM (g/kg) ash 98, N 30, NDF 518, acid-detergent fibre 305 and lignin 41. Samples of approximately 2·5 g and 1·0 g DM fresh silage were weighed into the bags for the determination of disappearance of feed constituents and particle-associated enzyme activities respectively. The silage was chopped to a length of approximately 5 mm. The bags were made of polyester (Polymon, Zürich, Switzerland) with ratios pore size (μm) : free open surface area of the total area (%) of 200 : 45, 41 : 33, 16 : 5, 10 : 2, 6 : 5 and 1 : 2 respectively. The external dimensions of the bags were 60 × 120 mm. The incubation periods were 0, 3, 6, 12, 24, 48 and 96 h. A total of thirty-six bags (six cloth types × six incubation periods) were inserted into the rumen of each cow at the same time and removed later at designated intervals. The incubations were repeated three times at 1-week intervals, incubations for particle-associated enzyme activities being made during the second week.

Immediately after withdrawal from the rumen the bags were washed in cold water for 30 min using an automatic household washing machine (programme based cold, two flushes, no spinning). After washing, the bags used for disappearance determinations were dried in a forced-draught oven (60°, 24 h). For enzyme activity assays, bags from the second set of incubations were thoroughly squeezed, and the content was quantitatively removed and then frozen at −20°.

Approximately 1 kg rumen contents was sampled for enzyme assays from different parts of the rumen on 4 d during the incubations, before feeding and 3, 6 and 9 h thereafter. The samples were thoroughly mixed and approximately 15 g was placed into nylon bags (41 : 33). The bags were washed using a procedure similar to that used for rumen-incubated bags. The residues were frozen, before enzyme assays and DM determination.

Chemical analyses and enzyme assays

Undegraded residues were analysed for total N by the Kjeldahl method and for NDF, acid-detergent fibre and lignin as described by Robertson & Van Soest (1981). Enzymes were extracted using the method of Nossal & Heppel (1966). Undegraded feed residues (quantitatively) or rumen contents (2 g) were mixed with 20 ml of 10 mM sodium phosphate buffer (pH 6·8), 2·5 ml CCl_4 and 1 ml lysozyme solution (50 mg/ml) and incubated for 3 h at 39°. After incubation the samples were centrifuged at 26 000 ***g*** at 4° for 15 min. Carboxymethylcellulose (sodium salt (low viscosity), Sigma no. C-8758; St. Louis, Missouri, USA) and xylan (from oat spelts, Sigma no. X-0376) hydrolysis were assayed in duplicate by measuring the formation of reducing sugars using 3,5-dinitrosalicylic acid reagent (Fisher & Kohtes, 1951). The procedures used for enzyme assays were as described by Groleau & Forsberg (1981). To remove xylan from the incubation media, samples for xylanase assay were centrifuged at 800 ***g*** for 5 min before absorbance measurements. Glucose was used as a standard for both CMCase and xylanase assays. The values were corrected for the release of reducing sugars from the undegraded feed residues or rumen particles released during the extraction of enzymes. Enzyme activities were expressed as μmol reducing sugars (glucose or xylose) released/g DM per min. To calculate the amount of DM of undegraded nylon bag residues used for enzyme assays, the values for DM disappearance were assumed to be similar to those determined by the other two replicate bags.

Calculations and statistical analyses

Data on disappearance of feeds from the bags and particle-associated enzyme activities were statistically analysed according to two-way ANOVA with the effects of the cloth type and animal in the model. As free surface area and pore size are confounded, the four last treatments (16 : 5, 10 : 2, 6 : 5 and 1 : 2 (μm:%)) were re-arranged 2 × 2 factorially to test the effects of pore size (small (1 and 6 μm) *v.* large (10 and 16 μm)) and open surface area (2 *v.* 5 %). The relationship between the particle-associated enzyme activities and disappearance of NDF from the bags was examined by regression analyses after natural logarithmic transformation of enzyme activities.

