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ROLE OF RUMEN PROTOZOA IN THE DIGESTION OF FOOD CELLULOSIC MATERIALS

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Although much is known about the cellulolytic activity of bacteria in cellulose digestion in the rumen, the role of protozoa in cellulose degradation is still widely debated (Hobson, 1976). Most studies carried out up to now have been essentially on cellulases and purified cellulose substrate digestion in protozoa cultures.

The use of adult sheep, whose rumen was either defaunated or inoculated with one or several types of ciliate, has enabled us to study *in vivo* the effect of protozoa presence in the rumen on the digestibility of cellulose, and on bacterial cellulolytic activity in the rumen. These parameters were studied according to the type of ciliate present in the rumen and according to the main carbohydrate in the diet.

Material and Methods

8 adult sheep fitted with a permanent rumen fistula were used. Two were defaunated according to the method previously described by Jouany and Senaud (1979) ; 4 others were inoculated, either with the strain « *Polyplastron multivesiculatum* » (2 sheep), or with the strain « *Entodinium sp* » (2 sheep), according to the technique described by Jouany (1978). Two sheep whose rumen contained at least 5

different genus of ciliates were also used. The sheep were kept in identical environmental conditions in individual pens, in separate and air-conditioned rooms.

Each sheep was initially given a control diet composed of meadow hay and groundnut meal (54.0 and 1.7 g/kg liveweight^{0.75} per day, respectively) (table 1). The lignocellulose content of hay was 32.0% of dry matter. Then they received consecutively 4 experimental diets, in which 65% of the hay in the control diet was replaced by barley, sugar beets, deproteinized whey or jerusalem artichokes, as sources of 4 carbohydrates : starch, sucrose, lactose and inulin. The lignocellulose content of these diets was respectively : 23, 18, 17 and 15% of dry matter.

The amounts of cellulose, starch, sucrose, lactose and inulin ingested were approximately 22 g/kg liveweight^{0.75}. Diets were complemented with groundnut meal to be isonitrogenous (14 % protein on dry matter basis).

Digestibility of the rations was determined at least twice in each animal and was measured by collecting all the faeces over a 5 day period. The percentage of lignocellulose in the rations and in the faeces was determined using the « acid detergent fibre » method, (Van Soest, 1963).

Cellulolytic activity of rumen bacteria was measured *in vivo* by a nylon bag technique by

Table 1. - Composition of different diets and dry matter intake (g/day)

Components	Diets				
	Cellulose	Starch	Sucrose	Lactose	Inulin
Hay	950	480	490	460	280
Barley	-	600	-	-	-
Sugar beets	-	-	620	-	-
Deproteinized whey	-	-	-	420	-
Jerusalem artichoke	-	-	-	-	620
Groundnut meal	30	140	90	120	90

Table 2. - Effects of rumen ciliates on lignocellulose digestibility (D. ADF) and cellulolytic activity of rumen bacteria (C.A.) in sheep fed different diets

Animals	Diets									
	Cellulose		Starch		Sucrose		Lactose		Inulin	
	D.(ADF)	C.A.	D.(ADF)	C.A.	D.(ADF)	C.A.	D.(ADF)	C.A.	D.(ADF)	C.A.
Defaunated	68.8±0.2 ^a	38.3±1.9 ^a	55.6±0.7 ^a	27.3±2.3 ^a	62.5±1.1 ^a	28.4±1.5 ^a	55.4±1.4 ^a	28.1±1.5 ^a	66.4±1.9 ^a	29.9±1.6 ^a
Contaminated only with										
E	70.1±0.1 ^b	41.3±3.6 ^b	60.3±1.8 ^b	24.5±1.8 ^b	66.4±3.1 ^b	26.7±2.5 ^b	54.7±1.8 ^b	26.2±1.7 ^b	68.3±1.2 ^{ab}	26.3±1.0 ^b
P	71.6±1.2 ^c	39.8±1.8 ^{ab}	61.4±0.6 ^b	25.4±0.9 ^b	67.1±4.6 ^b	26.0±1.6 ^b	58.6±2.4 ^b	26.8±0.7 ^b	68.2±1.3 ^{ab}	25.6±2.6 ^b
Faunated	71.1±0.8 ^c	41.0±0.9 ^b	60.0±1.2 ^b	21.1±1.0 ^c	69.2±0.8 ^b	26.8±1.4 ^b	58.9±1.8 ^b	23.2±0.7 ^c	68.7±1.2 ^b	26.2±1.3 ^b

