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Development of Gastric and Pancreatic Enzyme Activities and Their Relationship with Some Gut Regulatory Peptides in Grazing Sheep

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ABSTRACT : Forty-four Gansu Alpine Fine-wool lambs were used to study changes in the activities of three gastric and five pancreatic enzymes under grazing conditions between 0 and 56 days of age. The lambs were slaughtered on days 0, 3, 7, 14, 21, 28, 42 and 56, the abomasal contents, mucosa and pancreas were immediately removed and placed into liquid nitrogen and enzyme activities were determined. Gastric enzyme (chymosin, pepsin and pregastrc esterase) activities were relatively high at birth, especially chymosin, but decreased quickly between day 0 and 21. The activity of pepsin changed insignificantly with increasing age. There was no significant change in the pancreatic enzyme activities (trypsin, chymotrypsin, α-amylase, lipase and lactase). The activity of trypsin was relatively higher than that of the other pancreatic enzymes, and lactase activity was low. These ontogenic patterns might be under the control of many gut regulatory peptides, the plasma concentrations of which changed simultaneously. Some gastric and pancreatic enzymes were correlated with plasma concentrations of these gut regulatory peptides. (**Key Words :** Digestive Enzymes, Plasma Regulatory Peptides, Grazing Lambs)

INTRODUCTION

During the first post-natal months, the young ruminant is faced with three types of situations requiring physiological and digestive adaptation: adaptation to the extra uterine environment, maintenance in an extended preruminant stage and weaning (Thivend et al., 1980). The effects of age on pancreatic secretions have been more thoroughly investigated in monogastric animals (Corring et al., 1978; Kretchmer, 1985) than in ruminant species (Huber et al., 1961; Guilloteau et al., 1983, 1984, 1985). However, few studies report effects of age on gastric enzyme secretions in young mammals (Walker, 1959; Hartman et al., 1961; Henschel, 1973; Garnot et al., 1977; Foltmann et al., 1981). There are some reports on digestive physiology for non-grazing lambs; for example, it is generally acknowledged that the pre-ruminant abomasum characteristically secretes large amounts of chymosin, which, with pepsin and hydrochloric acid, coagulates milk casein. At 2 days of age there is a threshold of development of enzyme secretion potentiality in lambs. Quantities of gastric enzymes in relation to empty live weight increase between birth and 2 days, but that of chymosin then decreases, whereas pepsin does not change significantly. The evolution of pancreatic enzyme activity was usually the reverse of that of chymosin; however, trypsin activity was low at birth (Guilloteau et al., 1983). But very few studies report on digestive physiology for grazing lambs, and especially in grazing lambs distributed over a plateau at 2,600 m above sea level.

In China, lambs usually stay with their mothers until weaning of non-grazing lambs at the age of two months and of grazing lambs usually at the age of four months. In our experiment, the animals were Gansu Alpine Fine-wool sheep, which are distributed over the northern slope of the east Qilian mountains at the border of Gansu and Qinghai (101°45’E, 37°53’N). The area is a cold alpine pastoral range, and belongs to sub-alpine meadow and mountain rangeland. The altitude ranges from 2,600 m to 3,500 m, even to 4,000 m, and the cold season is longer and there is shortage of pasture forage. Gansu Alpine Fine-wool sheep are a special breed grazing on the plateau at 2,600 m above

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sea level. The study indicated that grazing lambs had a lot of grass in the rumen at 7 days post-partum, which means that at 7 days of age the lambs can eat solid food but are still maintained in the pre-ruminant state until day 28. Solid food enters the rumen, and an increasing amount of microbial digestion is observed together with increased development of the forestomachs. At the end of weaning (4 months old), lambs are no longer pre-ruminant but ruminant. Depending on the feeding conditions, i.e., milk or solid food, substantial differences in the enzyme activities along the digestive tract occur between these two quite different physiological situations. The lamb, therefore, is an interesting model for investigating the effects of development on digestive functions, and studies can result in useful parameters on digestive physiology for grazing lambs on the plateau, regardless of the nutritional substrates involved.

