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Development of rumen metabolism and epithelium in heifers during transition to pasture

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ABSTACT

After turn-out to pasture, total volatile fatty acid (VFA) concentrations in the rumen contents of heifers significantly decreased from 107.7 to 88.7 mmol.l^{-1} ($p < 0.01$). After subsequent increase the total VFA concentrations reached their highest value (117.0 mmol. l^{-1}) in the 8th week of grazing. The proportion of the molar acetic acid concentration in the rumen content increased insignificantly whereas that of propionic acid decreased insignificantly. In the molar proportion of butyric acid no significant differences could be stated. The energetic yield of VFA production in the rumen of heifers decreased insignificantly from 73.6 to 72.7 %, the acetate : propionate ratio revealed an insignificant increase from 3.66 to 4.18. Cellulolytic bacterial counts in the rumen contents significantly decreased from 8.08 to 7.61 log10.ml^{-1} ($P<0.01$) and then a significant increase to 8.39 log10.ml^{-1} was observed again in the 3rd week of grazing ($P<0.05$). During pasture, a significant increase in the counts of lactate-utilizing bacteria was recorded. As to the numbers of lactobacilli, a significant decrease ($P<0.05$) with a subsequent insignificant increase during grazing could be observed. Scanning electron microscopy revealed the surface of the rumen papillae during winter feeding to be smooth, cocci presenting the dominant morphotype of adhering rumen microflora. In the first phase after turning out to pasture (week 4) roughened sites on the sides and base of the papillae as well as an increase in the free surface of the epithelial cells could be observed. The numbers of adhering bacteria were increased and none of the morphotypes prevailed. In the second grazing cycle (week 8) circular depressions became visible on the larger epithelial cells which were colonized by high numbers of adherent rumen bacteria.

Keywords: heifer, pasture, rumen metabolism, microflora, vollatile fatty acids, rumen epithelium

INTRODUCTION

Grazing promotes the development of a massive and efficient digestive tract that is able to process and utilize nutrients from a large ration of voluminous feeds. Ruminal fermentation metabolites, VFA and microbial proteins present the basic nutrients supplied by roughage. In this transitory grazing period it is essential to guarantee the physiological processes of rumen fermentation (Kay, 1993).

At the time when animals are turned out from the stable (winter feeding) to graze, the young grassland contains increased amounts of nitrogen and decreased amounts of dry matter and fibre, thus affecting the level of rumen fermentation and metabolism in the grazing animals. As claimed by Jambor (1986), transition to pasture not only wasted valuable proteins but often lead to dietetic and metabolic disorders: the altered nutrient intake lead to changes in ruminal biochemism, decreased blood glucose and increased urea levels.

Žitňan et al. (1993) were concerned with ruminal fermentation in grazing cattle. Dougherty et al. (1989) observed the intake and digestion of pasture herbage in heifers during the grazing period. Van Vuuren et al. (1991) reported on the degradability of grassland herbage in the bovine rumen using the in sacco method. Ellis and Hill (1995) investigated the importance of dietary amino-acids, that appear to modulate forage intake, in the nutrition of grazing ruminants. Murphy et al. (1995) studied the interaction of concentrate intake and pasture development and observed differences in rumen metabolism that could be related to the composition of the supplementary feed which, in turn, may influence pasture intake. Concentrate supplements reduced molar proportion of acetate and increased the molar proportion of propionate in some studies (Sayers, 1999; García et al., 2000; Bargo et al., 2002). Two studies evaluated the effect of forage supplements such as corn silage (Elizalde et al., 1992) or hay (Reis and Combs, 2000) on ruminal

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fermentation of dairy cows on pasture.

This work focused on the changes of rumen fermentation and rumen epithelium in heifers in the period of transition from the winter feeding ration to pasture and in the subsequent grazing cycles.

MATERIALS AND METHODS

In this experiment 6 blackspotted heifers and 3 animals with rumen cannula aged 6 months with a mean body weight of 220 kg were included. Prior to turn-out to pasture, the winter feeding ration consisted of 6 kg maize silage, 4 kg green rye cuttings, 1 kg pasture hay and 1 kg barley straw. After the animals had been turned out to pasture, the basic part of their ration consisted of grass crops, the nutritive value of which during the single cycles of grazing is given in Tab. 1. In the first week of pasture, they were given additional 2 kg of maize silage and during the first month, 1 kg of grass hay per animal and day.

