Influence of the diet on the morphology of ruminal and intestinal mucosa and on intestinal carbohydrase levels in cattle

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ABSTRACT: This study examined the effects of extensive and intensive feeding on the morphology of the gastrointestinal tract as well as on the level of carbohydrase activity in the small intestine of growing cattle. Fourteen growing male bulls aged 5 months were divided into two feeding groups. The extensively fed animals were kept on pasture in the summer and in a stall in the winter whereas the intensively fed group was housed all the year long. The bulls were slaughtered 16 h after the last feeding at the age of 18 months. Rumen fluid samples and mucosa samples from the ventral ruminal sac and the intestinal tract (duodenum, jejunum, ileum) were subjected to analyses. Evaluation of rumen fermentation did not reveal significant differences between the groups, however, the molar proportions of propionic acid were increased in the intensively reared bulls. As to the activity of the individual carbohydrase enzymes (maltase, cellobiase, lactase) no significant differences could be stated between the groups. Comparison to the extensively reared group revealed that the length and width of papillae of the ventral ruminal sac was significantly increased in the intensively reared animals ($P < 0.001$) and so was the papillar surface per cm^2 of mucosa ($P < 0.001$). The length of duodenal villi in the intensive group was singnificantly increased ($P = 0.026$) whereas that of the jejuenal villi approached the limits of significance ($P = 0.052$) when compared to the extensive group. There were no significant differences in the depth of crypts, however, the crypts of the intensively reared animals were somewhat deeper. The length of jejunal villi positively correlated both with the length ($r = 0.658$; $P = 0.011$; $n = 14$) and with the absorption surface of the rumen papillae ($r = 0.636$; $P = 0.015$; $n = 14$). Our results confirm that high concentrate rations increase both the absorption surface of the rumen papillae and the height of villi in the small intestine of intensively fed cattle.

Keywords: cattle; rumen fermentation; carbohydrase enzymes; ruminal and intestinal morphology

In cattle, energy-rich feeding causes an increase in the size of rumen papillae and leads to considerable mucosa proliferation (Dirksen *et al.*, 1984). In animals fed low and high energy diets rumen mucosa revealed progressive reduction and intensive proliferation, respectively (Dirksen *et al.*, 1985). Intensity of rumen fermentation increases with the increasing intake of concentrate and simultaneously the occurring volatile fatty acids promote the structural development of the rumen epithelium (Kauffold *et al.*, 1977; Hofmann and Schnorr, 1982; Zitnan *et al.*, 1998).

So far, insufficient or no investigations at all have been carried out into the effects of the diet upon the development of small intestinal mucosa. According to Tivey and Smith (1989), changes in the development of enterocytes and in the structure of villi determine the digestive and absorptive capacity of the small intestine. Kreikemeier *et al.* (1990) studied the activity of carbohydrate digesting enzymes in Holstein and Longhorn bulls and observed it to be influenced by the type of diet and the level of feed intake. Mir *et al.* (1997) considered the length of villi and crypts and mucosal carbohydrase activity to be an important factor in nutrient absorption; they recorded differences in these parameters between different cattle breeds, of which Holstein bulls had the highest lactase activity and the longest villi in the middle part of the intestine.

The aim of this investigation was to examine the effects of extensive and intensive feeding on the morphology of the gastrointestinal tract as well as on the level of carbohydrase activity in the small intestine of growing bulls.

MATERIAL AND METHODS

Animals and nutrition

Fourteen growing male Black Pied bulls (9 Deutsche Holstein Frisian – DH, 5 Deutsche Fleckvieh – DF), 5 months of age and initially of about 134 kg body weight (BW) were divided into two groups for intensive and extensive rearing (4 DH + 3 DF and 5 DH + 2 DF, respectively). The intensively reared animals were stanchion-housed in a conventional stall with partitioned mangers for individual feeding. The experimental diet consisted of barley straw and a pelletted concentrate mixture as main components at a DM ratio of 28 : 72 (82.0 ± 0.5 MJ ME/d). The diet was offered twice daily in equal parts at 6.00 and 14.00 h with water available at all times. During summer, the animals of the extensively reared group grazed on a pasture and in winter, in particular from October to the middle of May, they were housed in a stall similarly to the intensively reared group. The experimental diet was based on meadow grass on pasture and on wilted silage in the stall, the mean roughage to concentrate DM ratio in the diet being 94 : 6 (67.8 ± 0.6 MJ ME/d). The mean composition and nutrient contents of the diets fed between month 5 and 18 of life are given in Table 1. On pasture, the feed intake of the animals was calculated by their daily body weight gain. Body weights were recorded at approximately the same time at 28-day intervals. The animals were slaughtered at 18 months of age and 16 h after the last feeding. The final mean BW of the cattle before slaughter was 616 ± 39 kg and 511 ± 52 kg in the intensively and the extensively reared group, respectively. The respective mean daily weight gains were 1 211 ± 99 and 960 ± 123 g.

