

## Effect of the microfauna composition on fermentation pattern in the rumen of sheep\*

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### ABSTRACT

Three Polish Merino sheep fed hay and ground barley were defaunated and then refaunated consecutively with only one (*Eudiplodinium maggii*), two (*Eudiplodinium maggii* and *Entodinium caudatum*) or three (*Eudiplodinium maggii*, *Entodinium caudatum* and *Dasytricha ruminantium*) ciliate species. Both the concentration and production rate of total VFA as well as the proportion of individual acids were estimated before the morning feeding and 4 h thereafter. The concentration of VFA varied from 8.2 to 11.6 mM/100 ml of rumen fluid and production rate from 29.1 to 34.1  $\mu$ M/g rumen digesta/h in relation to the time after feeding.

Molar proportion of acetate, propionate and butyrate in the total VFA in the rumen or produced *in vitro* was 65.1-74.6, 16.0-21.2 and 9.1-11.8 %, respectively. The concentration of VFA as well as production rate and molar proportion of individual acids were influenced by ciliates and composition of the fauna. Presence of *Eudiplodinium maggii* in the rumen resulted in the highest proportion of butyrate at 4 h after feeding. Establishment of ciliates in the rumen of sheep was accompanied by the drop in acetate proportions.

No effect of ciliates on pH values before feeding was found whereas increase in the acidity of rumen fluid after feeding was affected by the fauna composition.

KEY WORDS: ciliates, sheep, VFA production, molar proportion

### INTRODUCTION

Ruminant animals utilize volatile fatty acids (VFA) as the main source of energy in cellular metabolism. VFA are released from the cells of ruminal bacteria, fungi and protozoa as end products of their carbohydrate metabolism. They are

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intensively absorbed from the rumen and transported with the blood to the tissues and cells of the host. Investigations on pure species of rumen microbiota have shown large diversity in fatty acids formation (Stewart and Bryant, 1988; Williams and Coleman, 1997) but acetic, propionic and butyric acid were found to be the main acids in the rumen fluid at variety of feeding regimes (Owens and Goetsch, 1988).

Rumen ciliates play an important part in VFA production in the rumen (Michałowski, 1987) but carbohydrate metabolism of these organisms differ significantly among the species (Williams and Coleman, 1997). This suggest that microfauna composition could influence the fermentation pattern in the rumen, however more detailed information on the role of individual species is still lacking (Ivan et al., 2000)

The aim of this study was to examine the concentration and molar proportion of particular acids following refaunation of the defaunated sheep with selected species of ciliates as well as to estimate the production rate of VFA in relation to microfauna composition and time after feeding.

## MATERIAL AND METHODS

Three growing male Polish Merino sheep weighing 40-43 kg at the beginning of the experiment were used. The animals were fitted with permanent rumen fistulae of about 10 cm in diameter. They were kept in separate pens with the solid walls and fed 750 g hay and 130 g ground barley every 12 h and had free access to water. The sheep were defaunated by the methods of Michałowski et al. (1999) for at least 40 days before the experiment has begun.

The study composed of four experimental periods. The sheep were either ciliate-free (Period 1) or refaunated with *Eudiplodinium maggii* alone (Period 2), with the latter ciliate species and *Entodinium caudatum* (Period 3) or with the both mentioned species plus *Dasytricha ruminantium* (Period 4). Both species from the family *Ophryoscolecidae* originated from *in vitro* cultures. *Dasytricha ruminantium* ciliates were picked from the rumen fluid of cow and introduced immediately to the rumen of sheep.

Rumen fluid for pH and VFA concentration measurements was sampled just before the morning feeding (8 am) and 4 h thereafter. The samples of rumen digesta (about 200 g) were taken at the same time points to determined the production rate of VFA and the protozoal numbers. The sampling began not earlier than 3 weeks following the sheep defaunation (Period 1) or the establishment of each protozoal population (Periods 2, 3 and 4). The sampling was repeated three times in the case of each animal on three different days of experimental period. The samples for VFA measurements were fixed with formic acid (10 :1 v/v) and centri-

fuged at 20000 g for 30 min. Volatile fatty acids were determined qualitatively and quantitatively by gas chromatography methods according to Ziotecki and Kwiatkowska (1973) in the supernatant fraction of the centrifuged samples. The number of ciliates was determined microscopically according to Michalowski (1975) using the rumen digesta fixed with 4% formalin solution (1:1 w/v). The rumen fluid reaction was estimated using Beckman 390 pH-meter. Production rate of VFA was estimated by the modified method of Carrol and Hungate (1954). For this purpose the amples of rumen digesta (20 g) taken from the rumen and immediately diluted with 50 ml of a warm (40°C) buffer composed of (mg/100 ml):  $(\text{NH}_4)_2\text{SO}_4$  - 45;  $\text{KH}_2\text{PO}_4$  - 45;  $\text{K}_2\text{HPO}_4$  - 45;  $\text{NaCl}$  - 90;  $\text{MgSO}_4$  - 9;  $\text{NaHCO}_3$  - 750; cysteine hydrochloride - 25; casitone - 100; yeast extract -100; thioglicolic acid - 0.03 and sterilized rumen fluid - 30 ml. The samples were incubated anaerobically for 2 h at 40°C at a continuous mixing with a  $\text{CO}_2$  stream (60 ml/min). Concentration of VFA was measured just before start of incubation and then every 30 min. Sample fixation and fatty acid determination methods were the same as described above. Production rate was calculated from the increase in the acid concentrations and expressed as  $\mu\text{M}$  acids/g rumen digesta/h. Mean values were calculated from the obtained results whereas Students' t test was used to estimate the significance of differences between mean values.

