Effects of Nutritional Level on Digestive Enzyme Activities in the Pancreas and Small Intestine of Calves Slaughtered at Same Body Weight

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ABSTRACT : Six Holstein heifer calves weaned at 45 days-of-age were randomly allocated into high daily gain (1.1 kg/d, HDG) and low daily gain (0.56 kg/d, LDG) groups, and were slaughtered at 170 kg of live weight. Energy intake level in the feeding period was 2.4 × maintenance in 105 days for HDG and 1.4 × maintenance in 216 days for LDG calves. Total length of the small intestine was identical between groups, but both weights of the pancreas and of the small intestinal mucosa were greater ($p < 0.01$) for HDG calves. Alpha-amylase, lipase, proteinase, and trypsin activities of the whole pancreas were higher ($p < 0.05$) in HDG calves. Disaccharidase activity of the whole small intestinal mucosa was also higher ($p < 0.10$) for HDG than for LDG calves. However, the enzymatic activities, expressed as per gram or per protein of the pancreas and the small intestinal mucosa, were not affected ($p > 0.10$) by the plane of nutrition. These results suggest that the digestive enzyme activity in the small intestine varies primarily with the weight of tissues synthesizing the enzyme.
(Key Words: Nutritional Level, Digestive Enzyme, Calf)

INTRODUCTION

Ruminant digestion is characterized by microbial fermentation in the rumen, the first chamber of the stomach and main digestive site. However, digestion by host enzymes in the lumen of the small intestine is also indispensable for nutrient utilization. The enzymes related to the latter digestion originate in the pancreas and the mucosa of the small intestine. And while the influence of diet on the activities of these enzymes has been widely investigated in monogastric animals, it has only rarely been examined in ruminants (Harmon, 1992b). Kreikemeier et al. (1990) slaughtered steers of different body weights and found that enzymatic activity in their small intestines was affected by energy consumption level via weight changes in the tissues responsible for enzyme synthesis. However, as the tissue weight may vary with both energy consumption level and body weight, the direct influence of energy intake on enzymatic activity is unclear. In this study, therefore, calves were fed a high or low energy-level diet and slaughtered at the same body weight prior to examination of the enzymatic activity and tissue weights of the pancreas and samll intestinal mucosa.

MATERIALS AND METHODS

Animals and management

Six Holstein heifer calves weaned at 45 days-of-age (average live weight, 52 kg) were randomly allocated into a high daily gain group (HDG) or low daily gain group (LDG), each comprising three calves. HDG calves had access to concentrate and Italian ryegrass hay *ad libitum*. The same diet at the same ratio (concentrate : hay) was fed to LDG calves, but in a quantity allowing only half the body-weight gain of HDG calves. LDG calves were weighed weekly to adjust their feed supply. All calves were housed in individual calf hatches with free access to fresh water, and were fed in two equal installments at 09:00 and 17:00. The concentrate was purchased commercially, and had a stated proportion of ingredients as follows: 50% grains, 35% protein supplements, and 15% mineral supplements and other by-products. Crude protein in dry matter was 22.7% in the concentrate and 10.6% in hay. The whole mixed diet contained 71% of TDN, in air dry matter, which was measured by a direct digestion trial immediately before slaughter. The maintenance energy requirements of calves were as listed in the Japanese Feeding Standard (Agriculture, Forestry and Fisheries Research Council Secretariat, 1987).

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Received December 4, 1997; Accepted February 19, 1998

Slaughter and tissue collection

All calves were slaughtered at approximately 170 kg of live weight by bleeding under general anesthesia with sodium pentobarbital injection after 2 hours of diet deprivation. Evisceration was performed within approximately 20 min after slaughter. The alimentary tracts were removed and the weights of tracts and remaining digesta recorded. After the total length of the small intestine was measured, ten 30-cm segments were taken from the sites at 5, 15, 25, 35, 45, 55, 65, 75, 85, and 95% of the total length. Small intestine and the whole pancreas samples were placed in plastic bags, chilled on ice, and immediately transported to the laboratory for further preparation.

