Response of Pancreatic Exocrine Secretion in Sheep Fed Different Type and Amount of Hay

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ABSTRACT : Three wethers fitted with silastic catheters for collection of pancreatic juice, and cannulas located in the abomasum and the duodenum were used to investigate the effects of different hay and energy intake on pancreatic exocrine secretion. The wethers were fed Italian ryegrass hay or alfalfa hay at maintenance energy requirement and alfalfa hay *ad libitum*. High energy intake from alfalfa significantly increased abomasal flow of dry matter and both the concentration and daily secretion of α-amylase. The high energy intake also tended to increase daily secretion of lipase, trypsin and chymotrypsin through the large volume of pancreatic juice. Compared with Italian ryegrass hay, alfalfa hay at the maintenance decreased abomasal dry matter flow, but increased concentration of α-amylase in the pancreatic juice, and tended to increase daily secretion of α-amylase. The secretion of the other enzymes was not different between the two hays at maintenance intake. These results suggest that the kind of hay could change the concentration of α-amylase in the pancreatic juice, and that the intake level of alfalfa hay affects the α-amylase concentration and the juice volume secreted from the pancreas. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 8 : 1044-1049*)

Key Words : Sheep, Pancreatic Juice, Alpha-Amylase, Hay

INTRODUCTION

The digestive enzyme secreted from the pancreas in ruminants, as in non-ruminants, has been regarded as a key for the process of nutrient digestion in the small intestine. In slaughter experiments (Russell et al., 1981; Kreikemeier et al., 1990; Wang et al., 1998), high energy intake of cattle increased the digestive enzyme activity of the whole pancreas. Additionally, Kreikemeir et al. (1990) found that α-amylase activity of the pancreas was greater when cattle were fed a high-forage diet than when fed a high-grain diet, even though both groups were raised at the same level of energy intake. However, higher activity of digestive enzyme in the pancreatic tissue may not result in greater secretion of the enzyme, because pancreatic secretion also varies with diet (Harmon, 1992).

A physiological study indicated that dry matter flow to the post-rumen affected the pancreatic exocrine secretion of sheep (Magee, 1961). An increase in energy intake is accompanied by the increase in post-ruminal digesta flow. Furthermore, the extent of ruminal fermentation and the subsequent digesta flow to the duodenum varies with feed stuffs. At equal levels of energy intake, therefore, the kind of forage consumed by ruminants may affect the pancreatic exocrine secretion.

The objectives of this study were 1) to elucidate the influences of energy intake level from forages, at the maintenance and *ad libitum*, on the daily secretion of pancreatic exocrine in sheep fed alfalfa hay, and 2) to compare alfalfa hay with Italian ryegrass hay at the maintenance energy level in the relation to digesta flow to the abomasum.

MATERIALS AND METHODS

Animals and diets

Three Corriedale×Suffolk wethers, averaged 71 kg (SE: 2 kg) of body weight, were surgically fitted with two silastic catheters into the common bile duct and the bile duct, and two cannulas located in the abomasum and the duodenum immediately distal to the papillae of the common bile duct according to the procedures described by Kato et al. (1984) and Taniguchi and Obitsu (1994). Surgical and animal care procedures for the experiment were followed by the "Guide for the Care and Use of Animals" (Hiroshima University Animal Care Committee). Surgery took place more than 3 weeks before use, and was performed in a sterile environment under general anesthesia by the combination injection of intravenous pentobarbital and intramuscular xylazine. The wethers were kept in individual crates (1.6×0.7 m^2/head) in a temperature-controlled room at 19 to 21℃ with a 14 h light : 10 h dark cycle.

All three wethers received the dietary treatments in the following order: 1) chopped alfalfa hay *ad libitum* (AH); 2) alfalfa hay at maintenance energy level (AM); 3) chopped Italian ryegrass hay at the same maintenance level (IM) according to a feeding standard for sheep (Agriculture, Forestry and Fisheries Research Council Secretariat, 1996). The alfalfa hay contained 18.3% CP and 45.3% NDF in dry matter. The Italian ryegrass hay contained 8.1% CP and 64.8% NDF. When wethers were allowed free access to the Italian

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ryegrass hay after IM treatment, the increase in intake compared with IM was slight due to the low quality. Therefore, we did not measure the response of pancreatic exocrine secretion to *ad libitum* intake of Italian ryegrass hay. All hays were given in two equal portions daily at 08:30 and 18:30 h. All wethers had free access to fresh water and trace mineralized salt blocks. In order to measure digesta flow to the abomasum, 0.5 g of encapsulated chromic oxide, as an indigestible marker, was dosed orally at each feeding time, beginning two weeks before the first sampling.