The present work aimed to study the patterns of abomasal and pancreatic enzyme secretions in grazing lambs. The development of gastric and pancreatic digestive functions was tentatively correlated with changes in the plasma concentrations of five gut regulatory peptides.

MATERIALS AND METHODS

Study site description

The animals were from Huangcheng sheep breeding testing farm of Gansu, China. The farm was the first breeding farm for Gansu Alpine Fine-wool sheep which is located at Huangcheng District in the Sunan Yugu autonomous county of Gansu Province of China, which is on the northern slope of the east Qilian mountains at the border of Gansu and Qinghai (101°45’E, 37°53’N). The area is cold alpine pastoral range and belongs to sub-alpine meadow and mountain rangeland. The altitude is from 2,600 m to 3,500 m, even up to 4,000 m. The climate is variable over the year and 4 seasons are not clear. Plateau mainland climate, winds and dryness. The climatic characteristics during the year are drought and cold in winter, warm and humid in summer. Mean annual temperature is 0.6 to 3.8°C, the highest is 31°C in July, and lowest is -29°C in January. Annual sunshine hours are 2,272 h. The absolute frost-free period is 45 to 60 days. Mean annual rainfall is 361.6 mm, annual evaporation is 1,111.9 mm, mean annual relative humidity is 38% to 58%. The natural grass sprouts in April and dies in September, the wilt period of pasture is above 7 months. The soil type is kastanozem, mountain phaeozem and cinnamon soil. The soil is fertile, and water is abundant. The types of natural grassland are grass family, sedge family, weeds of grass family and sedge family and scrub weeds. The vegetation mainly consists of grass family and sedge family as well as a few pea family.

Animals and feeding

There were about 10,000 Gansu Alpine Fine-wool lambs in a breeding flock single born between April 22 and May 22 in 2007. Lambs suckled their dams and grazed on native grassland pasture, with no additional feed. Animals were penned at pasture at night without access to feed and water. There were no feed supplements, but a salt mineral mix was provided to the lambs throughout the year; animals had access to water only once a day. Lambs were not weaned until 4 months old, and therefore they remained with their dams throughout the lactation period. Forty-four single-born male lambs obtained at birth from the herd were randomly distributed into eight groups (Table 1). The experimental period was from May to June, and grass samples were collected according to pasture type, one pasture type from five sampling spots and each sampling area was 1 m^2. Grass kinds included bush cinquefoil (*Potentilla fruticosa Linn.*), linearleaf kobresia (*Kobresia capillifolia* (*Decne.*) *Clarke*), larch needlegrass (*Stipa grandis P.Smirn*), bellard kobresia (*Kobresia bellardii(All.) Degl.*) , and mountain willow (*Salix oritrepha Schneid*). The original samples were clipped. Then, the samples were divided into four equal parts and a randomly chosen part was used to analyze the composition. Nutrient composition

Table 1. Base status of the test lambs

Age (d)	Slaughter number (n)	Birth weight (kg)	Slaughter weight (kg)	Daily gain (g)	Withers height (cm)	Body length (cm)	Heart girth (cm)
0	6	4.02±0.175	4.02±0.175	-	36.00±2.828	29.83±1.472	34.50±2.739
3	8	3.97±0.283	4.57±0.488	229.58±118.127	36.38±2.973	32.88±1.553	36.50±1.690
7	5	3.86±0.498	5.11±0.244	178.86±49.54	38.20±1.095	35.20±1.643	38.60±2.302
14	5	3.59±0.397	6.84±0.996	232.14±66.047	39.80±1.924	36.60±1.673	43.40±2.191
21	5	4.19±0.394	7.01±0.415	134.19±27.376	41.80±1.924	38.80±0.837	42.40±1.517
28	5	4.14±0.397	8.86±1.841	168.57±72.464	39.00±3.674	40.20±1.789	47.40±3.362
42	5	3.83±0.303	10.20±0.837	151.67±21.716	47.20±1.924	44.20±2.588	49.40±2.074
56	5	4.11±0.292	16.44±2.674	220.18±46.73	52.60±2.793	51.40±2.302	61.60±3.715

* Values are means±SEM. ** Statistics is Tukey’test under One-Way ANOVA in SPSS12.0.