Sample preparation

The pancreas was trimmed of excess fat and debris, then weighed and diced. Two grams of pancreatic tissue were combined with 20 ml of cold 0.9% NaCl solution in a 50 ml plastic test tube and homogenized by a homogenizer (Nihon Seiki Kogyo Co., Tokyo) for 45 s. The resulting homogenate was centrifuged at 1,300 × g for 10 min. Supernatant of the homogenate was stored at −20℃ until determination of α-amylase, lipase, trypsin, chymotrypsin, and proteinase activities.

Small intestine were trimmed of excess fat, cut longitudinally, and their mucosa were rinsed with saline. After removing excess saline with a piece of filter paper, mucosa were scraped from the small intestine using a glass microscope slide. The scraped mucosa was weighed, and two grams were combined with 20 ml of cold 0.9% NaCl solution and homogenized in the same manner used for pancreatic samples. Samples were maintained under cold temperature throughout the preparation. Mucosa from each small intestinal site was used to assay for maltase, isomaltase, and lactase activities.

Analyses

Pancreatic zymogen was activated by enterokinase (E-5510, Sigma Chemical Co. St. Louis. USA) at 30℃ for 20 min in 15 mmol/L Tris buffer (pH 8.1). Trypsin and chymotrypsin activities were determined photometrically using Na-p-toluenesulfonyl-L-arginine methyl ester (Rick, 1974a) and N-benzoyl-L-tyrosine ethyl ester (Rick, 1974b), respectively. Proteinase was assayed by the method of Iwasiro et al. (1992). Alpha-amylase activity in the pancreas was measured by the method of Hall et al. (1970) using Starch Azure (S-7776, Sigma Chemical Co.) as substrate. Lipase activity was measured by the method of Whitaker (1973) using α-naphthyl palmitate as substrate. Maltase, isomaltase, and lactase activities were measured by the methods of Kreikemeier et al. (1990) and Bergmeyer et al. (1974). One unit of disaccharidase activity was defined as that which hydrolysed one μmol disaccharide per min at 37℃. The protein content of the homogenates was assayed by the method of Lowry et al. (1951).

A t-test was used to test the difference in enzyme activities of the pancreatic and small intestinal mucosa between each treatment. The effect of the treatment on the distribution pattern of disaccharidase activity along the small intestine was analysed by the method of split plot analysis using SASD (1990). Inter-treatment differences among intestinal site were a nalyzed by t-test. P value < 0.10 were considered significant.

RESULTS

Mean body weight at slaughter was 172 and 165 kg for HDG and LDG, respectively. HDG calves consumed more concentrate and hay, and showed twice the daily body-weight gain of LDG calves throughout the feeding period (table 1). Mean daily intake of TDN was 2.64 kg for HDG and 1.54 kg for LDG calves. LDG calves thus required twice as many days to reach the target live weight.

Table 1. Feed intake and performance of calves

Item	Calves		SEM	P
	HDG[1]	LDG[2]		
Feed intake[3] (kg/d)				
Concentrate	3.45	1.99	0.47	< 0.001
Italian ryegrass hay	0.26	0.18	0.03	< 0.004
Total	3.71	2.17	0.50	< 0.001
Energy level[4]	2.4 M	1.4 M	0.24	< 0.001
Daily gain (kg)	1.09	0.56	0.06	< 0.001
Feeding period (d)	105	216	25	< 0.001

[1] High Daily Gain.
[2] Low Daily Gain.
[3] Mean daily intake.
[4] Times of maintenance energy requirement to the mean body weight for the whole period.

The fresh weights of alimentary tracts and digesta are shown in table 2. Weights of the tissue of the liver, reticulo-rumen, omasum-abomasum, and small and large intestine were greater in HDG calves. Weight of the digesta of the small intestine was greater in HDG calves, but that of the large intestine was greater in LDG calves. Weights of the digesta of the reticulo-rumen and the omasum-abomasum were not affected by the nutritional level.

