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Use of nylon bags of different porosity to study the role of different groups of rumen ciliates in *in situ* digestion of hay in sheep*

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ABSTRACT

Three rumen fistulated Polish Merino wethers were used to study the digestion of hay *in situ*. The animals were either defaunated or faunated only with *Eudiplodinium maggii*, with *Eudiplodinium maggii* and *Entodinium caudatum* and with both entodiniomorphids plus *Dasytricha ruminantium*. The diet consisted of hay (750 g) and ground barley (130 g) per meal and was given every 12 h. An *in sacco* technique was applied to measuere the disappearance of dry matter, ADF and NDF from hay samples incubated for 12, 24 and 48 h in the rumen. The bags were either of 42 or 206 μm pore diameter. Numbers of *Entodinium caudatum, Eudiplodinium maggii* and *Dasytricha ruminantium* in the rumen contents were 52.4-55.5, 1.4-2.3 and 6.1 x 10^4/g, respectively. The concentration of *Entodinium caudatum* and *Dasytricha ruminantium* inside the bags was not influenced by pore size and varied in the range of 29.4-57.0 and 2.0-5.3 x 10^4/g, respectively. Number of *Eudiplodinium maggii* inside the bags of 42 and 206 μm pore size was 0.1-0.4 and 0.6-2.6 x 10^4/g. Colonization of hay by the fibrolytic bacteria was determined as the activities of particle associated CMC-ase and xylanase. They were the range of 1.6-8.1 and 10.1-51.8 μmol reducing sugars/g DM/min, respectively. Establishment of *Eudiplodinium maggii* alone or together with *Entodinium caudatum* increased colonization whereas appearance of *Dasytricha ruminantium* decreased it to the level observed in the ciliate-free sheep. Disappearance of DM, ADF and NDF from hay incubated *in sacco* varied in the range of 38.2-75, 18.4-57.6 and 20.8-68.9% in relation to the time of incubation and microfauna composition. *Eudiplodinium maggii* alone increased significantly the ADF disappearance after 12 and 24 h of incubation while the loss of DM and NDF was either not influenced or reduced. Microfauna composed of *Eudiplodinium maggii* and *Entodinium caudatum* supported ADF digestion after

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12 h of incubation, however, to a significantly lesser extent than *Eudiplodinium maggii* alone. Loss of DM and NDF was not influenced or diminished in relation to the time of incubation or porosity of the bags used. Microfauna consisting of the both entodiniomorphids and *Dasytricha ruminantium* significantly reduced disappearance of all three components independently of the incubation time. Most visible differences were, however, observed after 12 h of incubation. Enlargement in the pore size and thus in the numbers of *Eudiplodinium maggii* inside the bags did not increase neither DM nor ADF and NDF disappearance from the hay samples incubated *in sacco*.

KEY WORDS: rumen ciliates, digestion *in sacco*, DM, ADF, NDF, microfauna manipulation

INTRODUCTION

Forage diets given to ruminants are characterized by a high proportion of fibre consisting mainly cellulose and hemicellulose. Cellulose is a linear polymer of glucose while xylose and arabinose are the most frequent sugars in hemicellulose. Glucose and pentose residues are bound with the 1,4-β-D-glycosidic linkages in both of the polysaccharides and it is well known that ruminants, like other mammals, do not have the digestive enzymes capable of catalyzing cleavage of such bounds. In contrast to mammals a number of microorganisms are capable of synthesizing cellulases and hemicellulses enabling them to digest fibrous material. Some of them live in the rumen. Fibrolytic microorganisms include bacteria, fungi and protozoa. The most important bacteria are *Fibrobacter succinogenes, Ruminococcus albus, Ruminococcus flavefaciens* and *Butyrivibrio fibrisolvens* (Chesson and Forsberg, 1997). Of the rumen fungi *Neocallimastix frontalis, Piromyces communis* and *Orpinomyces* sp. (Fonty and Gouet, 1994; Trinci et al., 1994) are most often listed as participants of cellulolytic consortium. The most cellulolytic and xylanolytic species of ciliates seems to be *Eudiplodinium maggii, Ostracdinium dilobum* and *Epidinium ecaudatum* (Williams, 1988; Dehority, 1993; Michałowski, 1997; Michałowski and Harmeyer, 1998; Michałowski et al., 2001). It is well documented that rumen bacteria and fungi participate directly in fibre degradation in the rumen (Chesson and Forsberg, 1997). Conversely, role of protozoa is not well established. It has been found that *Eudiplodinium maggii, Epidinium ecaudatum* and some other large ophryoscolecids enhanced fibrolytic activity in the rumen (Coleman, 1985; Michałowski and Harmeyer, 1998). On the other hand the establishment of a mixed fauna in defaunated sheep was most often accompanied by increase in dry matter and fibre digestion, however, a reverse effect was also observed and difference in the microfauna composition were considered as possible causes of the observed effects (Ushida et al., 1991; Jouany et al., 1995). Mechanisms by which the rumen protozoa influence fibre degradation are poorly characterized but it is thought that they participate in cellulose and hemicellulose digestion and affect the fibrolytic microflora (Williams, 1988; Ushida et al., 1991). It can be assumed that large