Table 2. Nutrient component of pasture*

Month	DM	CP	EE	NDF	ADF	Ash	Ca	P
5	62.22	10.33	2.19	71.67	37.98	11.31	0.61	0.10
6	37.36	10.04	2.14	71.57	39.35	11.16	0.59	0.10

* They were expressed on dry matter bases but dry matter.
** DM = Dry matter; CP = Crude protein; EE = Ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber.

of grazed pasture is presented in Table 2.

Tissue sampling

On the day of slaughter at 3, 7, 14, 21, 28, 42 and 56 days of age the lambs were fed as usual; the lambs slaughtered at 0 d of age were fed nothing. Lambs were removed from their dams at approximately 09:00 h, transported to the research laboratory and final weights were obtained and recorded. Lambs were slaughtered by severing the jugular vein. The abdominal cavity was opened and the entire gastrointestinal tract was removed. The pancreas was carefully dissected free, cleaned of extraneous tissue, weighed, wrapped in pledget and immediately placed into liquid nitrogen. The abomasum was cut open, 100 to 200 g abomasal contents were collected and put into liquid nitrogen, then emptied of its contents, rinsed with ice-cold isotonic saline, gently blotted with filter paper, defatted, weighed and spread out onto a glass plate lying on ice. Finally, the abomasal mucosa of the cardiac, fundic and pyloric gland regions were scraped off with a glass slide, and put into liquid nitrogen. All the frozen samples were subsequently stored at -80°C until use.

Sample preparation

The abomasal mucosa and pancreas were all minced with scissors, and homogenized in 5 volumes of ice-cold 0.4 M KCl (W/V = 1:5) for 45 s in an ice-cold vessel (4°C overnight). The homogenate was centrifuged at 15,000 g (4°C for 20 min), and aliquots of the supernatant were stored at -80°C.

Enzyme analyses

The homogenate of abomasal mucosa was analyzed for chymosin activity using the method described by Arima (1967). Pepsin activity and pregastric esterase activity were determined by spectrophotometry using bovine haemoglobin and olive oil, respectively, as substrates. The activities of pancreatic trypsin and chymotrypsin were determined by spectrophotometry using benzoyl-L-arginine-p-nitroanilide and N-glutaryl-L-phenylalanine-p-nitroanilide, respectively, as substrates. Lipase activity was determined by spectrophotometry using olive oil as a substrate. The α-amylase and lactase activities were assayed using starch and ONPG (o-nitrophenyl-β-D-galactoside), respectively, as substrates.

The results are expressed as enzyme quantities per milligram of tissue protein content for all the enzymes.

Plasma gut regulatory peptide analysis

Blood was collected on EDTA (4%) from an external jugular vein at 9:00 h on the morning before slaughter. Plasma samples were stored at -20°C and concentrations of five gut regulatory peptides were subsequently determined by ELISA methods.

Statistical analysis

Variance analysis was used to assess the effects of age, and Tukey’s test was used to classify the means. Correlation coefficients were used to study the relationships between enzyme activities and plasma concentrations of the gut regulatory peptides. Values were considered to be significant at $p<0.05$.

RESULTS

Development of abomasum pH

pH either in abomasal contents or in gland region mucosa was highest at birth and then decreased with increasing age (except for a high value at 28 d in contents), but remained within the optimal range for enzyme activity. pH was generally similar in each gland region mucosa (Table 3).