Table 2. Fresh weights of tissue and digesta of alimentary tract of calves fed at different nutritional plane

Item	Calves		SEM	P
	HDG[1]	LDG[2]		
Tissue weight per body weight (g/kg)				
Reticulo-rumen	21.5	16.6	0.4	0.041
Omasum and abomasum	9.6	7.8	0.6	0.060
Small intestine	22.3	13.4	2.1	0.002
Large intestine	9.1	7.5	0.5	0.041
Liver	22.6	15.6	0.8	0.004
Digesta per body weight (g/kg)				
Reticulo-rumen	103	115	8.0	0.244
Omasum and abomasum	9.1	7.0	1.2	0.225
Small intestine	21.4	11.5	2.6	0.017
Large intestine	7.2	10.2	0.7	< 0.001

[1] High Daily Gain.
[2] Low Daily Gain.

Table 3. Weight and digestive enzyme activity of pancreas of calves fed at different nutritional plane

Item	Calves		SEM	P
	HDG[1]	LDG[2]		
Pancreas				
Wt (g)	169	141	3.3	0.007
Protein (mg/g)	78.1	64.3	6.3	0.168
Alpha amylase activity				
Units/g	1,135	1,015	41.4	0.161
Units/mg protein	14.6	16.4	0.9	0.191
Total Units[3] ($\times 10^3$)	191	142	11.7	0.003
Lipase activity				
Units/g	56.7	49.5	5.7	0.282
Units/mg protein	0.72	0.77	0.03	0.241
Total Units[3] ($\times 10^3$)	9.5	6.9	0.7	0.081
Proteinase activity				
mg/g	4,068	3,642	318	0.272
mg/mg protein	52.0	57.9	3.1	0.199
Total Units[3] ($\times 10^3$)	682	511	42.6	0.058
Trypsin activity				
Units/g	208	171	17.2	0.175
Units/mg protein	2.65	2.75	0.21	0.419
Total Units[3] ($\times 10^3$)	34.8	24.1	2.4	0.047
Chymotrypsin activity				
Units/g	213	206	18.6	0.432
Units/mg protein	2.74	3.28	0.21	0.137
Total Units[3] ($\times 10^3$)	35.8	29.3	2.7	0.137

[1] High Daily Gain.
[2] Low Daily Gain.
[3] Total activity was calculated by multiplying the activity per mucosa by total weight of pancreas.

The weight of the pancreas was greater in HDG calves, but the difference in protein content was not significant (table 3). Enzyme activities, expressed per gram or per protein content, did not differ between HDG and LDG calves. When enzyme activity was expressed as total pancreatic activity, however, α-amylase, lipase, trypsin, and proteinase activities were greater in HDG calves. Chymotrypsin also tended to be greater ($p < 0.14$) in HDG calves.

The small intestinal morphology and mucosal disaccharidase activity are shown in table 4. The length of the small intestine was the same for both groups, but mucosal weight was greater in HDG calves. When disaccharidase activity was expressed per gram of mucosa or protein content of mucosa, maltase, isomaltase, and lactase activities were not significantly different. When expressed as total activity or per centimeter of intestine, these disaccharidase activities were significantly increased in HDG calves.

Table 4. Morphology and disaccharidase activities of small intestine of calves fed at different nutritional plane

Item	Calves		SEM	P
	HDG[1]	LDG[2]		
Morphology				
Length (m)	26.9	26.5	1.7	0.902
Mucosal wt (g/cm)	0.73	0.44	0.03	0.006
Protein content (mg/g mucosa)	0.78	0.85	0.03	0.522
Enzyme activity				
Maltase				
mU/g mucosa	210	147	22.3	0.232
mU/mg protein	270	173	23.6	0.121
mU/cm intestine	156	59	16.5	0.044
Total activity[3]	467	159	52.0	0.042
Isomaltase				
mU/g mucosa	4.39	0.85	1.19	0.212
mU/mg protein	5.40	0.96	0.87	0.188
mU/cm intestine	3.31	0.34	0.13	0.089
Total activity[3]	9.65	1.22	2.44	0.079
Lactase				
mU/g mucosa	802	594	137	0.491
mU/mg protein	1,006	679	163	0.374
mU/cm intestine	602	220	46.9	0.016
Total activity[3]	1,801	615	128	0.018

[1] High Daily Gain.
[2] Low Daily Gain.
[3] Total activity was calculated by multiplying the activity per centimeter by total length of small intestine.