Secreted bile and pancreatic juices were collected in separate polyethylene bottles on crushed ice, and were continuously returned into the duodenum through the cannula with separate peristaltic pumps according to the secretion rate. The each volume of secreted bile and pancreatic juice was estimated from the speed of the pump and from the juice volume remaining in the polyethylene bottle.

Sample collection

Each experimental period for the three treatments consisted of 14 days for adaptation and 4 days for sample collection. Samples of hay, refusals, abomasal digesta, feces and pancreatic juice were taken during the collection periods. The samples of abomasal digesta (80 g) and pancreatic juice (1.0 ml) were collected 12 times over 4 days. Three sampling times were evenly spaced within each day with sampling times shifted ahead 2 h each day to allow one sample for every 2 h of a 24 h period for each wether during the collection period. The samples were pooled separately for each wether within each sampling period. The abomasal sample was later lyophilized. The pancreatic juice sample was stored at -20℃ until analysis of enzyme activity. All the refusals and feces were collected every morning during the collection periods, and were dried at 55℃ in a forced-air oven for 48 hours. Samples of hay, refusals, abomasal digesta and feces were ground through a 1 mm screen prior to analysis.

Analysis and calculation

Samples of hay, refusal, digesta, and feces were analyzed for dry matter and nitrogen according to the procedures of the AOAC (1975). NDF was determined by the methods of Robertson and Van Soest (1981). Gross energy was determined by adiabatic bomb calorimeter (CA-3, Shimadzu Seisakusho, Japan). Chromic oxide was determined colorimetrically (Yoshida et al., 1967). The sample of pancreatic juice was analyzed for the protein content by the method of Lowry et al. (1951). The activity of α-amylase in the pancreatic juice was measured as described by Hall et al. (1970). The lipase activity was measured by the procedures of Whitaker (1973) using naphthyl palmitate as substrate. 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, after activation by enterokinase (E-5510, Sigma Chemical Co. St. Louis. USA) at 30℃ for 20 min in 15 mmol/L Tris buffer (pH 8.1). The pH of pancreatic juice was determined at each sampling time.

Dry matter flow at the abomasum was calculated by dividing the amount of chromic oxide excreted in the feces by chromic oxide concentration in the digesta. Flow of each nutrient to the abomasum was then calculated by multiplying dry matter flow by the nutrient concentration in the digesta.

Paired t-test was used to analyze the difference between *ad libitum* and maintenance feedings of alfalfa hay, and between alfalfa hay and Italian ryegrass hay at the maintenance level. The comparisons were considered to be significant if probability was equal to or less than 0.10.

RESULTS

Flow and digestion of nutrients

All variables on intake, abomasal flow, fecal excretion and disappearance of dry matter, nitrogen and NDF were greater for AH than for AM (table 1). As a result, digestible energy intake of AH was 1.9 times higher than that of AM. The digestible energy intake for IM was not different from that for AM, but nitrogen intake was less, and the intakes of dry matter and NDF were greater for IM than for AM. Abomasal flows of dry matter, NDF and nitrogen were greater for IM than for AM.

The ruminal disappearance of dry matter was not different between IM and AM, but that of NDF was greater for IM. Nitrogen disappearance in the rumen for IM was negative, and less than that for AM. Disappearance of dry matter and nitrogen in the post-rumen was greater for IM than for AM. In the whole digestive tract, nitrogen disappearance was less for IM than AM.

Pancreatic exocrine secretion

In comparison with AM, AH had a greater volume and higher pH of pancreatic juice, but AM and AH did not differ in protein content of pancreatic juice (table 2). Alpha-amylase activity, expressed as concentration (unit per milliliter pancreatic juice volume), specific activity (unit per milligram protein) or daily secretion was greater for AH than for AM. Daily secretion of chymotrypsin for AH was also greater than for AM, and daily secretion of lipase and trypsin tended to be greater because of the greater pancreatic juice volume.