For the comparison of enzyme activities in the rumen ingesta and undegraded nylon bag residues, a weighted mean of activities measured at different incubation periods was used. The distribution of rumen ingesta from different meals was estimated from a dynamic two pool rumen model (Allen & Mertens, 1988) using POWERSIM® software version 2.01 (Powersim AS, Bergen, Norway). The values of 0·05, 0·04 and 0·06/h were used for rate of release of particles from the rumen non-escapable to escapable pool, rate of passage from escapable pool and for rate of NDF digestion respectively. Before feeding, the proportions of particles arising from the previous meals given at 12, 24, 36, 48 and 60 h earlier were 0·616, 0·244, 0·091, 0·033, 0·011 and 0·004 respectively. The areas under CMCase and xylanase activity curves were calculated

assuming linear changes in enzyme activities between the two rumen incubation periods.

Results

Disappearance of DM and neutral-detergent fibre

The disappearance of the silage DM and NDF from the bags, with the statistical effect of bag cloth type, are shown in Table 1. The bag cloth type had a highly significant ($P < 0.001$) effect on DM and NDF disappearance irrespective of the length of incubation. Differences between the cloth types of 200:45 and 41:33 were relatively small; only at 12 and 24 h of incubation more NDF disappeared from the 200:45 bags than from the 41:33 bags. The differences between the cloth types in DM disappearance diminished with increasing length of incubation period, and after incubation of 96 h only the disappearance from the 1:2 bags was considerably less than from the other bags. At 96 h of incubation NDF disappearance from the 10:2 and 1:2 bags was 50–60 and 120–130 mg/g lower than from the other bags. Despite the smaller pore size, the disappearance of DM and NDF was greater from the 6:5 bags than from the 10:2 bags. The cloth types 16:5 and 6:5 resulted in similar values of DM and NDF disappearance. Zero-hour washing losses decreased with the smaller pore size and/or small free surface area. The effects of the cloth type on the disappearance of N and other fibre fractions (acid-detergent fibre, cellulose and hemicellulose) were similar to those on DM and NDF disappearance (results not shown).

Particle-associated enzyme activities

Consistently with DM and NDF disappearance, particle-associated CMCase and xylanase activities decreased significantly with decreasing pore size and/or free open surface area of the bags (Table 2). Peak values of CMCase and xylanase activity occurred earlier for cloth types of 200:45, 41:33 and 16:5 (24 h) than for the other types (48 h). As judged from enzyme activities, the cloth type of 10:2, and especially that of 1:2, exhibited a slower colonization of particle-associated fibrolytic microbes than the other cloth types. The effect of free surface area on enzyme activities was generally more significant than the effect of pore size (Table 3). Although the statistical comparisons are not entirely unconfounded, consistently higher activities with the 6:5 bags than with the 10:2 bags, and similar activities with the 16:5 and 6:5 bags, demonstrate the importance of free surface area rather than pore size of bag cloth. The decrease in enzyme activities after 24 or 48 h may represent detachment of particle-associated microbes as the available substrates were exhausted. Random variation also increased with long incubation periods. Consequently differences in xylanase activity after 96 h incubation were not significant despite large numerical differences.

Comparison of enzyme activities in rumen ingesta and nylon bag residues

Particle-associated CMCase and xylanase activities were considerably higher in the rumen particulate material than in bag undegraded feed residues. The mean CMCase and xylanase activities in rumen particulate matter were 12·6 and 39·6 μmol/g DM per min. The values were highest before feeding and lowest at 3 h (CMCase) or 6 h (xylanase) after feeding. Direct comparison of the activities between rumen digesta and nylon bag residues is difficult because rumen ingesta consists of feed particles originating from different meals. Therefore a modelling approach (see p. 162) was used to estimate the contribution of different meals to rumen particulate matter. Although the calculations are based on many assumptions, it is evident that the activities were considerably lower within the bags than in the surrounding digesta. Calculated as weighted means, CMCase and xylanase activities in 200:45 bags were only 0·347 and 0·334 of those in rumen ingesta, and with the

Table 1. Disappearance (g/kg) of DM and neutral-detergent fibre (NDF) of grass silage enclosed in nylon bags with varying pore size and free surface area (μm:%) in the rumen of dairy cows