Abomasal enzymes

In lambs, the specific activity of chymosin in abomasal contents was highest at birth, but tended to decrease between 0 and 56 d, possibly due to dilution by the intake of milk and grass. There were more differences in enzyme activity of abomasal contents among individuals, possibly affected by enzyme secretion, milk and grass intake. At d28, d35 and d42 the enzyme activity was high for unknown reasons. Between days 0 and 14 the specific activity of chymosin was high in both contents and mucosa, and thereafter it decreased. Between days 0 and 14 the specific activity was lower in the mucosa of the fundic gland region than in the cardiac and pyloric gland regions, and was similar in all regions thereafter except for d28 in the cardiac gland region (Table 4).

Compared to chymosin, the specific activity of abomasal pepsin was lower. Pepsin activity was high at birth and increased little with increasing age. There was a

Table 3. Changes of pH of abomasal contents and mucosa

Age (d)	Slaughter number(n)	Contents	Cardiac gland region mucosa	Fundic gland region mucosa	Pyloric gland region mucosa
0	6	4.67±0.681[a]	4.74±0.896[a]	4.43±0.794[a]	4.36±0.745[a]
3	8	3.39±0.461[ab]	3.53±0.349[a]	3.54±0.362[ab]	3.49±0.326[a]
7	5	3.36±0.474[ab]	3.75±0.621[a]	3.39±0.602[ab]	3.27±0.815[a]
14	5	3.37±0.538[ab]	3.48±0.674[a]	3.62±0.536[ab]	3.52±0.488[a]
21	5	3.29±0.896[ab]	3.75±1.041[a]	3.30±1.070[ab]	3.35±0.905[a]
28	5	4.09±1.331[ab]	3.26±0.644[a]	3.16±0.717[ab]	3.32±0.750[a]
42	5	3.59±0.736[ab]	3.62±0.390[a]	2.86±0.789[b]	3.55±0.579[a]
56	5	2.96±0.184[b]	3.41±0.175[a]	2.94±0.485[b]	3.41±0.357[a]

* Values are means±SEM.
** Statistics is Tukey'test under One-Way ANOVA in SPSS12.0.

Table 4. Developmental changes of abomasal chymosin (U/mg protein)

Age (d)	Slaughter number (n)	Contents	Cardiac gland region mucosa	Fundic gland region mucosa	Pyloric gland region mucosa
0	6	43.00±34.430[a]	21.13±16.498[ab]	18.32±10.377[ac]	37.94±15.660[ab]
3	8	6.92±2.867[a]	52.41±16.556[a]	39.02±16.270[a]	58.60±28.920[a]
7	5	4.21±1.985[a]	49.27±22.490[ab]	55.60±27.100[abc]	14.55±1.328[ab]
14	5	6.64±2.991[a]	29.80±11.575[ab]	21.80±1.323[ab]	29.74±18.168[ab]
21	5	16.24±9.409[a]	12.61±7.702[b]	9.15±3.867[c]	6.92±5.440[b]
28	5	14.60±15.237[a]	23.28±11.671[ab]	8.57±3.117[c]	11.89±8.037[ab]
42	5	12.57±14.391[a]	7.04±3.041[b]	10.79±5.108[bc]	10.23±10.745[b]
56	5	5.35±3.601[a]	5.22±3.229[b]	3.43±2.327[c]	4.07±4.910[b]

* Statistics is Tukey'test under One-Way ANOVA in SPSS12.0.

little difference in different gland region, in fundic gland region mucosa which was highest, and in pyloric gland region was lowest;and in contents which was lowest because of milk and grass diluted (0.003 to 0.009 U/mg protein)(Table 5).

Pregastric esterase activity in abomasal contents was high at birth, and decreased thereafter by milk and grass diluted. In d28 it tended to increase. The enzyme is secreted by the tongue root. It seemed that the secretion capacity had little change from day 0 to 56 (Table 6).

Pancreatic enzymes

The specific activity of trypsin was higher than that of α-amylase, chymotrypsin and lipase. Lactase activity was low. The specific activity of all the tested pancreatic enzymes fluctuated with increasing age except that of chymotrypsin which tended to increase after d14.

