Journal of Animal and Feed Sciences, 14, Suppl. 1, 2005, 287–290

The distribution of fibrolytic activity in the rumen of ciliate-free and faunated sheep

T. Michałowski[C]

The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences
05-110 Jabłonna, Poland

ABSTRACT

The aim of this study was to determine the ruminal fibrolytic activity in defaunated and selectively faunated sheep. After defaunation, xylanase activity was 25-31 while carboxymethylcellulase (CMC-ase), 2.5 μM reducing sugars/g DM/min. As much as 73-81% of xylanase and 84-96% of CMC-ase in defaunated sheep originated from particle-associated bacteria. Ciliates increased the activity of xylanase to about 90-95 and CMC-ase to 4.7 μM reducing sugars/g. As much as 74-80% of total xylanase and 61-62% CMC-ase in faunated animals originated from the ciliate, *Eudiplodinium maggii*. Ciliates did not negatively influence the colonization of feed particles by bacteria.

KEY WORDS: rumen content, particulate matter, protozoa, β-endoglucanase, xylanase

INTRODUCTION

Fibre degradation in the rumen depends on microorganisms able to synthesize cellulolytic and hemicellulolytic enzymes. It is known that predominant fibrolytic bacteria and fungi colonize fibrous particles (Mc Allister et al., 1994) while ciliates do not (Williams and Coleman, 1992). It is also well documented that protozoa diminish the numbers of bacteria and fungal zoospores in the rumen fluid (Williams and Coleman, 1992). Thus they can influence the colonization of fibrous feed by two other groups of microorganisms. The aim of this study was to determine the activity of carboxymethylcellulsae (CMC-ase) and xylanase in whole rumen digesta, as well as in the particulate and liquid fractions with respect to the absence and presence of ciliates.

Corresponding author: e-mail: t.michalowski@ifzz.pan.pl

MATERIAL AND METHODS

Two adult female Black Head rumen-fistulated sheep were kept in separate pens and fed 150 g of pelleted concentrate (17% crude protein) at 8 and 16 h. Meadow hay and drinking water were given *ad libitum.* Samples of rumen contents were collected three times a day on the 10, 11 and 12 day after defaunation (Jouany and Senaud, 1979) as well as on the 22, 24, 26, 28 and 30 d after spontaneous refaunation of animals. The particulate fraction was prepared by rinsing rumen digesta with tap water on a screen of pore size 0.5 mm, while the liquid fraction, by squeezing the rumen content and centrifuging the obtained fluid for 4 min at 500 × g and 4ºC. The protozoa were purified and separated into *Entodinium maggii* and "small ciliates" groups by a sedimentation method (Michałowski, 1990). Crude enzymes were extracted with CCl_4 at 40ºC for 4 h according to Huhtanen and Khalili (1992). The activity of CMC-ase and xylanase was determined by quantification of the reducing sugars released from carboxymethylcellulose and xylan incubated at 40ºC for 1 h with crude enzymes. Reducing sugars were measured using 3,5-dinitrosalicylic acid (Miller et al., 1960). The protozoa were counted using a light microscope.

RESULTS AND DSCUSSION

The total fibrolytic activity in the rumen resulted either from the presence of bacteria or bacteria and the re-established protozoa, because fungi did not develop after defaunation of sheep. The spontaneously re-established ciliate

Table 1. Enzyme activities in the rumen content and its two fractions (μM released reducing sugars/g DM rumen content and particulate fraction or ml of liquid fraction) of defaunated and spontaneously refaunated sheep

Enzymes	Sheep 1		Sheep 2	
	defaunated	refaunated	defaunated	refaunated
Whole rumen content				
xylanase	30.7 ± 10.67	94.8 ± 8.37**	25.0 ± 4.11	90.3 ± 10.50**
CMC-ase	2.5 ± 0.50	4.7 ± 0.93**	2.5 ± 0.29	4.7 ± 0.87**
Particulate fraction				
xylanase	22.3 ± 5.53	22.1 ± 3.44	20.4 ± 3.27	20.4 ± 7.03
CMC-ase	2.4 ± 0.16	2.1 ± 0.24	2.1 ± 0.25	2.3 ± 0.55
Liquid fraction				
xylanase	0.5 ± 0.11	0.4 ± 0.07	0.3 ± 0.06	0.2 ± 0.05
CMC-ase	0.08 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	0.05 ± 0.01

** $P<0.01$

species were *Eudiplodinium maggii*, *Entodinium simplex* and *Dasytricha ruminantium*. The last two reappeared in the rumen of only one sheep. The significant increase in the activity of xylanase and CMC-ase resulted from the establishment of fibrolytic ciliates *Eudiplodinium maggii* (Coleman, 1985). These ciliates clearly determined the fibrolytic activity in the rumen of both sheep (Table 2). No differences were found between the particle-associated activity in the ciliate-free and refaunated sheep and this suggests that establishment of protozoa did not restrict the colonization of feed particles by fibrolytic bacteria (Silva et al., 1987), at least as far as particles larger than 0.5 mm are concerned. These results are contradictory to the findings of Newbold et al. (1989). However, the effect of fauna composition should be taken into consideration during such a comparison. The very low CMC-ase and xylanase activity in the liquid fraction of the rumen digesta confirms the opinion that the majority of fibrolytic bacteria is tightly associated with the particles of forages (McAllister et al., 1994).