	Cloth type						SEM	Statistical significance of difference between means (ANOVA): *P*
Incubation period (h)	200:45	41:33	16:5	10:2	6:5	1:2	(df 10)	
DM								
0	359	343	324	295	300	200		
3	394	407	351	332	340	239	8·9	***
6	465	464	370	353	375	256	12·1	***
12	641	606	542	424	527	298	12·0	***
24	744	722	651	561	641	374	22·7	***
48	798	788	726	648	733	527	8·5	***
96	807	826	817	779	807	673	8·6	***
NDF								
0	98	85	69	46	42	−37		
3	125	143	87	62	66	2	12·3	***
6	226	222	109	78	107	2	14·3	***
12	465	412	327	161	301	32	16·0	***
24	629	592	485	356	465	151	36·0	***
48	717	704	608	490	618	424	11·1	***
96	741	759	753	695	736	624	11·6	***

*** $P < 0.001$.

Table 2. Particle-associated carboxymethylcellulase (CMCase) and xylanase activities (μmol/g DM per min) extracted from undegraded residues incubated in nylon bags with varying pore size and free surface area (μm : %) in the rumen of dairy cattle

Incubation period (h)	Cloth type 200 : 45	41 : 33	16 : 5	10 : 2	6 : 5	1 : 2	SEM (df 10)	Statistical significance of difference between means (ANOVA) : *P*
CMCase								
3	1·58	1·58	1·32	1·15	1·23	1·03	0·041	***
6	2·41	1·77	1·42	1·12	1·35	0·90	0·079	***
12	4·08	3·01	1·79	1·34	2·15	1·00	0·267	***
24	7·40	7·87	4·26	2·81	3·93	1·60	0·557	***
48	5·34	7·39	4·11	3·03	4·46	2·27	0·496	***
96	5·06	5·01	2·48	2·38	2·28	1·62	0·727	*
Xylanase								
3	2·51	2·36	1·97	1·61	1·72	1·33	0·088	***
6	5·13	3·70	2·81	2·13	2·79	1·98	0·197	***
12	12·97	9·12	4·85	2·91	4·99	2·08	0·707	***
24	29·14	28·86	16·07	7·92	12·81	4·04	1·736	***
48	18·44	16·64	12·28	9·21	14·47	7·07	1·006	***
96	16·48	9·72	5·67	6·98	6·16	4·52	2·889	NS

* $P < 0.05$, *** $P < 0.001$.

Table 3. Statistical significance of the effect of pore size† (P) and free surface area‡ (F) of bag cloth on neutral-detergent fibre disappearance (NDFD) and particle-associated enzyme activities extracted from undegraded feed residues, which had been incubated in the rumen of dairy cows for 12, 24 and 48 h

Incubation period (h)	SEM (df 7)	P	F	F × P
12				
NDFD	12·3	**	***	**
CMCase	0·163	NS	**	NS
Xylanase	0·333	NS	***	NS
24				
NDFD	32·0	*	***	o
CMCase	0·301	NS	**	NS
Xylanase	1·30	o	**	NS
48				
NDFD	9·4	o	***	*
CMCase	0·094	NS	***	**
Xylanase	0·565	NS	***	*

o $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† 1 and 6 μm *v.* 10 and 16 μm.
‡ 2 *v.* 5 %.

cloth type of 2 : 1, activities inside the bag were less than 0·10 of those in rumen ingesta (Fig. 1). Comparison of peak values of activities inside the bags and in rumen ingesta revealed that the activities in the 200 : 45 and 41 : 33 bags were approximately 0·50 of that in rumen ingesta. This comparison may be justified, because the activities in the bags peaked in most cases at 24 h and the average age of rumen particles was estimated to be 19 h before the next meal. Even when compared with the mean values in rumen ingesta, the highest bag CMCase activity (41 : 33 at 24 h) was only 0·64 of that in rumen ingesta. The highest bag xylanase activity (200 : 45 at 24 h) was 0·74 of that in rumen ingesta.