Plasma concentrations of gut regulatory peptides

In lambs, considerable changes in the plasma concentrations of gastrin, GIP, CCK, and sectin (Table 8)

Table 5. Developmental changes of abomasal pepsin (U/mg protein)

Age (d)	Slaughter number(n)	Contents	Cardiac gland region mucosa	Fundic gland region mucosa	Pyloric gland region mucosa
0	6	0.008±0.005[a]	0.025±0.005[a]	0.027±0.004[a]	0.016±0.002[ab]
3	8	0.003±0.001[a]	0.025±0.002[a]	0.031±0.003[a]	0.017±0.003[a]
7	5	0.003±0.001[a]	0.027±0.003[a]	0.033±0.004[a]	0.013±0.002[ab]
14	5	0.004±0.002[a]	0.028±0.003[a]	0.034±0.001[a]	0.013±0.004[ab]
21	5	0.005±0.003[a]	0.032±0.006[a]	0.032±0.006[a]	0.011±0.003[ab]
28	5	0.005±0.001[a]	0.030±0.004[a]	0.030±0.013[a]	0.009±0.001[b]
42	5	0.009±0.010[a]	0.027±0.002[a]	0.029±0.004[a]	0.014±0.008[ab]
56	5	0.006±0.002[a]	0.026±0.004[a]	0.031±0.003[a]	0.013±0.004[ab]

* Statistics is Tukey'test under One-Way ANOVA in SPSS12.0.

Table 6. Developmental changes of abomasal pregastric esterase (U/mg protein)

Age (d)	Slaughter number(n)	Pregastric esterase activity
0	6	0.083±0.041[a]
3	8	0.034±0.008[a]
7	5	0.037±0.004[a]
14	5	0.040±0.010[a]
21	5	0.036±0.009[a]
28	5	0.052±0.013[a]
42	5	0.057±0.012[a]
56	5	0.077±0.019[a]

* Statistics is Tukey'test under One-Way ANOVA in SPSS12.0.

occurred with increasing age, especially between days 0 and 42, which tended to decrease. CCK concentration was sharply decreased by 1/15 between 3 and 42 d, but increased by 3-fold between d 42 and 56. The secretin and gastrin concentrations reached a peak at d 3, and thereafter which decreased and on d 42 were as 45% and 36% as of d 3. GIP concentration was high between 0 and 7 d and decreased at d 14 and d 28. At d 42 it decreased 9.2% of d 7. Increased plasma PP concentration was recorded between 0 and 42 d.

Correlation analyses

No significant correlation was found between plasma concentrations of CCK,secretin, PP, gastrin, GIP and chymosin activity in the contents ($p>0.05$). By contrast, significant positive correlations were observed between plasma concentrations of CCK, secretin, gastrin, GIP and the chymosin activity in the mucosa of the cardiac, fundic and pyloric gland regions ($p<0.01$ or $p<0.05$), while significant negative correlation was recorded between plasma concentration of PP and the chymosin activity in the mucosa of the fundic and pyloric gland regions ($p<0.01$ or $p<0.05$). Significant negative correlations were found between plasma concentrations of CCK, secretin, gastrin, GIP and pepsin activity in the contents ($p<0.05$). Only plasma concentration of GIP was positively correlated with the specific activity of pepsin in the mucosa of the pyloric gland region ($p<0.05$). The plasma concentration of PP was positively correlated with the specific activity of pepsin in the mucosa of the cardiac gland region while negatively correlated with the pyloric gland region ($p<0.05$). Significant negative correlations were recorded between the plasma concentrations of CCK, secretin, gastrin and pregastric esterase activity of the contents ($p<0.05$), and no significant correlation was observed between the plasma

Table 7. Developmental changes of pancreatic enzymes (U/mg protein)

