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Original article

Effect of anaerobic fungi on the ruminal proteolysis in gnotobiotic lambs

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Summary — The establishment of a fungal population composed of the main species usually found in ruminants in the rumen of gnotobiotic lambs did not significantly alter the *in sacco* digestibility of meat meal and soybean cake. The proteolytic activity of the rumen fluid against ^{14}C-casein was not affected by the fungi. Therefore, these microorganisms probably do not play an important role in the degradation of proteins in the rumen.

rumen / proteolysis / anaerobic fungi / chytridiomycetes

Résumé — Effet des champignons anaérobies sur la protéolyse ruminale chez des agneaux gnotobiotiques. *L'implantation dans le rumen d'agneaux gnotobiotiques d'une population fongique composée des principales espèces habituellement rencontrées chez les ruminants n'a pas amélioré la digestibilité* in sacco *de la farine de viande et du tourteau de soja. De même l'activité protéolytique du jus de rumen à l'égard de la ^{14}C-caséine n'a pas été significativement modifiée en présence des champignons. Il est par conséquent peu vraisemblable que ces micro-organismes jouent un rôle important dans la protéolyse ruminale.*

rumen / protéolyse / champignons anaérobies / chytridiomycètes

INTRODUCTION

It is now well established that anaerobic fungi are autochtonous members of the rumen ecosystem (Orpin and Joblin, 1988; Fonty and Joblin, 1991) and that they may play an important role in rumen fermentation (Fonty *et al,* 1990). There have been several recent *in vitro* studies of their cellulolytic and hemicellulolytic activities (Lowe *et al,* 1987; Williams and Orpin, 1987a,b; Hébraud and Fèvre, 1988; Bernalier *et al,* 1991, 1992). These functions have also been observed *in vivo* in gnotobiotic lambs (Fonty and Gouet, 1989; Fonty *et al,* 1992). In contrast, the proteolytic activity of the fungi is a matter of debate. Wallace and Joblin (1985) and Wallace and Munro (1986) reported that strain PNK_2 of *Neocallimastix frontalis* had a relatively high

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proteolytic activity, whereas, of the 7 strains studied by Michel *et al* (1993), only one, a *Piromyces* sp strain, exhibited hydrolytic activity against casein. Furthermore, the activity was very weak. All 7 strains, however, showed aminopeptidase activity.

The aim of this work was to study the effect of these chytridiomycetes on the ruminal proteolysis in gnotobiotic lambs reared in sterile isolators.

MATERIAL AND METHODS

Animals

Three naturally born lambs (A, B, C) were left with their dams for 24 h and then placed in sterile isolators. They received sterile cow's milk (UHT) until the age of 70 d. From 1 month of life they also had access to a sterile pelleted ration of dehydrated lucerne hay. The lambs were fitted with a permanent rumen cannula at 2.5 months of age (Fonty *et al,* 1988; Fonty and Gouet, 1989). Hence, they were isolated before the natural establishment of the fungi, which occurs around the age of 10 d (Fonty *et al,* 1987).

Table I. Composition of the fungal population inoculated in gnotobiotic lambs.

Strain	*Genus or species*	*Origin*
MCH3	*Neocallimastix frontalis*	Sheep rumen
8628	*Neocallimastix* sp	Sheep rumen
LM1	*Piromyces* sp	Cow rumen
4	*Piromyces* sp	Sheep rumen
8516	Not determined	Sheep rumen
Tp90.7	Polycentric strains	Sheep rumen
Tr92.4	*Caecomyces* sp	Sheep faeces

During the fifth week of life they received 3 separate administrations of a fungi-free bacterial inoculum of 1 ml of a 10^{-6} dilution of rumen contents taken from a conventionally reared sheep (period I). At the age of 4 months (period II) the lambs were administered a fungal inoculum composed of 7 strains of anaerobic fungi (table I) isolated from the rumen and faeces of sheep and cows. The activity of these strains has been previously studied *in vitro* (Michel *et al,* 1993). The establishment of the fungi and the development of their population were regularly monitored throughout the experimental period by zoospore counts in the rumen content according to the roll-tube method described by Joblin (1981).