Table 1: Nutritive value of the grassland in the single cycles of grazing (g.kg^{-1})

	Cycle of grazing		
	I.	II.	III.
Dry matter	199	229	259
Organic matter	180	208	230
Crude fibre	51	73	94
N-free extract	37	34	35

Samples of rumen contents were taken through a pharyngeal tube in six heifers and rumen epithelium of the three animals were obtained via the cannula. The first sampling from animals was carried out one week prior to driving the animals to pasture. On pasture, rumen contents were sampled at weekly intervals from Week 1 to Week 4 (May, June - Cycle I), then in Week 8 and 12 (July, August – Cycle II) and in Week 16 (September – Cycle III).

Volatile fatty acids in the rumen fluid were determined by gas chromatography using a Hewlett Packard (USA) gas chromatograph with a column containing 10% SP 1200 + 1% H_3PO_4 on Chromosorb W. Quantitation was carried out by the external standard method following injection of a VFA standard of known concentration. The energetic yield of VFA production was calculated in percentage of gross energy according to Orskov et al. (1968).

Strictly anaerobic bacteria were determined on selective media in Black's bottles in a protective CO_2 atmosphere. RGCA medium supplemented with microcristalline cellulose (6g.l^{-1}, treated with phosphoric acid) was used to isolate cellulolytic bacteria. MLH medium (Mackie and Heath 1979) was employed to state the total numbers of lactate-utilizing bacteria. For the isolation of lactobacilli Rogoza Agar (Oxoid) was used while streptococci were isolated on Base Agar No. 4 supplemented with 1% starch.

For scanning electron microscopy, tissue samples of 0.5 cm^2 were out from the ventral ruminal sac, placed in sodium cacodylate buffer and washed (Cheng et al. 1979). Tissue were fixed as outlined by Žitňan et al. (1998) and examined using a Joel Jem-100 CX II (Joel Ltd., Akishima, Japan) with an ASID-4D high resolutin scanning system at an accelerating voltage of 40 kV.

Statistical analyses were carried out using Student's t-test. The results are mean values ± SD.

RESULTS AND DISCUSSION

After turn-out to pasture, total VFA (volatile fatty acid) concentrations in the rumen contents of heifers significantly decreased from 107.7 to 88.7 mmol.l^{-1} ($P<0.01$; Tab. 2). Subsequently total VFA concentrations increased and reached their highest value (117.0 mmol.l^{-1}) in the 8th week of grazing. The proportion of the molar acetic acid concentration in the rumen contents increased insignificantly from 67.1 to 70.1 mol% whereas that of propionic acid decreased insignificantly from 18.4 to 16.8 mol%. In the molar proportion of butyric acid no significant differences could be stated. In the following weeks the molar proportions of acetic acid were rather balanced and ranged from 66.2 to 68.0 mol%; the molar proportion of propionic acid insignificantly increased in the 4th week (18.7 mol%). The molar proportions of butyric acid on pasture insignificantly increased in week 3 (15.2 mol%) and appeared to be balanced, acquiring later values between 14.0 and 14.8 mol%. The energetic yield of VFA production in the rumen of young heifers decreased insignificantly from 73.6 to 72.7%, the acetate-to-propionate ratio revealed an insignificant increase from 3.66 to 4.18 (Tab. 2).

The change of feeding after turn-out to pasture leads to an increase in the content of nitrogenous substances as well as a decrease of dry matter and crude fibre, thus affecting both supplementation and ruminal digestion (Bargo et al., 2003). In this experiment, the transition from winter feeding to grazing lead to a significant, one-week-lasting decrease of VFA concentrations in the rumen content of the heifers. Subsequently an increase of the total VFA levels occured during weeks 3 and 4 that approached the levels observed prior to driving the animals to pasture. On the basis of the total VFA dynamics it can be stated that the adaptation of the rumen ecosystem lasted 3-4 weeks. Supplementation with maize silage during the first week of grazing contributed to shortening of the adaptation period. Sayers (1999) found that supplementation with fiber-based concentrates