Table 2. Ciliate number in the rumen of the spontaneously refaunated sheep and their contribution to the total activity of CMC-aze and xylanase, % of total ruminal activity

Item	Sheep 1	Sheep 2
Total CMC-ase, μM reducing sugars/g DM/min	94.8 ± 8.3	90.3 ± 10.50
Eudiplodinium maggii numbers, × 10^3/g digesta	36.8 ± 12.01	35.5 ± 8.33
Eudiplodinium maggii xylanase, % of total value	73.7 ± 5.12	79.8 ± 4.91
Eudiplodinium maggii CMC-ase, % of total value	62.2 ± 3.72	61.1 ± 4.59
Small ciliate numbers, × 10^3/g digesta	0.0	198.4 ± 73.23
Small ciliate xylanase, % of total value	ND	1.5 ± 0.28
Small ciliate CMC-ase, % of total value	ND	3.1 ± 0.49

ND - not determined

The ciliates appeared spontaneously in the rumen as early as about 3 weeks after defaunation and this suggests that the applied defaunation procedure was unsuccessful. Of the re-established species, *Entodinium simplex* and *Dasytricha ruminantium* belonging to the group "small ciliates" were present in the rumen of only one sheep. The fibrolytic activity detected in these protozoa originated presumably from the engulfed bacteria since the both species are unable to digest cellulose (Williams and Coleman, 1992).

CONCLUSIONS

In the ciliate-free sheep, the activities of CMC-ase and xylanase originated predominantly from bacteria colonizing the particulate fraction of rumen contents while in the refaunated animals, from the fibrolytic ciliate, *Eudiplodinium maggii.*

The obtained data also suggest that the appearance of ciliates in the rumen of sheep did not diminish the colonization of feed particles by fibrolytic bacteria.

REFERENCES

Coleman G.S., 1985. The cellulase content of 15 species of entodiniomorphid protozoa, mixed bacteria and plant debris isolated from the ovine rumen. J. Agr. Sci. 104, 349-360

Huhtanen P.P., Khalili H., 1992. The effect of sucrose supplement on the particle-associated carboxylethylcellulase (EC 3.2.1.4) and xylanase (EC 3.2.1.8) activities in cattle given grass-silage-based diet. Brit. J. Nutr. 67, 245-255

Jouany J.-P., Senaud J., 1979. Defaunation du rumen de mounton. Ann. Biol. Anim. Biochem. Biophys. 19, 619-624.

McAllister T.A., Bae H.D., Jones G.A., Cheng K.-J., 1994. Microbial attachment and digestion in the rumen. J. Anim. Sci. 56, 1235-1244

Michałowski T., 1990. The synthesis and turnover of the cellular matter of ciliates in the rumen. Acta Protozool. 29, 47-92

Miller G.L., Blum R., Glennnon W.E., Burton A.L., 1960. Measurement of carboxymethylcellulase activity. Anal. Biochem. 2, 127-132

Newbold C.J., Griffin W.P., Wallace R.J., 1989. Interaction between bacteria and ciliate protozoa in their attachment to barley straw. Lett. Appl. Microbiol. 8, 63-66

Williams A.G., Coleman G.S., 1992. The Rumen Protozoa. Springer-Verlag. New York

STRESZCZENIE

Dystrybucja aktywności fibrolitycznej w żwaczu owiec wolnych od orzęsków i selektywnie faunowanych

Celem badań było określenie aktywności enzymów fibrolitycznych w żwaczu owiec zdefaunowanych i selektywnie faunowanych. Stwierdzono, że po defaunacji aktywność ksylanazy wynosiła 25-31, a karboksymetylocelulazy (CMC-azy) - 2.5 μM cukrów redukujących/mg s.m./min. Około 73-81% ogólnej aktywności ksykanazy i 84-96% aktywności CMC-azy pochodziło z bakterii kolonizujących cząstki stałe paszy. Orzęski spowodowały wzrost aktywności ksylanzay do 90-95 i CMC-azy do 4,7 μM cukrów redukujących/mg s.m./min. Stwierdzono, że 74-80% ogólnej aktywności ksylanazy w żwaczu i 61-62% aktywności CMC-azy pochodziło z komórek orzęsków *Eudiplodinium maggii*. Wykazano, że orzęski nie miały ujemnego wpływu na kolonizację cząstek paszy przez bakterie.