Biochemical analysis

During these 2 periods, we measured the DM disappearance of 2 protein-rich substrates (meat meal and soybean cake) in the 3 animals by the nylon bag method (Michalet-Doreau, 1990). Nylon bags containing meat meal (2.5 g dry matter/bag) were removed from the rumen after 2, 4 and 7 h of incubation and those with soybean cake (2 g dry matter/bag) after 24 and 48 h. Six bags were placed in the rumen of each lamb for each incubation time.The proteolytic activity against ^{14}C-casein (^{14}C-methylated casein, Sigma) in the rumen contents of lambs A and B was determined *in vitro,* during the 2 periods according to the method described by Wallace and Joblin (1985). Three samples from each lamb were used for these measurements. The rumen contents were withdrawn through the cannula and filtered on a double layer of cheesecloth.

Statistical analysis

The effect of fungi on DM disappearance was tested for each substrate by an analysis of variance using the SAS GLM procedure (SAS Institute, 1985).

RESULTS AND DISCUSSION

The fungi became established in the rumen of the 3 lambs soon after inoculation.

The fungal population, which reached a level of 10^3 zoospores per ml of rumen content 8 d after the last inoculation, ranged between 10^3 and 10^4 zoospores ml^{-1} throughout the experimental period. The relative proportions of the different strains were not determined but all the morphological types present in the inoculum were found in the roll-tubes (mono- and polycentric, rhizoidal and non-rhizoidal strains).

The results of the degradation of the meat meal and soybean cake in the nylon bags (table II) show that the establishment of anaerobic fungi in the rumen did not significantly alter the extent of DM disappearance or the rate of degradation. The fungi did not therefore enhance the degradation of the 2 protein-rich substrates.

The activity of the rumen fluid from lambs A and B varied widely between samples (table III), making it difficult to interpret the results. Neither of the animals showed variations in proteolytic activity in relation to the time of sampling. In the absence of fungi, although it was inoculated with the same bacterial flora and reared in the same conditions as lamb A, lamb B had slightly greater activity against ^{14}C-casein. This difference did not result in a more efficient digestibility *in sacco* of the meat meal and soybean cake.There was a slight average increase in activity in lamb A after establishment of the fungi but it was not significant. In animal B, however, the activity was slightly weaker in the presence of fungi, particularly before feeding, but again the variations were not significant. Although the fungal populations, as assessed by zoospore counts, reached comparable levels in the 2 animals, the relative proportions of the different strains may have been different. A higher population level of *Piromyces* LM 1 in lamb A, for example, could have been responsible for the slight increase in the proteolytic activity of the rumen fluid against ^{14}C-casein during period II, since this strain was the only 1 of the 7 making up the inoculum in which proteolytic activity was evidenced *in vitro* (Michel *et al*, 1993).

This study is the first published work on the role of anaerobic fungi in the *in vivo* degradation of proteins. The results support those obtained *in vitro* by Michel *et al* (1993), who showed that these micro-

Table II. Percentage of DM disappearance [a] of meat meal and soybean cake in nylon bags after incubation in the rumen of gnotobiotic lambs in the absence or presence of anaerobic fungi.

Substrate	*Period of incubation (h)*	*Percentage of DM disappearance*		
		Absence of fungi	*Presence of fungi*	*SEM*
Soybean cake	2	49.3	49.9	5.4
	4	51.7	55.5	4.1
	7	59.2	59.5	5.2
Meat meal	24	44.6	42.0	3.1
	48	44.8	44.0	2.3

a The animal effect was not significant ($P \geq 0.05$) and in consequence the nylon bags introduced in the 3 animals were pooled for the determination of the DM disappearance.

Table III. Amount of ^{14}C-casein (µg ± SD) (n = 3) degraded by 1 ml rumen fluid in 1 h in the presence or absence of anaerobic fungi.

	Absence of fungi		*Presence of fungi*	
	T_0 [a]	T_2 [b]	T_0 [a]	T_2 [b]
Lamb A	168 ± 22	161 ± 25	200 ± 18	182 ± 39
Lamb B	260 ± 62	232 ± 73	165 ± 52	172 ± 42

[a] T_0: rumen sample collected before feeding; [b] T_2: rumen sample collected 2 h after feeding.

organisms have no great proteolytic activity. However, they contrast the finding of Wallace and Joblin (1985) and Wallace and Munro (1986), who reported a relatively high activity *in vitro* in strain PNK2 of *N frontalis.*

In conclusion, this study indicates that anaerobic fungi probably do not play an important role in the degradation of dietary proteins in the rumen ecosystem.

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