Table 2: Products of rumen fermentation of grazing heifers

		Stable	Sampling on pasture at weekly intervals						
			1	2	3	4	8	12	16
Total VFA (mmol.l^{-1})	x̄	107.7	88.7*	96.5	103.2	114.8*	117.0	113.6	109.2
	SD	5.2	4.1	4.6	3.7	5.8	7.3	6.4	4.4
Acetic acid (mol %)	x̄	67.1	70.1	68.0	66.8	66.2	67.1	66.9	67.2
	SD	2.6	3.8	3.2	2.8	1.9	2.6	3.0	2.8
Propionic acid (mol %)	x̄	18.4	16.8	17.4	17.1	18.7	17.4	18.1	17.9
	SD	1.6	2.7	2.5	2.6	2.0	2.0	2.1	1.8
Butyric acid (mol %)	x̄	13.0	11.9	13.6	15.2	14.3	14.8	14.0	14.2
	SD	1.1	2.1	1.7	1.8	1.5	0.9	1.3	1.2
Acetate : propionate	x̄	3.66	4.18	3.91	3.9	3.53	3.84	3.69	3.76
	SD	0.43	0.57	0.63	0.6	0.4	0.55	0.48	0.52
Energetic yield of VFA (E %)	x̄	73.6	72.7	73.3	73.4	73.9	73.5	73.7	73.6
	SD	0.67	0.82	0.76	0.89	0.93	1.05	0.69	0.74

* p < 0,01 significance in comparison with the preceding value, (n = 6)

Table 3: Total numbers of bacteria in the rumen contents of heifers (log 10.ml^{-1})

Group of bacteria		Stable	Sampling on pasture at weekly intervals				
			1	2	3	4	8
Cellulolytic bacteria	x̄	8.08	7.61**	7.92	8.39*	8.43	8.38
	SD	0.07	0.21	0.31	0.19	0.22	0.2
Lactate ulitizing bacteria	x̄	7.54	8.95**	8.86	9.12	9.69*	9.58
	SD	0.31	0.12	0.27	0.12	0.38	0.36
Lactobacilli	x̄	4.05	3.03*	3.48	3.76	3.52	3.8
	SD	0.15	0.25	0.1	0.11	0.19	0.04
Streptococci	x̄	5.04	5.16	5.43	6.14*	5.93	5.5
	SD	0.22	0.16	0.21	0.19	0.31	0.19

* p < 0,05, ** p <0,01 significance in comparison with the preceding value, (n = 6)

increased the molar proportion of acetate and butyrate, and decreased the molar proportion of propionate. Khalili and Sairanen (2000) reported no changes in the molar proportion of any of the three major VFA. In this experiment, the transition to grazing was accopanied by an insignificant increase of the molar acetic acid concentrations and a decrease of propionic acid, whereas the proportion of butyric acid only decreased in the 1st week of grazing. The above molar proportions of VFA are reflected in the acetate:propionate ratio that increased after turn-out to pasture as well as in the energetic yield of VFA production that revealed a decrease during the transitory period. For several types of diets Orskov (1975) determined an energetic yield of VFA production between 73 and 87%. In this relation, the energetic yield of VFA production in our experiment fluctuated at the lower border of the abovementioned range.

Cellulolytic bacterial counts in the rumen contents significantly decreased from 8.08 to 7.61 log10.ml^{-1} (P<0.01) and then a significant increase to 8.39 log10. ml^{-1} was observed again in the 3rd week of grazing (P<0.05). During pasture, a significant increase in the counts of lactate-utilizing bacteria was recorded. As to the numbers of lactobacilli, a significant decrease (P<0.05) with a subsequent insignificant increase during grazing could be seen. Throughout the grazing season, the counts of streptococci reached values that surpassed those recorded during the winter feeding period (Tab. 3).

Our data concerning cellulolytic bacteria in the rumen contents of heifers coincide with those published by Leedle and Butine (1987) and Dehority et al. (1989) who reported the numbers of these bacteria in animals held on rations containing high proportions of bulk feeds. In comparison with the winter feeding, increased numbers of lactate-utilizing bacteria and streptococci as well as decreased numbers of lactobacilli were observed throughout the grazing period.