entodiniomorphids, like the species mentioned above, practice both mechanisms while small *Entodinia* and holotrichs seem to use only the second. The role of particular species of ciliates has rarely been examined due to difficulties which results from a necessity to posses defaunated and selectively faunated ruminants (Jouany et al., 1995). Some experiments can also be conducted *in vitro*. However, an experience in isolation and cultivation of rumen ciliates is necessary to perform such a study. An useful method to examine the role some groups of ciliates in digestive processes in the rumen colonized by natural protozoal fauna seems to be the *in sacco* technique as it provides the possibility to select the ciliate species penetrating the incubated material by manipulation the pore size of the bags. Taking this as starting point we undertook the experiment presented in this report.

Objective of the study was to determine the disappearance of dry matter (DM), ADF and NDF from hay incubated *in sacco* in the rumen of sheep in relation to the number and proportion of ciliate species penetrating the incubation bags.

MATERIAL AND METHODS

Animals and feed

Three male Polish Merino wethers 2 year old and weighing 73, 76 and 85 kg were used. The animals were fitted with permanent rumen fistulae of about 10 cm in diameter and were kept in separate pens without contact *per os* to any others. Diet composed of hay (750 g) and ground barley (130 g) given to animals every 12 h. The water was available all the day. The animals were fistulated by about 2 months before experiment started.

Nylon bags

Nylon bags of two different pore size were used. The diameter of small pores was 42 while this of large 206 μm. The bags measured 16 cm in length and 9 cm in width. A dacron material was used for preparation of the both kinds of bags. It was supplied by Surtex (Poland).

Hay samples

Well dried meadow hay was chopped by hand for the particles up to 1 cm in length and this was followed by a screening through a screen of pore size of 1 mm and the particles which did not pass through the screen were collected and stored at room temperature. Hay samples of 3.0 g of air dry mass were weighed, put into the bags and inserted into the rumen.

Experimental design

The study consisting of four experimental periods:

Period 1: defaunated sheep (free of protozoa)

Period 2: sheep refaunated with only one species of ciliates (*Eudiplodinium maggii*)

Period 3: sheep refaunated with two species of ciliates (*Eudiplodinium maggii* and *Entodinium caudatum*)

Period 4: sheep refaunated with three species of ciliates (*Eudiplodinium maggii*, *Entodinium caudatum* and *Dasytricha ruminantium*).

Disappearance of dry matter and fibrous components following the *in sacco* incubation of the hay samples in the rumen was examined in each period. Four bags of the small and four of large pore size were incubated simultaneously in the rumen. Each set of bags was connected to a separate weight (165 g) and put by hand into ventral sack in the rumen. The bags were given into the rumen just before the morning feeding of sheep and incubated for either 12, 24 or 48 h. The bags from one set were incubated for the same time period.

The sacs were withdrawn from the rumen at the end of incubation period and treated on different way. Two of them were rinsed in a washing machine and hay residues were used for chemical analysis. Hay residues from the third bag were rinsed by hand on a screen using a stream of tap water and used for measurement of activity of the particle associated enzymes. Hay samples from the last bag were used for protozoa counting.

The program applied to rinse the bags with tap water in the washing machine consisted of two rinse cycles, while each cycle lasted for 17 min. The rinsed bags were dried for 48 h at 40 °C and allowed to remain at room temperature for the next 2 days. Air dry weight of hay residues was then determined. The weighed material was given to the plastic containers and stored at ambient temperature till the use for chemical analysis.