Age (d)	Slaughter number(n)	α-amylase	Lactase	Lipase	Trypsinase	Chymotrypsin
0	6	0.267±0.016[a]	0.006±0.002[b]	0.041±0.007[a]	1.607±0.317[a]	0.105±0.032[b]
3	8	0.244±0.018[ab]	0.010±0.002[a]	0.030±0.004[a]	1.475±0.407[a]	0.124±0.031[ab]
7	5	0.249±0.017[ab]	0.007±0.001[ab]	0.038±0.010[a]	1.325±0.322[a]	0.105±0.012[b]
14	5	0.259±0.021[a]	0.010±0.002[ab]	0.039±0.004[a]	1.644±0.080[a]	0.158±0.032[ab]
21	5	0.209±0.037[b]	0.008±0.002[ab]	0.037±0.010[a]	1.617±0.129[a]	0.157±0.031[ab]
28	5	0.261±0.031[a]	0.010±0.003[ab]	0.038±0.006[a]	1.534±0.201[a]	0.166±0.028[a]
42	5	0.259±0.015[a]	0.009±0.002[ab]	0.035±0.009[a]	1.556±0.195[a]	0.156±0.024[ab]
56	5	0.248±0.013[ab]	0.010±0.006[ab]	0.037±0.004[a]	1.579±0.121[a]	0.156±0.040[ab]

* Statistics is Tukey'test under One-Way ANOVA in SPSS12.0.

Table 8. Development changes of plasma regulatory peptide concentrations

Age (d)	Slaughter number (n)	CCK (pmol/L)	Secretin (ng/ml)	PP (ng/ml)	Gastrin (ng/ml)	GIP (ng/ml)
0	6	28.128±10.164[ab]	36.440±14.221[abc]	0.223±0.057[b]	9.108±1.921[ab]	0.441±0.125[a]
3	8	53.801±17.396[a]	54.496±7.731[a]	0.308±0.047[ab]	12.494±1.873[a]	0.425±0.192[a]
7	5	51.624±26.714[abc]	52.522±9.701[ab]	0.342±0.024[ab]	11.872±2.852[ab]	0.467±0.315[ab]
14	5	27.985±25.671[abc]	36.895±14.235[abc]	0.427±0.068[a]	7.051±3.513[ab]	0.167±0.084[ab]
21	5	43.454±32.693[abc]	43.970±17.876[abc]	0.403±0.127[ab]	9.724±4.906[ab]	0.161±0.116[ab]
28	5	20.582±20.512[abc]	31.282±12.29[bc]	0.440±0.087[ab]	6.300±3.388[ab]	0.068±0.051[b]
42	5	3.543±2.799[c]	24.820±8.593[c]	0.585±0.347[ab]	4.476±2.093[b]	0.043±0.051[b]
56	5	10.032±14.654[b]	29.251±8.349[bc]	0.437±0.098[ab]	5.090±2.320[b]	0.062±0.049[b]

* Statistics is Tukey'test under One-Way ANOVA in SPSS12.0.

** CCK = Cholecystokinin; PP = Pancreatic polypeptide; GIP = Gastric inhibitory peptide; same as following.

Table 9. Correlation between plasma regulatory peptide concentrations and abomasal enzyme activities

		CCK	Secretin	PP	Gastrin	GIP
Chymosin	Cardiac gland region	0.633**	0.530**	-0.230	0.595**	0.607**
	Fundic gland region	0.616**	0.570**	-0.343*	0.598**	0.691**
	Pyloric gland region	0.495**	0.377*	-0.438**	0.500**	0.676**
Pepsin	Cardiac gland region	-0.061	-0.106	0.309*	-0.153	-0.222
	Fundic gland region	0.064	0.201	0.234	0.089	-0.050
	Pyloric gland region	0.217	0.199	-0.326*	0.271	0.471*
Pregastric esterase	Contents	-0.346*	-0.350*	0.018	-0.345*	-0.200

* Statistics is Pearson in SPSS12.0.

concentrations of PP, GIP and pregastric esterase activity ($p>0.05$).