Table 1. Intake, abomasal flow and digestion of dry matter, nitrogen and neutral detergent fiber by sheep fed hays

Item	Treatment			SEM[4]	Probability	
	AH[1]	AM[2]	IM[3]		AH vs AM	AM vs IM
Dry matter (g/d)						
Intake	1,769	975	1,121	150	0.049	0.086
Flow to abomasum	896	462	604	78	0.055	0.072
Fecal excretion	600	339	378	51	0.042	0.139
Apparently digested						
In rumen	873	513	517	76	0.053	0.467
In intestine	296	123	226	31	0.078	0.058
In total tract	1,168	636	743	137	0.053	0.109
Nitrogen (g/d)						
Intake	53.8	27.8	14.5	6.3	0.042	0.002
Flow to abomasum	30.7	15.3	18.3	2.7	0.048	0.077
Fecal excretion	11.2	5.9	6.8	1.1	0.059	0.130
Apparently digested						
In rumen	23.1	12.4	-3.8	4.2	0.093	<0.001
In intestine	19.5	9.4	11.5	1.8	0.050	0.066
In total tract	42.6	21.9	7.7	5.1	0.038	<0.001
Neutral detergent fiber (g/d)						
Intake	805	441	727	69	0.048	0.013
Flow at abomasum	453	232	293	42	0.068	0.063
Apparently digested in rumen	352	209	433	37	0.021	0.008
Digestible energy						
Intake (kcal/d)	4,797	2,527	2,788	431	0.050	0.179

[1] Alfalfa hay *ad libitum*.
[2] Alfalfa hay at maintenance energy.
[3] Italian ryegrass hay at maintenance energy.
[4] Standard error of the mean.

Table 2. Activity of digestive enzyme secreted from the pancreas in sheep fed hays

Item	Treatment			SEM[4]	Probability	
	AH[1]	AM[2]	IM[3]		AH vs AM	AM vs IM
Pancreatic juice						
pH	7.77	7.62	7.68	0.02	0.038	0.356
Volume, ml/d	409	320	304	28	0.032	0.810
Protein content, mg/ml	54.6	55.4	51.3	1.2	0.660	0.274
Alpha amylase activity						
Units/ml	803	612	435	55	0.016	0.018
Units/mg protein	14.7	11.0	8.5	0.9	0.014	0.005
Daily secretion, kilo units/d	329	195	130	32	0.024	0.165
Lipase activity						
Units/ml	145	98	85	13	0.222	0.712
Units/mg protein	2.66	1.80	1.63	0.24	0.224	0.789
Daily secretion, kilo units/d	59.7	31.1	28.4	6.9	0.122	0.843
Trypsin activity						
Units/ml	708	646	692	33	0.440	0.072
Units/mg protein	12.9	11.6	13.5	0.5	0.444	0.145
Daily secretion, kilo units/d	293	210	211	26	0.149	0.973
Chymotrypsin activity						
Units/ml	476	460	422	13	0.505	0.216
Units/mg protein	8.7	8.3	8.2	0.2	0.405	0.311
Daily secretion, kilo units/d	194	148	132	16	0.054	0.656

[1] Alfalfa hay *ad libitum*.
[2] Alfalfa hay at maintenance energy.
[3] Italian ryegrass hay at maintenance energy.
[4] Standard error of the mean.

Compared with AM, IM did not show significant difference on the variables of the pancreatic exocrine secretion except for α-amylase. The concentration and the specific activity of α-amylase was less for IM than for AM. The daily secretion of α-amylase also tended to be less for IM.

Daily volume of bile juice was greater for AH (3,120 ml) than AM (1,935 ml), but did not differ between AM and IM (1,979 ml).

DISCUSSION

In a previous study (Wang et al., 1998), when the energy level of calves was elevated from 1.4 to 2.4 times of maintenance, the enzyme activity in the whole pancreas was increased by about 1.3 times across all enzymes due to the increased weight of the pancreatic tissue. In the present study, when sheep ingested 1.9 times maintenance (AH) compared with maintenance (AM), the daily secretion of pancreatic juice, trypsin and chymotrypsin was increased by 1.3 times of AM, whereas that of α-amylase and lipase was 1.7 and 1.9 times, respectively. Thus, energy intake level affects both the enzyme activities of the pancreatic tissue and of the secreted juice. It is speculated, however, that a proportion of the enzyme secreted from the pancreas to the same enzyme presented in the pancreas as zymogen is different among the enzymes (Reynolds and Heath, 1981).

The pancreatic response to a dietary stimulus in non-ruminant has been divided into four: cephalic, gastric, intestinal and humoral phases, depending on the site (Solomon, 1994). From a physiological point of view, the mechanism regulating pancreatic exocrine secretion is uncertain due to complex interactions between regulators such as autonomic nervous system, gastro-intestinal hormones and circulation nutrients. Thus, many factors influencing the secretion response of the pancreatic exocrine to energy intake could be involved. From a nutritional point of view, nevertheless, it is clear that high energy intake results in the increases in the pancreatic tissue weight and the pancreatic exocrine secretion. The response of pancreatic secretion to increased intake differs among pancreatic enzymes; the secretion response of trypsin and chymotrypsin to energy intake is less sensitive than α-amylase and lipase.