A screen of pore diameter of 0.5 mm was used to rinse hay residues under a continuous stream of tap water. Hay residues were rinsed for 3 min and stored at -30°(Microscopic examination of hay residues showed no protozoa in the examined material. They were performed immediately after the rinsing).

To determine the ciliate number penetrating the bags in the rumen of refaunated sheep the samples of hay from the bags were fixed with 4% formaldehyde solution (1:1 w/v) and stored at room temperature before counting. Samples of rumen content were fixed by the same method and used as control.

Each experiment started not earlier than 3 week after defaunation or development of population of the ciliate species successively inoculated into the rumen.

Sheep defaunation and refaunation

The sheep were defaunated by evacuation of the rumen and reticulum contents and washing the rumen, reticulum and omasum. The protozoa in the evacuated digesta were killed by heating up to 70°C and this was followed by a treatment of digesta with Aerosol OT in the proportion of 1 g/kg digesta. The procedure was described in detail by Michałowski et al. (1999).

The ciliates *Eudiplodinium maggii* and *Entodinium caudatum* were isolated from the rumen fluid of other animals as described by Michałowski et al. (1991) and cultured as one species population using a continuous culture system (Michałowski, 1979). The cultured protozoa were then used to refaunate the sheep. Ciliates *Dasytricha ruminantium* were isolated from the rumen fluid of cow and introduced directly to the rumen of sheep used in this study.

Chemical analysis

ADF and NDF were determined quantitatively according to AOAC (1990) procedure. Dry matter (DM) of hay residues in nylon bags were determined following drying of the samples at 105°C for 48 h. Similar determination were performed in the samples of hay following similar rinsing as described above.

CMC-ase and xylanase activities associated with pariculate matter were determined following extraction of enzymes with an use of carbon tetrachloride. The extracted enzymes were incubated with carboxymethylcellulose of low viscosity (Sigma, No. C-5678) and xylan from birch wood (Sigma, No. X-0502) for 1h at 40°C in a 0.02 M sodium phosphate buffer, according to Groleau and Forsberg (1981) and Huhtanen and Khalili (1992). The reducing sugars released from appropriate substrates were measured using dinitrosalycilic acid reagent (Miller et al., 1960), while glucose and xylose were used as standards. Enzyme activities were expressed as μmol glucose or xylose released/g DM/min.

Ciliate counting

To estimate of the ciliate number, the fixed samples of both the rumen and bag contents were analyzed not less than three times. All ciliates present in the sample of 0.1 ml in volume were counted using light microscope according to Michalowski (1975) while the fixed material was diluted if necessary. Ciliates were identified according to Dogiel (1927) and Grain (1966).

Statistical analysis

Student's "t" test was used to compare the mean values (Ruszczyc, 1970).

RESULTS

Characteristics of the microbial populations

The sheep were ciliate-free during the first period of the study and this was followed by the successive inoculation of *Eudiplodinium maggii, Entodinium caudatum* and *Dasytricha ruminantium* into the rumen of sheep in the consecutive periods of experiment, respectively. Large day to day and animal to animal variations in the numbers of ciliates were observed. The population densities of *E. caudatum*, *Eud. maggii* and *D. ruminantium* varied in the range of 14.7-102.5, 0.03-4.4 and 1.5-11.6 x 10^4/g rumen digesta, respectively. Mean values are given in Table 1. It was found that the appearance of *Entodinium caudatum* in the rumen of sheep (period III) resulted in a decrease in the population density of *Eudiplodinium maggii* already existing there since the beginning of the period II ($P<0.01$). On the other hand the establishment of *Dasytricha ruminantium* (period IV) was accompanied by the increase in the number of *Eudiplodinium maggii*, while *Entodinium caudatum* did not change significantly ($P>0.05$)

TABLE 1

Numbers of ciliates in the rumen contents (x10^4/g) of sheep in particular periods of experiment. Mean values; n = 27

Ciliates	Experimental periods				
	I	II	III	IV	SD
Eud. maggii	0.0	2.3^a	1.4^b	1.9^{ab}	1.06
E. caudatum	0.0	0.0	52.4^a	55.5^a	20.62
D. ruminantium	0.0	0.0	0.0	6.1	2.65

values in the row with different letters differ significantly at $P<0.05$

Large variations were also observed in the number of protozoa penetrating the bags. Mean values are given in Table 2. It was found that the number of *Eudiplodinium maggii* harboring the bags of 206 μm pore size was by 6-9 times higher than that of pore diameter 42 μm. Passage of *Entodinium caudatum* and *Dasytricha ruminantium* from the rumen digesta into the bags was not influenced by the porosity of bags ($P>0.05$).