Sampling and chemical analysis

Rumen fluid was taken from the perforated rumen immediately after slaughtering. For enzyme activity and morphometrical determinations samples were obtained within 30 min after slaughter. The duodenal samples (about 10 cm length) were taken from a site 50 cm distal of the pyloric sphincter, the jejunal ones from the mid-jejunum (approximate centre of the jejunum). Ileal samples were obtained 50 cm proximal of the ileo-caecal junction. Mucosa was obtained by scraping intestinal tract samples with a glass slide. Samples of the rumen wall intended for morphological examination were obtained from identical sites of the ventral ruminal sac (approximately 5 cm caudal of the *pila cranialis*).

For carbohydrase activity determination the intestinal samples were rinsed with a cold saline solution (0.9% wt/vol sodium chloride solution), frozen in liquid nitrogen and stored at –80°C till analysis. Lactase, cellobiase and maltase activity were measured according to Mir *et al.* (1987). In detail, mucosa samples (200 mg) were homogenised for 3 min with 1 ml saline solution. The homogenate was transferred to a test tube together with 2.5 ml (2 × aliquot) saline solution. Three reaction tubes were filled with 100 µl of the homogenate and placed in a 37°C water bath, then 400 µl of 56 mmol lactose, maltose or cellobiose in citrate buffer (pH 6.6, 0.01 mmol) were added, respectively. After shaking and incubation for 30 min enzyme activity was stopped in boiling water. The reaction tubes were centrifuged at 2 000 × g (10 min, 5°C) using a Varifuge 3.0R (Heraeus Sepatech GmbH, Osterode, Germany). Galactose and glucose in the supernatant were determined by the UV-method (UV-Test Lactose/D-Galactose No. 176303 and D-Glucose No. 716251, Böhringer Mannhein, Germany). Enzyme activity was expressed as mg of galactose or glucose hydrolysed per hour per mg of crude protein. For the latter nitrogen content was determined by an elemental analyser (Leco, CNS-2000; St. Joseph, USA) and the obtained value was multiplied with 6.25.

Light microscopy and morphometry

Ruminal as well as intestinal samples (1 cm^2) were fixed in 4% formalin solution. After rinsing with water, the samples were dehydrated in a graded series of absolute ethanol (30%, 50%, 70%, 90%), cleared with benzene, saturated with and embedded in paraffin. Sections of 7 µm thickness (10 slices of each sample) were stained with haematoxylin/eosin and observed under a light microscope. The length of 10 villi and depth of 10 crypts were determined by the computer operated *Image C* picture

analysis system (Intronic GmbH, Berlin, Germany) and the IMES analysis programme, using a colour video camera (Sony 3 CCD) and a light microscope (Axiolab, Carl Zeiss Jena, Germany). The same system was used to observe the length and width of rumen papillae and to estimate their number per cm^2 of mucosa. Total surface of papillae per cm^2 mucosa was determined as length × width × 2, multiplied by the number of papillae per cm^2.

Determination of nutrient and ruminal parameters

Dry matter (DM), crude protein (CP), crude fibre (CF) and ash in the experimental diets were determined according to the Weender standard procedure (Naumann and Basler, 1993). The energy content was calculated by the predicting equation of metabolizable energy in combined feeds (Kuhla and Weissbach, 1996). SCFA concentrations in samples of rumen fluid were determined by gas chromatography with *i*-capronic acid as internal standard (Geissler *et al.*, 1976) using a Shimadzu GC-14A with an FFAP 25m × 0.25 mm i. d. column. pH was measured with a glass electrode and ammonia concentration was determined by the microdiffusion method (Voigt and Steger, 1967).