## RESULTS AND DISCUSSION

The animals were ciliate-free during the first experimental period (Table 1). Rumen ciliate fauna composed of only *Eudiplodinium maggii* during the second period and of *Eudiplodinium maggii* and *Entodinium caudatum* during the third. *Dasytricha ruminantium* and two latter species represented the protozoa during the last period of experiments. Of the species established in the rumen of sheep the first belongs to the group of strongly fibrolytic organisms whereas the second is starch preferring (Coleman, 1986; Dehority, 1993; Michałowski, 1997). In oppo-

TABLE 1

Ciliate numbers in consecutive periods of experiments ( $\times 10^3/\text{g}$  rumen content)

Item	Experimental period			
	1	2	3	4
<i>Eudiplodinium maggii</i>	0.0	22.8 ± 9.9	11.8 ± 4.0	10.8 ± 4.4
<i>Entodinium caudatum</i>	0.0	0.0	268.7 ± 66.1	285.0 ± 52.4
<i>Dasytricha ruminantium</i>	0.0	0.0	0.0	53.4 ± 31.1
Other ciliates	not present	not present	not present	not present

± standard deviation (n=9)

site to the both ophryoscolecids *Dasytricha ruminantium* utilize only soluble carbohydrates (Van Hoven and Prins, 1977). Apart from difference in nutritional behaviour the species inoculated subsequently into the rumen of sheep differ significantly when carbohydrate fermentation pattern is taken into account (Williams and Coleman, 1997)

To study the changes in fermentation pattern the ciliates *Eudiplodinium maggii*, *Entodinium caudatum* and *Dasytricha ruminantium* were consecutively established in the rumen of each sheep and the total VFA concentration as well as production rate and the proportion of individual acids were examined. It was found that the concentration of VFA and molar proportion of individual acids was influenced by the time after feeding and composition of the ciliate fauna established in the rumen. The concentration of total VFA in defaunated animals increased significantly after feeding while the proportions of the acids did not change significantly (Table 2). In opposite to that the increase in the concentration of volatile fatty acids in refaunated animals was always accompanied by the decrease in molar proportion of acetate and by increase in this of propionate. A tendency to increase in the proportion of butyrate was also observed there but the statistically significant change was only noted when *Eudiplodinium maggii* was present in the rumen as the only ciliate species (Period 2). In other study the proportions of acetate and butyrate but not total VFA were influenced by the fauna composition (Ivan et al., 2000). Rumen fluid reaction before feeding was not affected by the presence of ciliates. However, the drop in pH value at 4 h after feeding was the smallest when only *Eudiplodinium maggii* was present in the rumen (Table 2).

Production rate of VFA varied between 29 and 34  $\mu\text{M/g}$  rumen digesta/h. It tended to increase at 4 h after feeding but statistically significant change was

TABLE 2  
Total concentration (mmol/100 ml) and molar proportions (%) of particular acids in the rumen of defaunated and differently refaunated sheep just before (0 h) and 4 h after feeding (n=9)

Item	Ciliate-free		<i>Eud. maggii</i>		<i>Eud. Maggii</i> <i>Ent. caudatum</i>		<i>Eud. maggii</i> , <i>Ent. caud.</i> <i>D. ruminantium</i>	
	0h	4h	0h	4h	0h	4h	0h	4h
Total VFA	8.3 <sup>a</sup>	10.4 <sup>a***</sup>	7.6 <sup>a</sup>	10.0 <sup>a***</sup>	10.0 <sup>b</sup>	11.6 <sup>b**</sup>	8.2 <sup>a</sup>	9.6 <sup>a**</sup>
Acetate	68.9 <sup>a</sup>	67.1 <sup>a</sup>	71.9 <sup>ab</sup>	65.1 <sup>a**</sup>	71.8 <sup>a</sup>	68.9 <sup>a***</sup>	73.4 <sup>b</sup>	71.3 <sup>a*</sup>
Propionate	19.9 <sup>a</sup>	21.2 <sup>a</sup>	17.6 <sup>ab</sup>	21.1 <sup>ab**</sup>	17.5 <sup>ab</sup>	19.9 <sup>ab**</sup>	17.4 <sup>ab</sup>	18.9 <sup>b</sup>
Butyrate	11.2 <sup>a</sup>	11.7 <sup>a</sup>	10.5 <sup>ab</sup>	13.8 <sup>b***</sup>	10.7 <sup>a</sup>	11.2 <sup>a</sup>	9.2 <sup>b</sup>	9.8 <sup>ac</sup>
pH	6.86 <sup>a</sup>	6.19 <sup>a</sup>	6.85 <sup>a</sup>	6.59 <sup>b</sup>	6.78 <sup>a</sup>	6.15 <sup>a</sup>	6.89 <sup>a</sup>	6.23 <sup>a</sup>

