

## MICROBIAL POLYSACCHARIDASE ACTIVITIES ASSOCIATED WITH RUMEN PARTICULATE MATERIAL AND FEED PARTICLES INCUBATED IN NYLON BAGS IN THE RUMEN

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### Introduction

Most of rumen bacteria are associated with feed particles during the degradation process. The more rigid cell walls that are digested require a firm adherence before degradation (Akin and Barton, 1983). It has been suggested that the reduced attachment is involved in the depression of fibre digestion with moderate decreases in rumen pH (Hoover, 1986). The degree of the colonization of cellulolytic microbes can be measured indirectly by measuring the activity of carboxymethylcellulase (CMCase) associated with feed particles incubated in nylon bags in the rumen (Silva et al., 1987). The results indicated that this method was very sensitive in assessing variation in rumen environment that affects the rate of fibre digestion. The purpose of this study was to investigate the effects of sucrose supplements in respect of their effect on rumen pH (without or with  $\text{NaHCO}_3$ ) and feeding method (twice daily or continuous infusion) on particle-associated polysaccharidase activities in cattle given a grass silage based diet. A further objective was to compare enzyme activities associated with rumen particulate material (RPM) or with particles incubated in nylon bags (NBP).

### Materials and Methods

A 4 x 4 Latin square experiment was carried out with cattle (liveweight  $344 \pm 5$  kg) fitted with a rumen and duodenal cannula. The control diet (diet C), offered at the level of 5.2 kg/d, comprised (DM basis) grass silage (700 g/kg), barley (240 g/kg) and rapeseed meal (60 g/kg). The other three diets were supplemented with 1 kg of sucrose per day given in two meals (diet S), in two meals with 250 g of  $\text{NaHCO}_3$  (diet B) or as a continuous intraruminal infusion (diet I). The animals were fed twice daily at 12 h intervals.

CMCase and xylanase activities were extracted from microbes attached to RPM sampled before feeding and 3 h after feeding, and from microbes attached to hay and silage particles incubated in nylon bags in the rumen for 12, 24 and 48 h. The silage was finely chopped and the hay was hammer-milled to pass a 2 mm-screen. Immediately after the withdrawal, the bags were washed in cold water by a house-hold washing machine. The duration of the washing procedure was 30 min, consisting of 5 rinsing cycles. RPM was also placed in the nylon bags and washed using a similar procedure. Enzyme extraction and assays were made as described by Silva et al. (1987). The results were analysed by a split-plot or split-split-plot analysis of variance.

### Results and Discussion

Sucrose supplements reduced ( $P < 0.05-0.01$ ) particle associated CMCase and xylanase activities extracted either from RPM or NBP (table 1). Increasing rumen pH with  $\text{NaHCO}_3$  almost completely eliminated the depressive effect of sucrose supplements on particle-associated enzyme activities. Dietary variation on CMCase and xylanase activities was accompanied by corresponding differences in fibre digestion *in vivo* or *in sacco* ( $R^2 > 0.90$ ). Enzyme activities were higher ( $P < 0.001$ ) in silage than in hay NBP and increased ( $P < 0.001$ ) with increasing incubation times.

CMCase and xylanase activities associated with RPM were markedly higher than the corresponding activities associated with NBP. After incubation of 24 h, CMCase and xylanase activities in silage NBP were only 43 and 59 % of those associated with RPM. The average xylanase:CMCase ratio was lower in RMP than in NBP (1.39 vs. 1.68). These differences suggest that microbial population attached to NBP differed from that attached to RPM both in quantity and

TABLE 1. CMCase AND XYLANASE ACTIVITIES ( $\mu$ MOL REDUCING SUGARS/G DM/H) EXTRACTED FROM MICROBES ATTACHED TO RUMEN PARTICULATE MATTER (RPM) AND FEED PARTICLES INCUBATED IN NYLON BAGS (NBP)

	Diet				SEM	Forage <sup>a</sup>		SEM
	C	S	B	I		S	H	
<b>NBP</b>								
CMCase	601	441	641	477	45*	719	361	20***
Xylanase	1313	701	1336	817	93**	1207	876	21***
<b>RPM</b>								
CMCase	1760	1203	1629	1275	97**			
Xylanase	2916	1725	2503	1860	156**			

<sup>a</sup>Forage incubated, silage (S), hay (H).

quality. On the basis of the lower polysaccharidase activities in NBP than in RPM the validity of nylon bag method in the estimation of the actual rate of degradation of cell wall carbohydrates may be questioned. This possibility requires, however, more investigation.

The reasons for the lower polysaccharidase activities in NBP are uncertain but some points should be considered. First, it should be noted that the same silage was incubated in nylon bags and given to the animals, and the washing procedures were similar. Second, sites for microbial attachment are mainly the damaged cell walls (Latham et al., 1978); therefore, the number of sites for attachment could be greater in masticated rumen digesta. Lower activities in milled hay residues as compared with chopped silage residues do not, however, support this concept. Third, the porosity of the bags used (41 $\mu$ m) may prevent some of the rumen protozoa from being attached to the feed particles in nylon bags. Fourth, the relatively small open surface area in the bags (33 %) may limit liquid inflow and outflow, and therefore the microenvironment within the bag may be different from that of the whole rumen

with respect to the availability of essential nutrients.

(Key Words: Sucrose, Enzymes, Rumen Particles).

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