The effects of feeding treatment on the disaccharidase activities and distribution patterns along the small intestine are shown in figure 1. On the whole, all disaccharidase activities were higher in HDG calves, especially at the site of 25% of the small intestine. Maltase activity was observed in all parts of the small intestine. The pattern of distribution of maltase activity along the small intestine was not much affected by the nutritional level. Isomaltase activity for both treatments was located mainly in the upper part of the small intestine. A maximal activity of lactase was located at the site of 5% and 25% of the small intestine length for LDG and HDG, respectively.

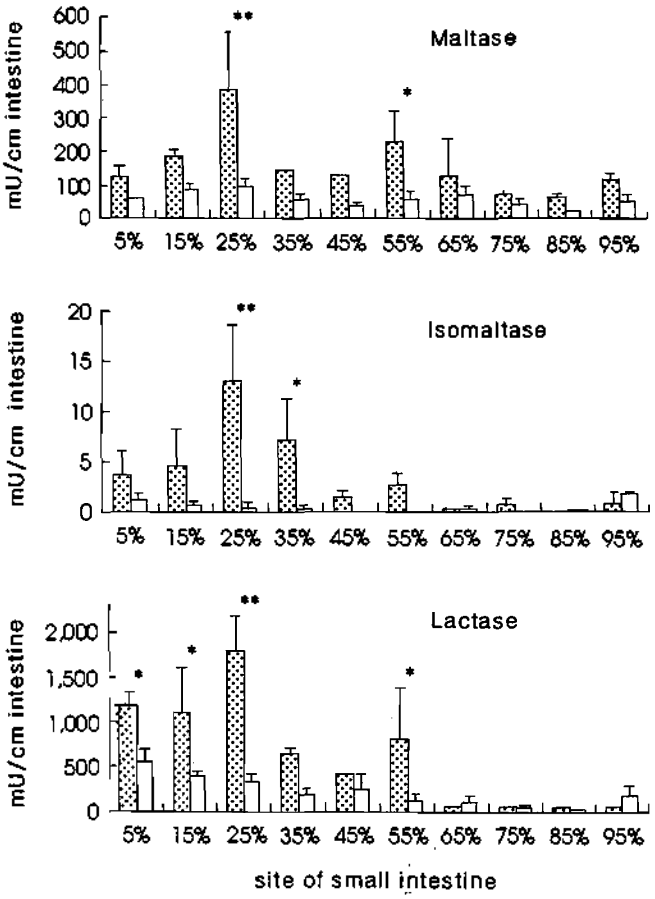

Figure 1. Distribution of disaccharidase activity along the small intestine for high daily gain (▨) and low daily gain (□) calves. Vertical bars show standard error (n = 3). * : $p < 0.05$, ** : $p < 0.01$.

DISCUSSION

Digestibility of starch in the small intestine of ruminants is known to be inferior to that of monogastric animals, and to vary with diet (Harmon, 1992a). Activities of pancreatic α-amylase and disaccharidase of the small intestinal mucosa affect the degree of starch digestion. In short term experiments, α-amylase secretion of the pancreas has been shown to be stimulated by various nutrients presented in the duodenum via gastric hormones, such as cholecystokinin in monogastric animals (Brannon, 1990). In ruminants, however, the mechanism of stimulating α-amylase release is unclear, although it may involve stimulation of pancreatic exocrine by short-chain fatty acids (Katoh and Yajima, 1989). In long term experiments, pancreatic α-amylase activity has been shown to increase not only with age of calf (Siddons, 1968; Morrill et al., 1970), but also with increased energy intake (Russell et al., 1981; Kreikemeier et al., 1990). In this study, the observed increase of total α-amylase activity with energy intake was consistent with the results of Kreikemeier et al. (1990). However, α-amylase activity per gram or protein content of pancreas did not differ between HDG and LDG calves, although other studies have reported that α-amylase per gram (Kreikemeier et al., 1990) and per protein (Russell et al., 1981) also increased with energy intake. The main cause of this discrepancy between this and other studies is probably the difference in cattle body weight at slaughter: we determined α-amylase activity using calves of the same body weight, versus the different body weights used by previous authors.