The plasma concentrations of CCK, secretin, PP, gastrin and GIP were all positively or negatively correlated with the specific activity of chymotrypsin ($p<0.01$ or $p<0.05$). The plasma concentrations of CCK and GIP were negatively correlated with the activity of lactase ($p<0.05$). Moreover, negative correlation was observed between α-amylase activity and the plasma concentration of secretin ($p<0.05$). No significant correlation was found between the plasma concentrations of CCK, secretin, PP, gastrin, GIP and the lipase, trypsin activities ($p>0.05$).

DISCUSSION

Grazing condition and grazing pasture

Gansu Alpine Fine-wool sheep grazes on alpine cold pastoral range which belongs to sub-alpine meadow and mountain rangeland. The altitude is from 2,600 m to 3,500 m, even to 4,000 m. The climate is plateau mainland climate, winds and dryness. The climatic characteristics in a year are drought and cold in winter, warm and humidity in summer. Mean annual temperature is 0.6 to 3.8°C, the highest is 31°C in July, and lowest is -29°C in January. Annual sunshine hours are 2,272 h. The absolute frost-free period is 45 to 60 days. Mean annual rainfall is 361.6 mm, annual evaporation is 1,111.9 mm, mean annual relative humidity is 38% to 58%. The natural grass sprouts in April and dies in September, the wilt period of pasture is above 7 months. The soil type is kastanozem, mountain phaeozem and cinnamon soil. The types of natural grassland are grass family, sedge family, weeds of grass family and sedge family, scrub weeds. The vegetation mainly consists of grass family and sedge family as well as a few pea family.

Lambs suckle their mothers and graze on native grassland pasture, with no additional feed. Animals are penned at pasture at night without access to feed and water. There are no feed supplements, but a salt mineral mix is provided to lambs throughout the year, animals have access to water only once a day. Lambs are not weaned until 4 months old.

The abomasal pH

The pH changed from 2.96 to 4.7 in the abomasal contents and from 2.8 to 4.8 in the mucosa.The pH was relatively low in the mucosa of the fundic gland region. The pH of pancreas didn’t change with increasing age. The results agree with previous findings in young ruminants (Gorrill et al., 1967). But these are the first for lambs on pasture in China.

Change of abomasal enzyme activities

All the enzyme activities recorded in the abomasum were high at birth, agree with the results reported by previous reports (Huber, 1969; Era Berinkovd, 1988). Pepsin activity was lower than that of chymosin.This finding confirmed the importance of chymosin in milk clotting, because its activity level was 100-200% higher than that reported for pepsin (Raymond et al., 1973). Pepsin activity tended to increase with increasing age, but chymosin activity reversed. After d14 chymosin activity in the mucosa decreased sharply. The results agree with the

Table 10. Correlation between plasma regulatory peptide concentration and pancreatic enzyme activities

	CCK	Secretin	PP	Gastrin	GIP
α-Amylase	-0.264	-0.298*	0.048	-0.219	-0.065
Lactase	-0.305*	-0.196	0.228	-0.294	-0.353*
Lipase	-0.190	-0.238	0.221	-0.183	-0.194
Trypsinase	0.008	0.016	0.147	-0.008	-0.007
Chymotrypsin	-0.303*	-0.307*	0.514**	-0.363*	-0.532**

* Statistics is Pearson in SPSS12.0.

most reports about chymosin of calves abomasal mucosa (Andren, 1980; Valles, 1980), contents (Garnot et al., 1977), gastric juice (Alais, 1963; Hill et al., 1970; Kirton et al., 1971; Hagyard and Davey, 1972) and lambs abomasal mucosa (Guilloteau, 1983), but disagree with Garnot (Garnot et al., 1977). The decreasing chymosin activity may be the increasing grass intake and decreasing milk intake after d14, which weaken the stimulus of abomasum resulting in the decrease of chymosin secretion. According to the previous reporting, milk (most probably its casein fraction) is responsible for the activation of chymosin secretion (Garnot et al., 1977).