Relationship between particle-associated enzyme activities and neutral-detergent fibre disappearance

Within each incubation period, CMCase and xylanase activities were highly correlated with NDF disappearance (results not shown). Generally, NDF disappearance was better correlated with xylanase than CMCase activity. When the data from four incubation periods (6, 12, 24 and 48 h) were combined, cumulative CMCase and xylanase activities measured as areas under the curve (AUC) were highly correlated with NDF disappearance from the bags (Fig. 2). Also in this case, xylanase was more closely correlated to NDF disappearance than CMCase. When the relationships were calculated separately for each cloth type, the regression coefficients were very similar between the cloth types (CMCase: 128–144 × ln(AUC CMCase); xylanase: 115–128 × ln(AUC xylanase). However, the intercept became more negative with decreasing pore size or free surface area (from −6 to −206 for CMCase, from −57 to −301 for xylanase). The R^2 for values calculated separately were higher than those calculated for combined data (CMCase: 0·74–0·95; xylanase: 0·86–0·98).

Discussion

Degradation of feed components and enzyme activities in relation to cloth type

Pore size of the bags is one of the most important factors affecting the disappearance of feed constituents from the bags (see Lindberg, 1985; Nocek, 1988; Michalet-Doreau & Ould-Bah, 1992). It should be small enough to minimize the loss of particles from the bags and prevent the inflow of rumen particles. At the same time, it should allow free passage of all relevant microbes into the bags and outflow of fermentation end-products. Obviously not all cloth types used in the present study fulfilled these requirements. Soluble material and small particles disappeared from the bags without microbial degradation in zero-hour washing. This fraction was much smaller for bags of small pore size and/or free surface area (especially 1 : 2) than for 200 : 45 and 41 : 33 bags, suggesting that these cloth types prevented free exchange of water and disappearance of soluble material and microbes. On the other hand, the smaller disappearance of DM at 96 h incubation (807 *v.* 827 mg/g) and smaller increases in DM disappearance between 48 and 96 h incubation (9 *v.* 28 mg/g) from the 200 : 45 bags