Statistical analysis

The results were statistically analysed by Statistica-Software of StatSoft Inc. (version 6.0). A two-factorial ANOVA with interactions (feeding group and breed) was used to determine the significance of differences between the feeding groups. The results are presented as means ± SD.

Table 1. Composition of diets (g DM/animal and day) and nutrient content (g/kg DM)

Diet	Intensive rearing	Extensive rearing
Ingredients		
Concentrate mixture*	5 531	398
Meadow hay	30	42
Wilted silage	376	5 877
Barley straw	1 652	
Meadow grass		720
Chemical composition		
Organic matter	940	894
Crude Protein	133	137
Crude fibre	205	268
ME (MJ/kg DM)	10.8	9.6

*Composition of the concentrate mixture: barley 44.8%, sugar beet pulp 36.9%, soya extracted meal 13.7%, molasses 3.0%, mineral-vitamin mixture 1.8%

Table 2. Parameters of rumen fermentation in cattle (mean ± SD, $n = 7$)

Treatment	Intensive rearing	Extensive rearing	*P* values
pH-Value	7.22 ± 0.21	7.15 ± 0.19	0.60
VFA (mmol/l)	48.0 ± 20.5	56.1 ± 24.5	0.72
C2 (mol%)	66.4 ± 4.6	68.7 ± 1.5	0.37
C3 (mol%)	18.6 ± 7.2	16.4 ± 0.9	0.61
C4 (mol%)	9.6 ± 1.9	10.2 ± 0.5	0.65
C2 : C3	4.05 ± 1.51	4.19 ± 0.30	0.94
NH_3 (mmol/l)	9.34 ± 2.48	9.68 ± 1.39	0.89

RESULTS AND DISCUSSION

Rumen fermentation and rumen mucosa

When evaluating the level of rumen fermentation in bulls it is important to take into account that the animals were killed 16 h after the last feeding. This fact became evident in the statistical evaluation of the individual parameters of rumen fermentation in which no significant differences could be stated (Table 2). Total volatile fatty acid (VFA) and acetic and butyric acid levels in the rumen contents of extensively reared bulls were increased. Propionic acid levels in the extensively reared animals were lower than in the intensively reared ones (16.4 vs. 18.6 mol%). Though the above values differed numerically, this difference was of no statistical significance. In the intensively reared bulls the acetate to propionate ratio was also decreased. Ammonia levels in the rumen contents were high in both groups but slightly higher in the extensively managed animals.

In the intensively reared group the length and width of rumen papillae of the ventral ruminal sac were significantly increased ($P < 0.001$) when compared to the extensive group of cattle (Table 3). There was no significant difference between the numbers of papillae per cm^2.

The papillary surface per cm^2 of mucosa in the intensively reared cattle reached 1 677 mm^2 and was significantly increased ($P < 0.001$) when compared to the extensive group (1 044 mm^2).

The feeding-dependent differences in rumen fermentation influence the development of the rumen mucosa. It is known from literature that concentrate-rich diets cause an increased VFA (mainly propionic and butyric acid) production thus stimulating the metabolism of the rumen epithelium, the structural development and resorptive activity of the latter. (Kauffold *et al.*, 1975; Hofmann and Schnorr, 1982; Jesse *et al.*, 1995; Lane and Jesse, 1997; Zitnan *et al.*, 1998). We could not observe any significant differences in the level of rumen fermentation since the animals had been killed 16 hours after the last feeding, however, the resorption surface of rumen papillae per cm^2 of mucosa was stated to be increased in the intensively reared group of animals which received higher amounts of the concentrate.

Length of villi and depth of crypts

The length of duodenal villi in the intensive group was singnificantly increased ($P = 0.026$) whereas that of the jejunal villi approached the limits of significance ($P = 0.052$) when compared the extensive group. No significant differences could be observed in the length of ileal villi (Table 4). In the intensive group the crypts were deepest in the duodenum (309 μm), this value approaching the limits of significance ($P = 0.065$) when compared to the extensive group (285 μm). In the jejunum and ileum the differences were very small, being, however, increased in the intensive group (Table 4).