Population density of the hay colonizing fibrolytic bacteria was determined by the measurement of activity of the particle associated enzymes according to Silva et al. (1987) and Huhtanen and Khalili (1992). The activity of CMC-ase associated with hay particles, which were incubated *in sacco* and then rinsed on a screen to eliminate the protozoa and not attached bacteria, is presented in Table 3. Faunation of the ciliate free sheep with *Eudiplodinium maggii* resulted in an increase in the activity of this enzyme independently of time of incubation and porosity of the bag wall ($P<0.01$). No increase was only found following incubation of hay for 24 h in the bags of 206 μm pore diameter ($P>0.05$). Establishment of the second species

in the rumen, i.e. *Entodinium caudatum* was not accompanied by further changes in CMC-ase activity with, however, an exception for hay incubated for 12 h in the bags with the small pores. In this case a significant decrease was observed ($P<0.01$). Appearance of the third species in the rumen, i.e. *Dasytricha ruminantium* was followed by a significant decrease in CMC-ase activity ($P<0.05$).

TABLE 2

Numbers of ciliates ($x10^4$/g) inside the bags in relation to the porosity of the bag wall (A, B) and time of incubation (h). Mean values; n = 9

Experimental period No.	Ciliates	Time of incubation					
		12		24		48	
		A	B	A	B	A	B
I	Protozoa	0	0	0	0	0	0
	E. caudatum	0	0	0	0	0	0
II	*Eud. maggii*	0.2	1.5**	0.4	1.9***	0.3	2.6***
	D. ruminantium	0	0	0	0	0	0
	E. caudatum	29.4	30.6	42.2	57.0	43.7	46.2
III	*Eud. maggii*	0.1	0.6**	0.2	1.2**	0.2	1.9***
	D. ruminantium	0	0	0	0	0	0
	E. caudatum	44.5	42.3	45.5	46.9	46.3	50.4
IV	*Eud. maggii*	0.2	0.7**	0.2	1.2***	0.4	1.3*
	D. ruminantium	2.8	2.0	5.3	5.0	4.1	4.6

A - pore diameter 42 μm, B - pore diameter 206 μm, * $P<0.05$, ** $P<0.001$, *** $P<0.0001$

TABLE 3

The activity of particle–associated CMC-ase (μM glucose/g DM/min) extracted from residues of hay incubated *in sacco* in the rumen in relation to the time of incubation (h) and pore diameter (μm) of bags. Mean values; n = 9

Pore diameter	Incubation time	Experimental periods				SD
		I	II	III	IV	
42	12	2.4^a	8.1^b	4.7^c	2.0^a	1.66
	24	3.4^a	6.1^b	7.8^b	2.9^a	1.21
	48	2.0^a	3.2^b	3.3^b	2.3^a	0.39
206	12	2.2^a	7.4^b	6.2^b	2.2^a	1.57
	24	4.0^a	5.6^{ab}	6.1^b	2.8^c	0.90
	48	1.6^a	4.4^b	4.2^b	2.3^a	1.01

values in the row with different letters differ significantly at $P<0.05$

TABLE 4

The activity of particle–associated xylanase (μM xylose/g DM/min) extracted from residues of hay incubated *in sacco* in the rumen in relation to the time of incubation (h) and pore diameter (μm) of bags. Mean values; n = 9

Pore diameter	Incubation time	Experimental periods				SD
		I	II	III	IV	
42	12	12.9[a]	51.8[b]	35.0[b]	10.1[a]	14.88
	24	22.8[a]	42.5[b]	50.7[b]	20.8[a]	9.81
	48	21.5[a]	26.1[a]	44.1[b]	23.8[a]	7.47
206	12	15.2[a]	46.6[b]	38.4[b]	12.2[a]	9.61
	24	30.5[a]	41.5[ab]	54.7[b]	20.6[c]	8.40
	48	20.3[a]	33.1[ab]	50.8[b]	23.7[a]	9.41

values in the row with different letters differ significantly at $P<0.05$

Changes in CMC-ase activity with the time of incubation were also found. The highest activity was found either after 12 (period II) or 24 h (periods I, III and IV) of incubation and this was followed by a decrease ($P<0.05$). No effect of pore size on the activity of CMC-ase was found ($P>0.05$).