Means followed by different superscripts are significantly different ($P \leq 0.05$)

E = *Entodinium* sp. ; P = *Polyplastron multivesiculatum*

using milled oat straw as substrate ; 4 bags were used for each animal. Measurements were repeated at least twice for each type of fauna studied. A nylon material in which the size of the mesh ($< 10\mu$) did not allow the protozoa to penetrate, was chosen. Microscopic examination of the contents of one of the bags taken from the rumen during each measurement showed that the ciliate protozoa could not penetrate into the bags.

Results and Discussion (table 2)

For all the diets studied, the presence of ciliate protozoa in the rumen gave a significant increase in the digestibility of lignocellulose (between 3% and 10%) and consequently of organic matter. This increase was especially high in animals contaminated with rumen

fauna including the strain *P. multivesiculatum*. The addition of other strains to the single ciliate *Polyplastron* did not improve the digestibility of lignocellulose ; no significant difference between animals inoculated with *Polyplastron* alone and those with a complete fauna, was found in the digestibility of lignocellulose. Given that lignin is not digested during its passage through the digestive tract, the quantity of cellulose which disappeared daily in the tract was increased by ciliate presence in the rumen by 30 g in the « cellulose » diet, 20-24 g in the « inulin », « starch » and « sucrose » diets, and 7 g in the « lactose » diet. The results prove the importance of ciliates and more particularly *P. multivesiculatum* in cellulose digestion.

Changes in lignocellulose digestibility induced by the presence of the single strain *Entodinium* in the defaunated rumen varied accor-

ding to the type of diet, or were even non-existent when the animals were fed the « lactose » or « inulin » diets. This corresponded to an increase of 1 - 5 g of digestible cellulose per day. On the other hand, when the animals received the « starch » and « sucrose » diets the increase was higher 7 - 20 g per day.

Cellulolytic activity of rumen bacteria was modified by the presence of ciliates, but in a manner which varied according to the main carbohydrate in the diet. Bacterial cellulolytic activity was greater (+ 8%) when ciliates were present in animals on the « cellulose » diet. In contrast, it was greatly reduced (8-14%) by inoculating one or more types of ciliates into the rumen of animals with an intake of readily fermentable carbohydrates (starch, sucrose, lactose, inulin) inducing a lower pH of rumen content (6.0 vs 6.8 on hay diet). The type of fauna has little effect on the decrease of cellulolytic activity.

From these results, the role of protozoa in lignocellulose, and therefore cellulose digestion in the rumen, can be clarified. It can be hypothesized that ciliates participate directly in

cellulose degradation when the diet has a low cellulose content (15% dry matter) because, simultaneous to an increase in lignocellulose digestion, there is a decrease in bacterial cellulolytic activity after protozoa inoculation into the rumen. The latter can probably be explained by a reduction in the total bacteria numbers, and therefore in cellulolytic bacteria in the inoculated animals ; pH of rumen content probably also inhibited cellulolytic strains. The existence of cellulases in rumen ciliate protozoa, and more particularly in *P. multivesiculatum* (Abou Akkada, 1965) is confirmed by our experiments, because it is in the presence of this strain that lignocellulose digestibility is greatest. When the diet has a higher cellulose content (30% dry matter), the improvement in digestion following an inoculation of ciliates into the rumen is probably due to a protozoal and bacterial synergetic effect. The cellulolytic action of the ciliates, previously described, adds to that of the bacteria which is increased in faunated animals. In this case, the stimulation of bacterial metabolism could be explained by the predatory action of protozoa on bacteria (Kurihara *et al.*, 1968).

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