Some correlation coefficients proved to be rather interesting. The length of jejunal villi positively

Table 3. Morphometrical parameters of the papillae of the ventral ruminal sac

	n	Length (mm)	Width (mm)	Number per cm^2	Surface (mm^2/cm^2)
Intensive rearing	7	6.54 ± 0.56	2.33 ± 0.24	55 ± 6	1 677 ± 191
Extensive rearing	7	5.23 ± 0.40	1.82 ± 0.14	55 ± 3	1 044 ± 80
P values		0.0002	0.001	0.85	0.00002

Table 4. Height of villi and depth of crypts in the small intestine

	n	Villi (μm)			Crypts (μm)		
		duodenum	jejunum	ileum	duodenum	jejunum	ileum
Intensive rearing	7	550 ± 44	585 ± 44	509 ± 42	309 ± 21	296 ± 23	303 ± 15
Extensive rearing	7	501 ± 58	527 ± 56	502 ± 32	285 ± 21	282 ± 22	302 ± 23
P values		0.026	0.052	0.54	0.065	0.104	0.83

Table 5. Carbohydrase activity in the small intestine (n = 7)

	Intensive rearing	Extensive rearing	P values
Maltase activity (μmol maltose hydrolysed mg CP/h)			
duodenum	2.48 ± 0.21	2.48 ± 0.25	0.97
jejunum	2.58 ± 0.25	2.55 ± 0.18	0.91
ileum	2.73 ± 0.29	2.64 ± 0.54	0.36
Cellobiase activity (μmol cellobiose hydrolysed mg CP/h)			
duodenum	0.36 ± 0.13	0.36 ± 0.16	0.98
jejunum	0.28 ± 0.16	0.30 ± 0.12	0.87
ileum	0.30 ± 0.13	0.28 ± 0.08	0.72
Lactase activity (μmol lactose hydrolysed mg CP/h)			
duodenum	0.61 ± 0.33	0.34 ± 0.27	0.16
jejunum	0.11 ± 0.07	0.09 ± 0.05	0.70
ileum	0.03 ± 0.03	0.02 ± 0.01	0.18

correlated both with the length (r = 0.658; P = 0.011; n = 14) and with the resorption surface of the rumen papillae (r = 0.636; P = 0.015; n = 14).

Mir *et al.* (1977) reported the lengths of the villi in the mid-intestine of different breeds to be comparable, however, he supposed the relatively longer villi in the Holstein breed to be able to influence the total absorption of nutrients. Kreikemeier *et al.* (1990) presumed the absorptive surface to represent the mucosal aspect of villi available for nutrient translocation. These authors observed the greatest absorptive surface in the proximal area of the bovine small intestine and they also recorded the absorptive surface to increase with increasing grain intake. The latter finding coincides with our results which revealed higher duodenal and jejunal villi in animals fed increased concentrate diets (intensively reared group).

Carbohydrase activities

In the experimental period the groups observed revealed no statistically significant differences in the activity of the individual carbohydrase enzymes (maltase, cellobiase, lactase; Table 5). Differences between the values proved to be minimal, the only exception being the activity of lactase in the duodenal mucosa (0.61 and 0.34 μmol lactose hydrolyzed mg CP/h in the intensively and extensively reared animals, respectively), which, however, was neither significant (P = 0.16). A positive correlation was observed between maltase activity and the depth of ileal crypts (r = 0.588; P = 0.027; n = 14).

In contrast to our findings Mir *et al.* (1997) noted differences in lactase acitivity. These differences might have resulted from the relatively longer villi in some breeds which might have influenced overall nutrient absorption in the animals. On the other hand, Kriekemeier *et al.* (1990) reported mucosal maltase activity to depend on feed intake and sampling site. Similarly to our findings the latter authors also observed mucosal lactase activity to be higher in the proximal segments of the small intestine. Lactase expression could also be affected by increases in crypt cell proliferation (Miller *et al.*, 1986; Ratcliffe *et al.*, 1989). On the contrary, Shirazi-Beechey *et al.* (1991) found intestinal digestive enzymes to be not regulated by diet which coincides with our results.

CONCLUSION

In intensively reared animals receiving higher amounts of concentrates not only the absorption surface of rumen papillae but also the height of duodenal and jejunal villi were seen to increase. This fact was confirmed by the positive correlation between the morphometric parameters of ruminal and intestinal mucosa. The activity of mucosal carbohydrase in the intestine was not influenced.

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