The activity of xylanase associated with hay particles incubated in the bags is presented in Table 4. The relationships between enzyme activity, time incubation as well was pore size and microfauna composition were similar to those described for CMC-ase.

Characterization of hay digestion

Hay samples were 3 g of air dry weight and contained 2.70 g DM, 1.02 g ADF and 1.72 g NDF on average. The results describing loss of dry matter during the *in sacco* incubation of hay in the rumen of sheep are presented in Table 5. The loss of DM varied from 38.2 to 75% of initial value in relation to the time of incubation and composition of microbial population. The largest part of DM disappeared during the first 12 h of incubation (38.2-46.1%) while the smallest during the second day (7.4-9.9%). Appearance of the consecutive populations of ciliates in the rumen was accompanied by a successive decrease in the loss of DM from the hay samples. The most abundant decrease was observed after appearance of the population of *Dasytricha ruminantium* (period IV). No effect was only found when ciliates *Eudiplodinium maggii* were present as a sole protozoal population in the rumen of sheep (period II) and the hay samples were incubated not longer than 12 h ($P>0.05$). Porosity of the bags and thus the number of *Eudiplodinium maggii* penetrating the incubated samples of hay did not affect disappearance of DM ($P>0.05$).

The quantities of ADF which disappeared from hay following it's incubation *in sacco* in the rumen is presented in Table 6. The loss of ADF varied from 18.4 to

TABLE 5

Disappearance (mg) of dry matter from hay incubated *in sacco* in the rumen of defaunated (period I) and differently refaunated sheep (periods II-IV) in relation to the time of incubation (h) and pore diameter (μm) of bags. Mean values; n = 9

Incubation time	Pore diameter	Experimental periods				SD
		I	II	III	IV	
12	42	1152^{a}	1166^{a}	1130^{a}	1078^{b}	62.0
	206	1245^{a}	1178^{ab}	1145^{b}	1030^{c}	93.5
24	42	1728^{a}	1657^{b}	1626^{bc}	1613^{c}	66.5
	206	1758^{a}	1664^{b}	1657^{b}	1613^{b}	72.4
48	42	1997^{a}	1931^{bc}	1953^{ab}	1876^{c}	63.5
	206	2024^{a}	1941^{b}	1920^{b}	1812^{c}	86.0

values in the row with different letters differ significantly at $P<0.05$

TABLE 6

Disappearance (mg) of ADF from hay incubated *in sacco* in the rumen of defaunated (period I) and differently refaunated sheep (periods II-IV) in relation to the time of incubation (h) and pore diameter (μm) of bags. Mean values; n = 9

Incubation time	Pore diameter	Experimental periods				SD
		I	II	III	IV	
12	42	240^{a}	358^{b}	318^{c}	228^{a}	69.9
	206	258^{a}	352^{b}	330^{b}	220^{c}	69.4
24	42	533^{a}	563^{b}	544^{b}	456^{c}	50.8
	206	530^{a}	543^{a}	550^{a}	442^{b}	51.5
48	42	679^{a}	680^{a}	692^{a}	689^{a}	25.4
	206	682^{a}	661^{ab}	642^{b}	655^{ab}	34.4

values in the row with different letters differ significantly at $P<0.05$

57.6% of the initial amount in relation to the time of incubation and experimental period. Nearly 37-47% of ADF disappeared from hay samples during the first 24 h of incubation and only 11-17% during the second. Establishment of population *Eudiplodinium maggii* in the rumen of defaunated sheep was accompanied by significant increase in the digestion of ADF during the first 12 h of incubation ($P<0.01$). A similar effect was observed after incubation of hay for 24 h in the bags with the small pores. Establishment of the second population of ciliates, i.e. *Entodinium caudatum* (period III) did not change the described relationships while appearance of *Dasytricha ruminantium* was accompanied by significant reduction in disappearance of ADF from hay incubated for both 12 and 24 h independently of pore size. No differences in the quantities of ADF which disappeared from hay were practically observed after 48 h of incubation. However, loss of this component

of fibre observed after establishment of both entodiniomorphid species (period III) was significantly lesser than in absence of ciliates (period I). It was found that disappearance of ADF from the bags of 42 μm pore diameter was significantly greater than from these of 206 μm in the diameter ($P<0.05$) when incubation was continued for 48 h and the both *Eudiplodinium maggii* and *Entodinium caudatum* were present in the rumen. No effect of the number of *Eudiplodinium maggii* inside the bags on ADF digestion was observed.