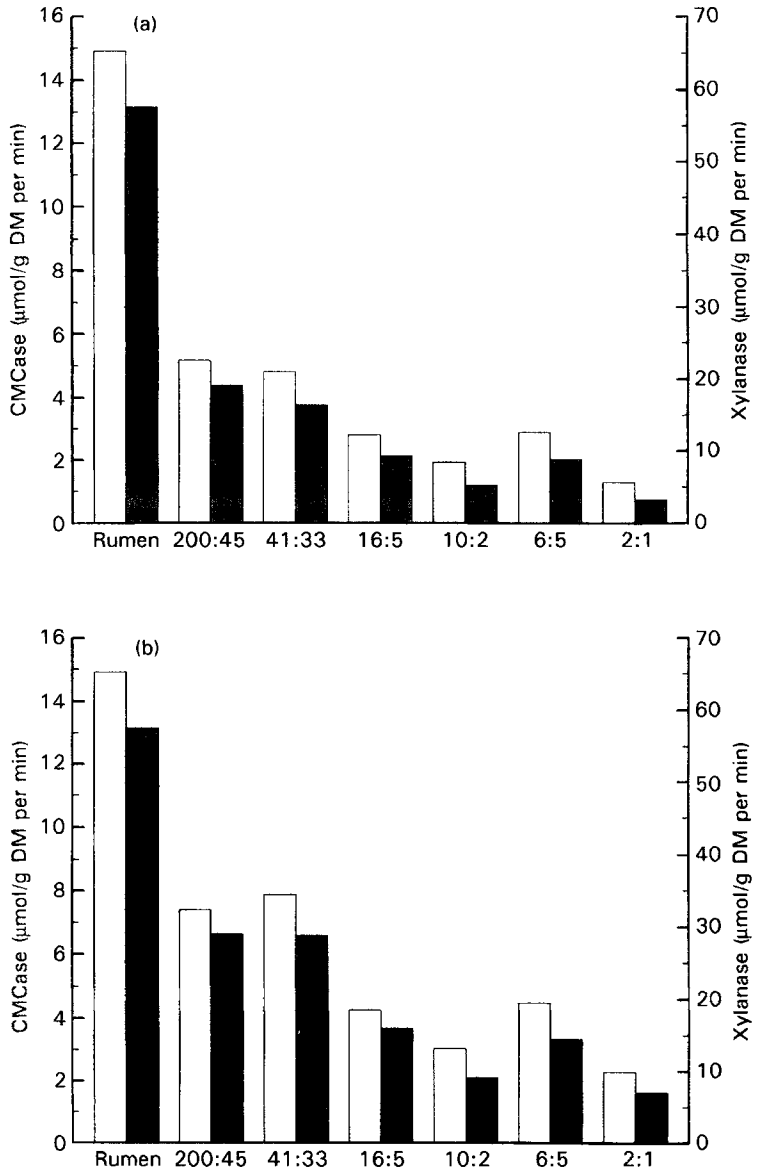

Fig. 1. Comparison of particle-associated carboxymethylcellulase (□; CMCase) and xylanase (■) activities extracted from rumen particulate matter or from undegraded nylon bag residues. (a) Rumen particulate matter before feeding and a weighted mean of nylon bag residues, and (b) rumen particulate matter before feeding and the highest values from nylon bag residues.

compared with the 41:33 bags may indicate a greater influx of rumen ingesta into 200:45 bags.

The present results are in agreement with those of Lindberg & Varvikko (1982), which suggest that the extent of digestion is not markedly influenced by bag cloth type but that both the lag time and rate of digestion are greatly modified by cloth type. Only with the cloth type 1:2 did the extent of digestion remain markedly lower than with the other cloth types, but it is likely that with extended incubation period the extent of digestion could have approached that obtained with the other bags. Although the extent of digestion may be estimated accurately with different cloth types, the rate of digestion is an important intrinsic characteristic of forages and together with passage rate of feed particles dictates the true availability of nutrients.

Disappearance of DM and NDF was consistently less from 10:2 bags than from 6:5 bags, but similar for 16:5 and 6:5 bags. Re-analysing the values 2 × 2 factorially also revealed that free surface area affected disappearance of DM and NDF more than the pore size, in agreement with the results on lucerne (*Medicago sativa*) DM and N by Meyer & Mackie (1986). Using the same four cloth types used in the present study in a mobile-bag study, Varvikko & Vanhatalo (1990) showed that free surface area influenced the disappearance of feed components in the intestines more than the pore size of the bags. In spite of this, the *in situ* method is widely used to determine the