values in a row with different letters (normal or bold, respectively) differ significantly  
significance of difference between the values of the same experimental period is marked by asterisk  
\* P<0.05 \*\* P<0.01 \*\*\* P<0.001

found only following development of *Eudiplodinium maggii* in the rumen of defaunated sheep (Table 3). The proportion of acetate produced tended to decrease after feeding. No significant differences were, however, found there. Proportion of propionate increased in defaunated and refaunated with *Eudiplodinium maggii* and *Entodinium caudatum* sheep. An increase by about 29% in the proportion of butyrate was found following the development of *Eudiplodinium maggii* in the rumen of defaunated sheep. This finding correlate well with high proportion of butyrate produced by pure cultures of this protozoan *in vitro* in absence of bacteria (Michałowski, 1997). Similar changes in proportion of butyrate in VFA produced by the microfauna composed of three species of ciliates were also found and they were accompanied by a decrease in acetate proportion. This last relationship seems to be the result of development of population of the ciliate species inoculated in the rumen.

TABLE 3

Production rate of total VFA (µM/g rumen digesta/h) and molar proportion of particular acids (%) produced during *in vitro* incubation of digesta taken just before (0 h) or 4 h thereafter from the rumen of defaunated and differently refaunated sheep (n=9)

Acids	Ciliate-free		<i>Eud. maggii</i>		<i>Eud. Maggii</i> <i>Ent. caudatum</i>		<i>Eud. maggii</i> , <i>Ent. caud.</i> <i>D. ruminantium</i>	
	0h	4h	0h	4h	0h	4h	0h	4h
Total VFA	31.8 <sup>a</sup>	33.4 <sup>a</sup>	30.4 <sup>a</sup>	34.1 <sup>a*</sup>	29.1 <sup>a</sup>	32.7 <sup>a</sup>	30.7 <sup>a</sup>	33.1 <sup>a</sup>
Acetate	72.8 <sup>ab</sup>	69.0 <sup>ab</sup>	70.4 <sup>a</sup>	68.0 <sup>a</sup>	73.2 <sup>ab</sup>	70.3 <sup>ab</sup>	74.6 <sup>b</sup>	72.1 <sup>b</sup>
Propionate	16.0 <sup>a</sup>	20.0 <sup>ab*</sup>	17.8 <sup>a</sup>	17.2 <sup>a</sup>	16.0 <sup>a</sup>	19.0 <sup>ab*</sup>	16.3 <sup>a</sup>	16.5 <sup>b</sup>
Butyrate	11.2 <sup>a</sup>	11.0 <sup>a</sup>	11.8 <sup>a</sup>	14.8 <sup>b***</sup>	10.8 <sup>a</sup>	11.7 <sup>a</sup>	9.1 <sup>b</sup>	11.4 <sup>a***</sup>

values in a row with different letters (normal or bold, respectively) differ significantly  
 significance of difference between the values of the same experimental period is marked by asterisk  
 \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

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## STRESZCZENIE

### Wpływ składu mikrofauny na przebieg fermentacji w żwaczu owiec

Trzy owce Merynosa Polskiego, żywione sianem i śrutą jęczmienną zdefaunowano, a następnie refaunowano pierwotniakami z gatunku *Eudiplodinium maggii*, *Eudiplodinium maggii* i *Entodinium caudatum* lub *Eudiplodinium maggii*, *Entodinium caudatum* i *Dasytricha ruminantium*. U tak przygotowanych zwierząt mierzono stężenie lotnych kwasów tłuszczowych (LKT), tempo ich produkcji oraz proporcje molowe poszczególnych kwasów. Pomiarów dokonywano przed porannym karmieniem i w 4 godziny po odpasie. Stwierdzono, że stężenie LKT wahało się od 8.2 do 11.6 mmol/100 ml płynu żwacza, a tempo ich produkcji od 29.1 do 34.1  $\mu$ mol/g treści żwacza/h w zależności od czasu po karmieniu. Proporcje molowe: octanu, propionianu i maślanu w LKT płynu żwacza oraz w LKT produkowanych podczas inkubacji treści *in vitro* wynosiły odpowiednio 56.1-74.6, 16.0-21.2 i 9.1-11.8%. Zarówno stężenie LKT jak i tempo produkcji oraz proporcje molowe kwasów zależały od obecności orzęsków i składu fauny żwaczowej. U owiec z rozwiniętą populacją *Eudiplodinium maggii* stwierdzono największy udział kwasu masłowego i najmniejszy octowego w próbach pobieranych 4 h po karmieniu. Rozwojowi populacji orzęsków towarzyszył spadek stężenia octanu w płynie żwacza. Nie stwierdzono wpływu obecności pierwotniaków na pH płynu żwacza przed karmieniem zwierząt. Wzrost kwasowości płynu po karmieniu zależał od składu fauny żwaczowej.