Data concerning the quantities of NDF which disappeared from hay samples incubated *in sacco* in the rumen of sheep are given in Table 7. The loss of NDF reached about 21-69% of it's level in hay before the experiment was started and was related to the time of incubation and microfauna composition. Establishment of ciliates in the rumen of defaunated sheep was accompanied by a decrease in the disappearance of this component of fibre from hay samples. No effect was only observed in the presence of *Eudiplodinium maggii* and *Entodinium caudatum* (periods II and III) when the samples were incubated in the rumen for 12 h. Presence of *Dasytricha ruminantium* in the rumen (period IV) diminished the loss of NDF when hay samples were incubated either for 12 or 24 h. The restricting effect was, however, the largest after 12 h of incubation ($P<0.001$). No effect of the microfauna composition was observed at the longest time of incubation ($P>0.05$). Pore size and thus the numbers of *Eudiplodinium maggii* inside the bags did not affect the quantity of disappearing NDF ($P>0.05$).

TABLE 7

Disappearance (mg) of NDF from hay incubated *in sacco* in the rumen of defaunated (period I) and differently refaunated sheep (periods II-IV) in relation to the time of incubation (h) and pore diameter (μm) of bags. Mean values; n = 9

Incubation time	Pore diameter	Experimental periods I	II	III	IV	SD
12	42	492[a]	520[a]	514[a]	358[b]	85.6
	206	547[a]	497[a]	508[a]	372[b]	88.5
24	42	981[a]	914[b]	885[b]	743[c]	91.0
	206	957[a]	900[b]	924[a]	755[c]	88.3
48	42	1186[a]	1124[b]	1140[b]	1121[b]	44.4
	206	1181[a]	1107[b]	1108[b]	1066[b]	62.3

values in the row with different letters differ significantly at $P<0.05$

DISCUSSION

According to Dogiel (1927) and Williams and Coleman (1992) cell length and width of *Eudiplodnium maggii* varied between 115-212 and 73-143, of *Entodinium caudatum* between 48-74 and 34-56 and of *Dasytricha ruminantium* 35-75 and 20-40 μm, respectively. Thus, to make possible the passage from the rumen contents into the incubated hay samples either for all three ciliate species or for only the small protozoa there were chosen the bags of either pore diameter of 206 or 42 μm. In fact, however, small pores reduced only the number of *Eudiplodinium maggii* which were passing through (Table 1). To explain this not expected findings the measurements of ciliates *Eudiplodinium maggii* from the rumen of experimental sheep were done additionally. Cell length and width of protozoa were 116.0 ± 11.38 and 82.4 ± 10.05 μm, respectively. The results of measurements show that dimensions of *Eudiplodinium maggii* harboring the sheep's rumen were visibly smaller than of those given by Dogiel (1927). The width of their cells was, however, two times larger than the diameter of small pores. Thus, the abundant elasticity of the nylon material used for preparation of bags, and resulting form this a modification in pore dimensions could, perhaps, be used for explanation of this findings.