Similar effects by energy status of calves were observed on other enzyme activities of the pancreas. The total activities of pancreatic lipase, proteinase, and trypsin increased for HDG calves, but specific activities of these enzymes were not affected by the energy intake. These results suggest that the influence of energy intake on pancreatic exocrine occurs through changes in pancreatic weight, not through the change of synthesis per gram or protein content of pancreas at a same body weight.

The specific activity of maltase in the small intestinal mucosa has been indicated to change little with age (Siddons, 1968; Coombe and Siddons, 1973). Our results agree with those of Russell et al. (1981), who found that energy intake did not affect maltase-specific activity. Nevertheless, our HDG calves showed higher maltase activity, expressed as per centimeter of the small intestine, than our LDG calves due to the greater mucosal weight of the former. A similar influence of energy intake on maltase activity has been observed by Kreikemeier et al. (1990). The greater mucosal weight in HDG calves may result from the greater weight of the small intestinal digesta, as shown in table 2.

A tendency similar to that for maltase activity was found for isomaltase activity, both per centimeter of the small intestine and by total activity. Because cattle age

has no affect on isomaltase activity (Coombe and Siddons, 1973), it is undoubtful that energy intake affected the isomaltase activity in this study. However, Kreikemeier et al. (1990) reported that changes in energy intake did not affect isomaltase activity per centimeter in steers slaughtered at different body weights. Thus, different body weight at slaughter may mask the influence of energy intake on both isomaltase and other enzyme activities. In this study, the increase in isomaltase activity in HDG calves was restricted to the upper site of the small intestine. Such restricted distribution of isomaltase, unlike that of maltase, may limit starch digestion in the small intestine. Mayes and Ørskvo (1974) have suggested that the limiting enzyme of digestion of starch infused into the abomasum of sheep might be isomaltase, based on the α-glucoside fraction of the terminal ileal digesta.

It is well known that intestinal lactase activity of calves is highest at birth and decrease with age (Siddons, 1968; Miyashige and Yahata, 1980). In this study, the differences in lactase activity between treatments, expressed as per gram or per protein of mucosa, were not statistically significant. However, lactase activity per centimeter and total lactase activity were greater in HDG calves. Whether this difference was related to the age or nutritional level of the calves is unclear.

That the maximal distribution of disaccharidase activity in the small intestine occurred in the upper site is consists with other results (Sir Elkhatim and Osman, 1983; Kreikemeier et al., 1990). However, most previous studies have roughly divided the length of the small intestine into a few segments. Using a division of ten segments, we found that disaccharidase activity per centimeter was most significantly influenced by the nutrition level at the cranial site, 25% of the total length.

Kreikemeier et al. (1990) have reported an increase in energy intake from a maintenance level to the twice elevated α-amylase activity (126%) of the whole pancreas, as well as maltase (74%), isomaltase (62%), and lactase (50%) activity of the whole small intestinal mucosa, and suggest that the increase of disaccharidase activity was largely a function of the greater length of small intestine. In our experiment, when energy intake was 2.4 times the maintenance requirement in HDG versus 1.4 times in LDG calves, total α-amylase activity in the pancreas increased 34%, and the activities of maltase, isomaltase, and lactase in the total small intestinal mucosa increased 193%, 690%, and 194%, respectively. On the assumption that total activity of pancreatic α-amylase and disacchridase of the small intestinal mucosa reflects the ability of an animal to digest starch, it could be concluded that HDG calves were able to digest a much greater quantity of α-glucoside compounds. The adaptation of digestion capacity to different amounts of feed consumption seems to vary with weight increase of tissues synthesizing the enzyme, under condition of small intestine of the same lenght.

In conclusion, digestive enzyme activities of the pancreas and the small intestine of calves was influenced by nutritional level due to the different tissue weights. The distribution of disaccharidase along the small intestine was strongly affected at the peak site of the activity by the nutritional level.

ACKNOWLEDGEMENTS

The authors thank Mr. H. Yamashiro and H. Kubota of the Experimental Farm of Hiroshima University for their help with the animal management.

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