In the period of winter feeding the surface of rumen papillae, as exposed by scanning electron microscopy (Fig. 1), was smooth with average keratinization and desquamation of the surface cells. Rather great numbers of adherent rumen microflora were seen to colonize the base and mainly the sides of the papillae, cocci, partly also in pairs, being the predominant morphotype. The morphological changes of the rumen epithelium in young heifers during the first cycle of grazing (week 4) were characterized by roughened spots on the sides of the papillae and an enlarged intercellular space between the individual epithelial cells (Fig. 2). The morphotypes observed in this period were rather different

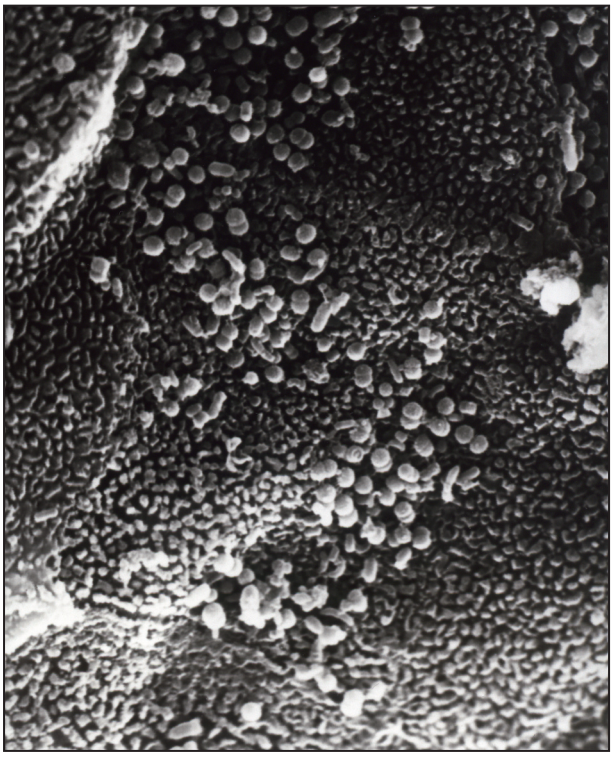

Fig. 1: Rumen epithelium of heifer during the winter feeding period – SEM
(scanning electron micrograph; **x3000)**

Fig. 2: Rumen epithelium of heifer in the first cycle of grazing - SEM (x1500)

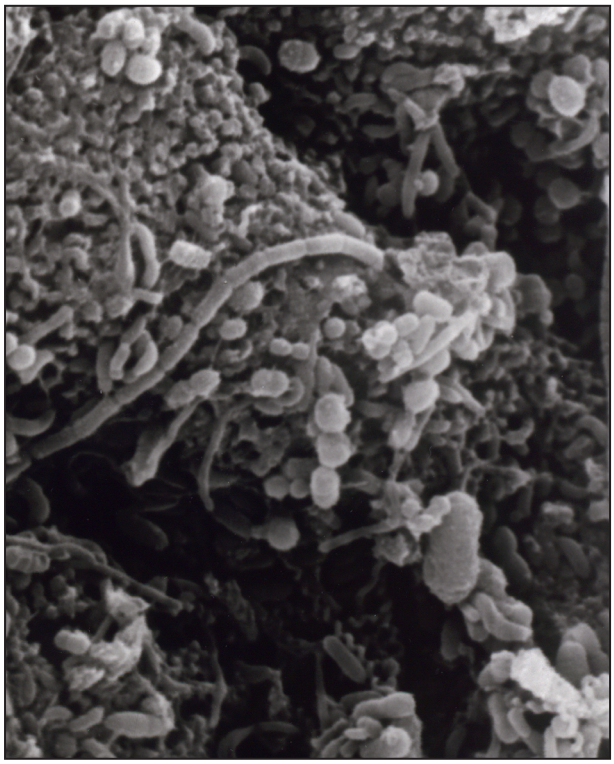

Fig. 3: Adhering rumen microflora of heifer in the first cycle of grazing - SEM (x6000)