As described in the section of "Material and Methods," the sheep were either ciliate-free (period I) or faunated with only fibrolytic *Eudiplodinium maggii* (period II), or *Eudiplodinium maggii* and starch preferring *Entodinium caudatum* (period III) or both ophryoscolecids and the soluble sugars utilizing *Dasytricha ruminantium*. Results obtained showed that the establishment of population *Eudiplodinium maggii* in the rumen of defaunated sheep enhanced disappearance of ADF from hay incubated *in sacco* for 12 and 24 h. Contrary to that loss in dry matter and NDF were reduced except after the shortest time of incubation. Appearance of population of the second species, i.e. *Entodinium caudatum* was accompanied by the significant diminishing or tendency to the lowering in the loss of ADF after 12 h of incubation comparing the proper period. Disappearance of NDF was reduced only after incubation for 48 h while loss of DM was related to the time of incubation and pore size. Finally the appearance of the representative of holotrich ciliates, i.e. *Dasytricha ruminantium* in the rumen of sheep being already faunated with *Eudiplodinium maggii* and *Entodinium caudatum* resulted in a visible decrease in the loss of dry matter and both components of fibre from the bags to the lowest level observed during the all experiment. No data describing the influence of the species used in this study on DM and fibre digestion *in situ* or *in sacco* were found in available literature. Jouany et al. (1995) have, however, found that refaunation of the defaunated rumen either with *Isotricha prostoma* or type B microfauna increased digestion of DM and ADF *in sacco* while NDF was not affected. Contrary to that Ushida et al. (1991) presented results showing that the ciliates from the family *Ophryoscolecidse* were more effective in the stimulating of NDF than

ADF digestion. A negative effect of holotrich protozoa on fibre digestion was also discussed there. This last finding is in good accordance with the results presented in this report when effect of *Dasytricha ruminantium* is considered.

It was expected that an improvement in hay digestion inside the bags of large pore size might result from increased number of *Eudiplodinium maggii* exhibiting fibrolytic activity (Dehority, 1993; Michałowski, 1997). In fact, a visible improvement was found in relation to the disappearance of dry matter after 12 h of incubation, unfortunately it was observed in the absence of ciliates (Table 5). In general, no relationship was found between the number of *Eudiplodinium maggii* inside the bags and the loss of the examined components of fibre. One of possible explanation for these findings could be a presence of ground barley in the diet. Due to this the protozoa engulfed readily starch grains before they penetrated the bags and did not ingest fibrous material thereafter. Of importance seems to also be the fact that rumen ciliates digest food inside their cells (Williams and Coleman, 1992) and hay particles incubated *in sacco* were, perhaps, to large to be ingested by protozoa. On the other hand a flow of particulate matter into the bags of large pore size was observed by Van Hellen and Ellis (1977) and this phenomenon, if existed in this study, could mask the positive effect of protozoa. Contrary to that, however, Nocek (1985) found that an increase in the porosity of bags was accompanied by increase in DM digestion. Thus, this finding seems to be more complex and needs further investigation.

Entodinium caudatum satisfies its carbohydrate requirements with starch (Coleman, 1980; Wiliams, 1988), while *Dasytricha ruminantium* utilize only soluble sugars (Van Hoven and Prins, 1977; Williams and Coleman, 1997). Ciliates from the both species were able to easily pass from the rumen content to the bags. They did not participate in the digestion of hay (Wiliams, 1988) due to inability to elaborate the fibrolytic enzymes. Both of them were, however, able to influence this process affecting the colonization of hay particles by fibrolytic bacteria. The extent of a such colonization can be expressed as the activity of particle associated CMC-ase and xylanase (Silva et al., 1987; Huhtanen and Khalili, 1992). Obtained results suggest that *Entodinium caudatum* supported while *Dasytricha ruminantium* restricted the colonization of hay particles during incubation *in sacco* in the rumen (Tables 3 and 4). The effect of *Eudiplodinium maggii* on the colonization of hay particles by bacteria inside the bags was similar to that of *Entodinium caudatum*. Thus, the increment in ADF disappearance observed after establishment of either *Eudiplodinium maggii* (period II) or *Eudiplodinium maggii* and *Entodinium caudatum* (period III) in the rumen resulted presumably from more dense colonization of hay in the bags by bacteria or/and higher fibrolytic activity of this microflora. Conversely, *Dasytricha ruminantium* reduced colonization of hay and this resulted in decrease in fibre digestion inside the bags during the last period of experiment. This explanation is in good accordance with the opinion of

Ushida et al. (1991) concerning the mechanisms by which *Entodinia* and holotrich protozoa influence the fibre digestion in the rumen. It is also noteworthy to say that the sporangia of rumen fungi were only rarely observed. This suggested that an involvement of these organisms in feed digestion in the rumen of the experimental sheep was rather poor.