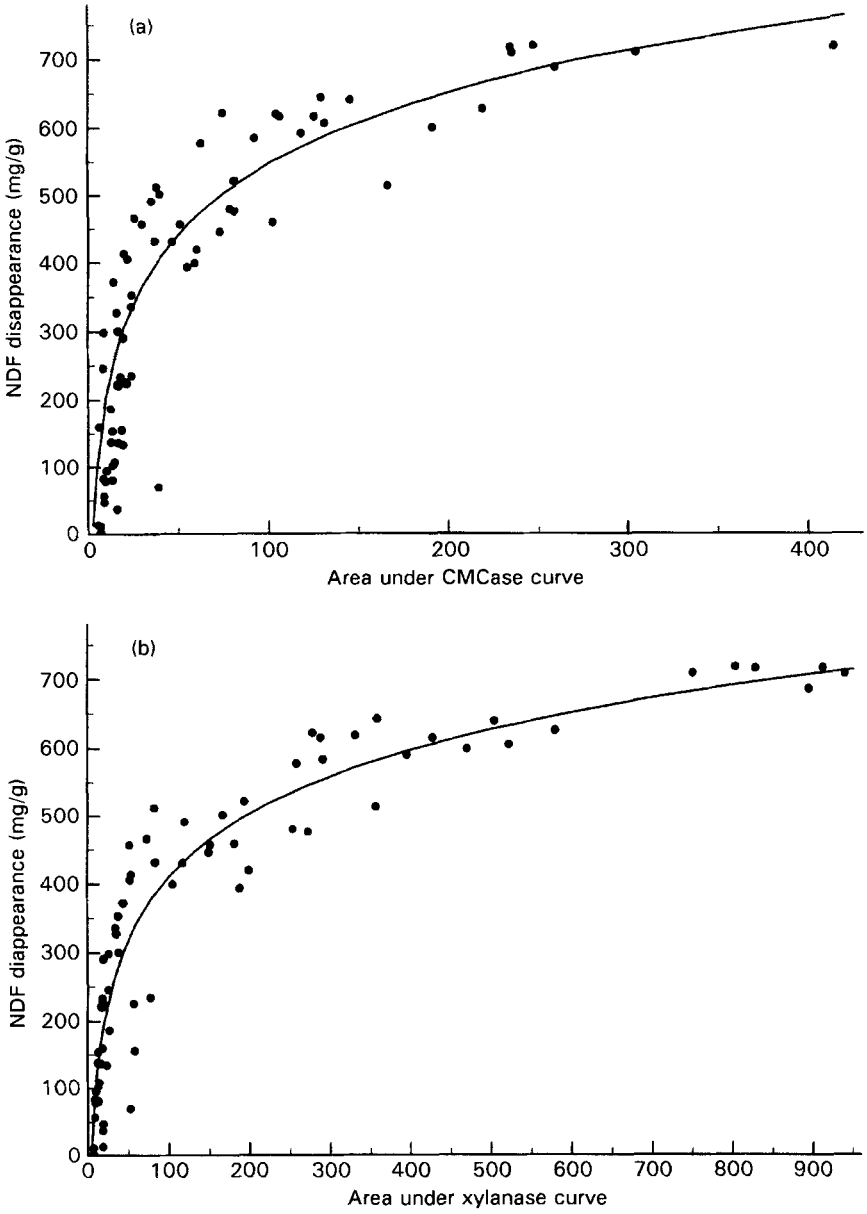

Fig. 2. Relationship between the area under the carboxymethylcellulase (CMCase) (a) or xylanase (b) curve and neutral-detergent fibre (NDF) disappearance from nylon bags incubated in the rumen of dairy cows for 6, 12, 24 and 48 h. Regression equations were:
NDF disappearance = $-141 + 149{\cdot}5 \times \ln$(CMCase) ($R^2$ 0·792);
NDF disappearance = $-210 + 134{\cdot}8 \times \ln$(xylanase) ($R^2$ 0·880).

digestion kinetics of N and NDF, and only a few studies have reported the bag free surface area. In addition to defined surface area and pore size, the pore size should not change with mechanical stress as pointed out by Trabalza Marinucci *et al.* (1992). Thus it is of critical importance to take into account the proportion of free surface area of cloth material used.

Particle-associated enzyme activities decreased with decreasing pore size or free surface area of the bags. Our findings are in agreement with the results of Meyer & Mackie (1986), who reported similar effects of bag cloth type on bacterial and protozoal number within the bags. Free surface area rather than pore size appeared to be a more important determinant of particle-associated enzyme activities within the bags. Closed surface area probably restricts the free inflow into the bags, hence reducing the number of microbes attached to feed particles, and consequently reducing enzyme activity within the bags.

Pore size may be a more important determinant of the number of protozoa than the bacteria entering the bags, and consequently on protozoal enzyme activities within the bags. With bags of pore size less than 10 μm, only small *Entodiania* spp. can enter (Kayoli *et al.* 1984; Meyer & Mackie, 1986). Large protozoa, such as *Polyplastron* spp. were unable to enter bags with a 53 μm pore size (Meyer & Mackie, 1986). Although more protozoa were probably

able to invade 16:5 compared with 6:5 bags, no differences were observed either in particle-associated enzyme activities or NDF disappearance. This is, however, consistent with the absence of fibrolytic activity in small protozoa, mainly *Entodinia* spp. (Coleman, 1985).

While the colonization of plant cell walls by bacteria and protozoa begins within 5 min after ingestion of feeds (Bonhomme, 1990), maximal concentrations of adherent microbes or enzyme activities appear much later. In the present study the highest particle-associated enzyme activities were detected after 24 h and 48 h rumen incubations. However, enzyme activities varied significantly due to cloth type showing that in addition to dietary forage:concentrate ratio (Silva *et al.* 1987) and rate of forage degradation (Bowman & Firkins, 1993) maximum colonization appears to depend on bag cloth type.