As expected, the increased energy intake from alfalfa was associated with an increase in post-ruminal nitrogen flow. Daily secretion of protease as trypsin and chymotrypsin tended to increase with increased alfalfa intake. Although high protein diets increase protease synthesis in the pancreas of non-ruminant (Brannon, 1990), abomasal casein infusion does not increase daily protease secretion from the pancreas of sheep (Wang and Taniguchi, 1998). In the present study, therefore, an increase in the pancreatic tissue with the high energy intake might induce the response of protease secretion.

Conversely, the secretion response of lipase for AH compared with AM could be attributable to the increase in abomasal flow of dry matter or nitrogen rather than energy intake per se. In a review, Kato et al. (1991) reported that the flow rate of pancreatic juice of ruminants is reduced to about 40% by fasting for 36 to 48 h or reduced by 30% by prevention of digesta entry into the duodenum, while secretion of lipolytic activity is reduced to 60%. When we infused casein into the abomasum of sheep, the daily lipase secretion increased by 6-fold compared with no casein infusion (Wang and Taniguchi, 1998). On the other hand, when Johnson (Harmon, 1993) infused vegetable oil duodenally, the pancreatic secretion of lipase decreased in sheep.

In ruminants, volatile fatty acids can stimulate the pancreatic α-amylase secretion (Harada and Kato, 1983). Possibly, the greater fermented dry matter in the rumen for AH compared with AM produced more volatile fatty acids which in turn increased α-amylase secretion. In comparison of alfalfa hay with Italian ryegrass hay, only α-amylase secretion increased by 1.5 times. This resulted from the increased concentration of α-amylase in the pancreatic juice without any change in the juice volume. Other studies reported that the post-ruminal flow of dry matter (Magee, 1961) and protein (Wang and Taniguchi, 1998) positively affects α-amylase secretion of sheep, but these factors do not explain the difference between AM and IM.

Increased pancreatic secretion of α-amylase for AH over AM, or for AM over IM may be attributable to different flow of calcium to the abomasum. Because legume hay generally contains calcium more than twice of grass hay, the higher content of calcium in alfalfa hay compared with Italian ryegrass hay might cause the greater secretion of α-amylase. Inoue et al. (1985) found that divalent cations such as calcium and magnesium infused into the duodenum of dogs increased release of cholecystokinin (CCK); CCK is a major hormonal mediator of pancreatic enzyme secretion, and produced by endocrine cells located in the intestine. Reynolds and Heath (1981) observed that CCK administered into the portal vein of sheep did not affect the secretion of juice, protein and chymotrypsin, but increased trypsin slightly and α-amylase appreciably. Furthermore, calcium ions may be an important messenger in acinar cells for amylase release, and the increase in their intracellular concentration is assumed to be derived from both extra- and intracellular sources (Kato et al., 1991). However, because calcium availability in alfalfa hay may be low due to the form in calcium oxalate, further research on pancreatic α-amylase by calcium intake in ruminants will be needed.

As an alternative explanation, the greater NDF flow for IM vs AM to the abomasum may depress α-amylase secretion. Taniguchi et al. (1991) reported that starch digestibility in the small intestine is related to the NDF flow to the post-rumen negatively. Additionally, we found that pancreatic α-amylase secretion is decreased by starch infusion into the abomasum (Wang and Taniguchi, 1998). In the intestinal lumen of ruminants, thus, polysaccharides such as starch or NDF may disturb the stimulation of other nutrients, as amino acids and fatty acids which are quantitatively much less than polysaccharides, to the CCK release cell.

Such speculation, however, does not exclude the possibility of other hormonal influence on α-amylase secretion (Le Huerou et al., 1992), because daily secretion of bile juice (which is strongly regulated by CCK) was not different between alfalfa hay and Italian ryegrass hay in this study.

An increase in α-amylase secretion in ruminants fed high or all-forage diets does not have any nutritional significance on the small intestinal digestion of starch because forages contain little if any starch. However, Taniguchi et al. (1991) observed that the digestibility of starch in the small intestine of sheep varies with the kind of forage and the ratio of concentrate and forage. The type and amount of forage used for a dietary combination with grains, therefore, may influence the post-ruminal digestion of nutrients via the pancreatic exocrine response.

In conclusion, an increase in energy intake from alfalfa hay resulted in an increase in the daily secretion of digestive enzymes from the pancreas of sheep, and α-amylase secretion, even though at same energy intake, was greater with alfalfa hay than with low quality Italian ryegrass hay.

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