Disappearance of fibre components from the bags changed with time and was distinctly lower during the second day of incubation than during the first one. Similar changes were observed in the activities of the particle associated enzymes. These last findings show that colonization of hay inside the bags by fibrolytic bacteria diminished during the second day of incubation in the rumen and this was accompanied by reduction in the digestion intensity of examined components of hay. Contrary to the bacteria no such relationship was concerning the ciliates. This supports a suggestion that bacteria were mainly responsible for the digestion of dry matter and fibre components in this experiment whereas ciliates introduced into the rumen affected this process rather indirectly influencing, perhaps, the colonization of hay particles by fibrolytic microflora. On the other hand, however, an increase in the colonization of hay observed in the second and third period of experiment when *Eudiplodinium maggii* alone or together with *Entodinium caudatum* were present in the rumen was accompanied by increase in digestion neither DM nor NDF. Thus some other factor should also be taken into account when digestion of a fibrous feed *in situ* is considered.

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STRESZCZENIE

Zastosowanie woreczków nylonowych o różnej wielkości porów w badaniach roli różnych grup orzęsków żwaczowych w trawieniu *in situ* siana u owiec

Doświadczenie przeprowadzono na trzech trykach rasy merynos polski z trwałymi przetokami żwacza. Zwierzęta otrzymywały dawkę złożoną z siana (750 g) i śruty jęczmiennej (130 g), podawanej co 12 godzin. Owce były pozbawione orzęsków (zdefaunowane) lub refaunowane jedynie *Eudiplodinium maggii* lub *Eudiplodinium maggii* i *Entodinium caudatum* oraz obydwoma gatunkami *Entodiniomorhida* i *Dasytricha ruminantium*. Mierzono ubytki suchej masy, ADF i NDF z próbek siana inkubowanego *in sacco* w ciągu 12, 24 i 48 godzin w żwaczu. Średnica porów w woreczkach wynosiła 42 lub 206 mikrometrów. Liczebność *Entodinium caudatum*, *Eudiplodinium maggii* i *Dasytricha ruminantium* w treści żwacza wynosiła 52,4-55,5, 1,4-2,3 i 6,1 x 10^4/g, odpowiednio. Gęstość populacji *Entodinium caudatum* i *Dasytricha ruminantium* w treści woreczków nie zależała od wielkości porów i wahała się od 29,4 do 57,0 i od 2,0 do 5,3 x 10^4/g, odpowiednio. Liczebność orzęsków *Eudiplodinium maggii* w woreczkach o średnicy porów 42 i 206 mikrometrów wynosiła 0,1-0,4 lub 0,6-2,6 x 10^4/g. Kolonizacja cząstek siana w woreczkach przez fibrolityczne bakterie była mierzona jako aktywność związanej z cząstkami stałymi CMC-azy i ksylanazy. Aktywności te wahały się od 1,6 do 8,1 i od 10,1 do 51,8 μM cukrów redukujących/g s.m./min. Ustalenie się populacji *Eudiplodinium maggii*, jako jedynej populacji orzęsków lub w koegzystencji z *Entodinium caudatum*, stymulowało kolonizację cząstek siana przez bakterie, natomiast pojawienie się *Dasytricha ruminantium* miało skutek odwrotny. Ubytki s.m., ADF i NDF z siana inkubowanego *in sacco* wahały się od 38,2 do 75,0; 18,4-57,6 i 20,8-68,9% w zależności od czasu inkubacji i składu mikrofauny. Pojawienie się *Eudiplodinium maggii*, jako jedynego gatunku orzęsków w żwaczu, zwiększało ubytki ADF po 12 i 24 godzinach inkubacji. Ubytki s.m. i NDF nie zmieniły się lub ulegały redukcji. Mikrofauna złożona z *Eudiplodinium maggii* i *Entodinium caudatum* zwiększała ubytki ADF po 12 godzinach inkubacji, lecz w mniejszym stopniu niż samo *Eudiplodinium maggii*. Ubytki s.m. i NDF nie zmieniły się lub ulegały redukcji, w zależności od czasu inkubacji i wielkości porów woreczków. Mikrofauna złożona z obydwóch przedstawicieli *Entodiniomorphida* i *Dasytricha ruminantium* zmniejszała ubytki badanych składników, niezależnie od czasu inkubacj, a różnice te uwydatniały się najwyraźniej po 12 godzinach inkubacji. Zwiększenie średnicy porów zwiększało liczebność *Eudiplodinium maggii* penetrujących woreczki, ale nie miało wpływu na wielkość ubytków s.m., ADF i NDF z siana inkubowanego *in sacco*.