Relationship between enzyme activities and neutral-detergent fibre disappearance

At 6, 12, 24 and 48 h of incubation, particle-associated CMCase and xylanase activities were highly correlated with NDF disappearance, in agreement with the observations of Silva *et al.* (1987) and Huhtanen & Khalili (1992). The method was highly sensitive in detecting differences in enzyme activity between bag cloth types, which were associated with differences in NDF disappearance. In contrast, the total number of bacteria measured as particle-associated glutamate dehydrogenase (*EC* 1.4.1.2) activity was not related to the ability to degrade substrates in a previous study (Silva *et al.* 1987). In the present study, the relationship presented saturation type kinetics, consequently natural-logarithm-transformed enzyme activities showed a better relationship with NDF disappearance than a linear regression. In all cases xylanase exhibited a better correlation to NDF disappearance than did CMCase as found earlier by Huhtanen & Jaakkola (1994). Cumulative enzyme activities measured as the area under CMCase or xylanase activity curves (Fig. 2) were highly correlated with NDF disappearance. This relationship also held when data from different incubation periods (6–48 h) and cloth types were combined. This is in agreement with the observations of Bowman & Firkins (1993), who calculated the corresponding relationship for each forage separately. In the present study, similar slopes of regressions for each cloth type indicate that NDF disappearance was similarly related to microbial activity inside the bags irrespective of the cloth type. On the other hand, more negative intercepts with bags of smaller pore size and/or open surface area suggest that more enzyme activity was needed before the initiation of NDF disappearance, or alternatively, the same washing procedure was less efficient for removing loosely associated microbes from these bags compared with bags of larger pore size and surface area.

Enzyme activities within rumen ingesta v. *within bags*

The lower particle-associated enzyme activities within bags than in rumen ingesta have been reported earlier (Huhtanen & Khalili, 1992; Huhtanen & Jaakkola, 1993; Nozière & Michalet-Doreau, 1996). However, in all previous studies the diets also contained concentrates, whereas in the present study, the same silage which was fed was also incubated in nylon bags. All these observations are consistent with those of Meyer & Mackie (1986), who reported considerably smaller bacterial and protozoal counts within bags than in rumen ingesta (at maximum 0·60 of those in rumen digesta). In the present study, the highest activities inside the bags were only about 0·35 of that in rumen ingesta when calculated as a weighted mean of activities measured at different incubation periods. Even the highest single activities inside the bags were less than 0·75 of the mean activity in rumen ingesta. From these observations it can be concluded that the critical assumption of microbial activity within the bag being similar to that in the rumen ingesta cannot be justified, and therefore values of digestion kinetic parameters determined by the *in situ* method are at best biased. This may partly explain the large variation associated with this technique (see e.g. Madsen & Hvelplund, 1994).

In addition to the lower microbial counts, reduced pH (Trabalza Marinucci *et al.* 1992; Nozière & Michalet-Doreau, 1996) may explain the lower microbial activities inside bags than in rumen ingesta. Lack of mastication during ingestion and rumination may reduce the amount of damaged fibre, which is the major site for microbial attachment (Bauchop, 1980). However, mastication had no effect on microbial contamination of *in situ* residues or NDF disappearance (Olobobokun *et al.* 1990).

The lower microbial activities inside bags compared with rumen ingesta may explain the slower rates of NDF digestion observed with the *in situ* method than those estimated from rumen evacuation (Tamminga *et al.* 1989; Huhtanen & Jaakkola, 1993; Huhtanen *et al.* 1995). The use of digestion kinetic parameters estimated by the *in situ* method resulted in considerably lower estimates of ruminal NDF digestibility than values based on rumen evacuation (Huhtanen *et al.* 1995; Rinne *et al.* 1997). However, the magnitude of differences in ruminal NDF digestibility between estimates based on the *in situ* method and those determined *in vivo* did not depend on the type of feed (Archimède *et al.* 1995) suggesting that the *in situ* method may be used for relative comparisons between the feedstuffs.

In summary, the results of our present study indicate that the disappearance of feed components from nylon bags is greatly influenced by bag cloth type with free surface area being a more important factor than pore size. Differences in particle-associated CMCase and xylanase activities explained a large proportion of the variation in NDF disappearance. However, the present results indicate that the general assumption of the *in situ* technique, that the same micro-environment exists inside the bag and in the surrounding ingesta, is not justified. The lower activity within the bags explains the slower rate of NDF digestion measured by the *in situ* technique compared with values derived from rumen evacuation. Therefore care should be taken when interpreting the results for feed evaluation demand from the *in situ* technique. The method may be adequate for ranking the relative differences between feeds when incubated in host animals given the same diet and for

ranking associative effects between diet components. However, the method does not allow quantitative estimation of digestion rates, which are necessary for predicting feeding values.

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