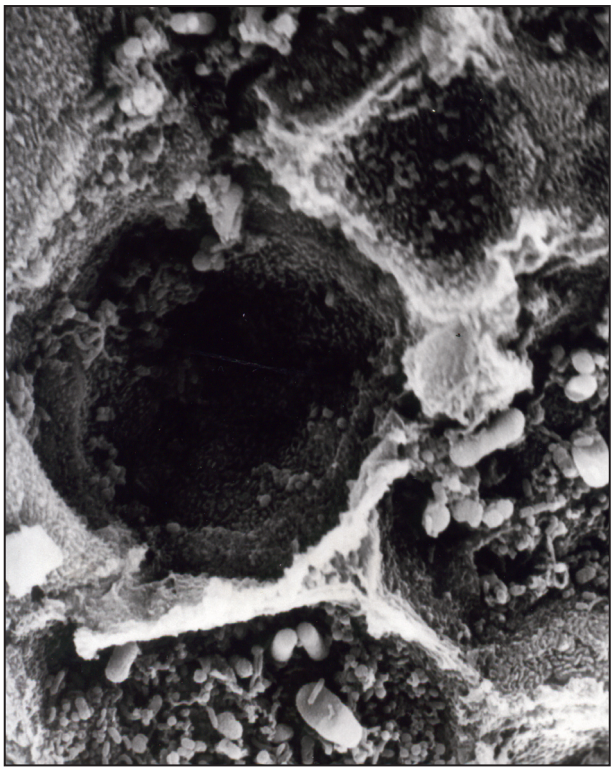

Fig. 4: Rumen epithelium of heifer in the second cycle of grazing - SEM (x2000)

and no one predominated. Both solitary and paired small and large cocci were seen as well as straight rods with rounded ends, curved rods with pointed ends, rods in chains and spiral microorganisms (Fig. 3). The rumen epithelium of young heifers in the second cycle of grazing (week 8) was characterized by circular depressions on the larger epithelial cells (Fig. 4). These depressions were colonized by large numbers of adhering rumen bacteria that were rather similar to those observed in the first grazing cycle. The surface of the rumen papillae in the third cycle of grazing (week 16) was characterized by increased keratinization and desquamation of the epithelial cells. Similarly to the second cycle of grazing, circular depressions could be observed in the epithelial cells that were mainly colonized by cocci together with other morphotypes (Fig. 5). According to these observations keratinization and desquamation is succeeded by recycling of the epithelial cells towards the end of the grazing period.

The transition from winter feeding to grazing had an adverse affect upon rumen fermentation which required a certain adaptation phase in order to normalize. In our experiment this adaptation phase took 3 weeks.

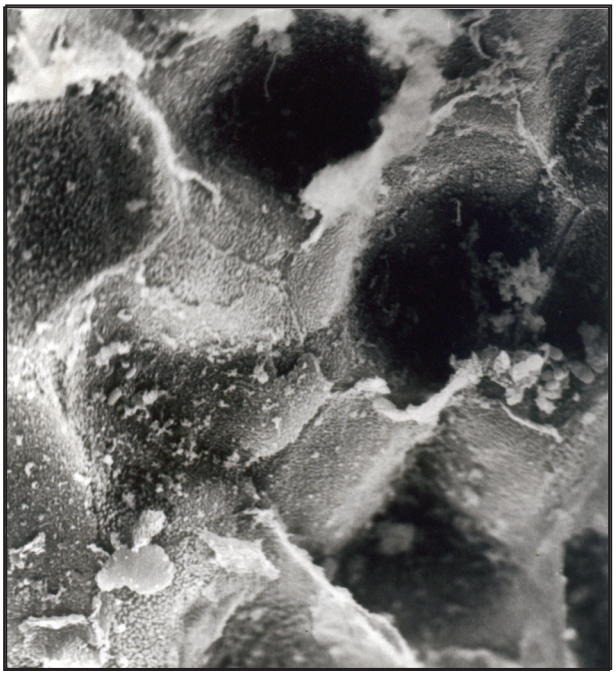

Fig. 5: Rumen epithelium of heifer in the third cycle of grazing - SEM (x1500)

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