Pepsin activity in abomasal contents was relatively high and decreased to the lowest at d3, possibly because of dilution by milk intake, and thereafter it began to increase. The development of secretion potentiality of abomasum appeared to depend on age and intake. In the testing, pepsin activity in the mucosa of the cardiac and fundic gland regions was higher than that in the pyloric gland region. As a whole, the changes of pepsin activity agree with previous findings in young ruminants (Guilloteau et al., 1984; Huber et al., 1961; Isabelle et al., 1992). Pregastric esterase activity was high at birth, at d 3 which decreased by dilution of milk intake and increased a little after d 28. It appeared that the secretion potentialities of root of tongue changed little between d 0 and 56.

Change of pancreatic enzyme activities

Trypsin, chymotrypsin are all secreted by pancreas. It also secretes carboxypeptidases, nucleic acid enzyme and so on. All those enzymes flow into small intestine through bile duct along with pancreatic liquid. It reported that pancreatic proteolytic enzyme contributes approximately 72% of the protein in pancreas. Trypsin and chymotrypsin activities were 6-fold of the summation of α-amylase, lactase as well as lipase, and erepsin secreted by small intestine epithelia, so the digestibility of protein was very strong. The pancreas was relatively mature at birth, but the relative weight (% body weight) of it was still growing, and the function was increasing after d 21. Trypsin activity changed little with increasing age,but chymotrypsin activity tended to increase after d 14. Lipase activity existed at birth but change little with age. Under grazing conditions, the dietary contain more fat only in the period of suckling. The fat in the grass is about 1% to 4%, which is hydrolyzed by rumen microorganism into digestible fatty acid (salt) and flows into small intestine. Lipase activity in pancreas and small intestine mucosa increased little with increasing age, but still digested the fat in the diet. Pancreatic lactase activity was high at birth, but was lower than that in small intestine mucosa. Disaccharidases (lactase and maltase) are mainly secreted by small intestine mucosa and epithelium.

α-Amylase is secreted by pancreas and small intestine. According to the reports, in young pigs and calves, α-amylase was low at birth and increased with age. The dietary of weaning animals doesn't add starch generally. In the present study, the animals were grazing lambs, starch in the dietary was very little, but α-amylase activity was observed in pancreas at birth. Pancreatic activity was greater than that in small intestine mucosa. Pancreatic α-amylase activity had insignificant change with increasing age.

Relationships with gut regulatory peptides

Significant change was observed in plasma concentrations of gut regulatory peptides. High concentrations of gastrin, CCK and secretin at d3, and GIP at 7 d in plasma were observed. Thereafter they decreased and at d 42 reached the lowest point. By contrast, the concentration of plasma PP was relatively low at birth and increased with age and by d42 it reached the highest. The changes of all plasma gut regulatory peptides tested were consistent with the change of the rumen function. Adaptation of chymosin activity to dietary changes under the grazing conditions may be influenced by the decrease of CCK, secretin, gastrin, GIP, or increase of PP concentrations. In the present study, all the tested plasma concentrations of gut regulatory peptides seemed to be involved in the regulation of abomasal mucosa chymosin activity, because it was highly correlated with abomasal mucosa chymosin activity. And the change of CCK, secretin, gastrin and GIP were consistent with the change of chymosin activity. All of them decreased with increasing age, while PP reversed. On the contrary, all the tested plasma concentrations of gut regulatory peptides did not seem to be involved in the regulation of pepsin and pregastric esterase activities, because they had little correlation with the specific activity of them. This disagreed with previous reporting (Daviccon et al., 1980; Pierzynowski et al., 1991), maybe influenced by the management system (grazing) or the time of blood collection. The plasma concentrations of all the tested plasma gut regulatory peptides may have decisive, but opposite, influences on pancreatic chymotrypsin is supported by the correlations observed with the pancreatic chymotrypsin. But they did not seem to be involved in the regulation of pancreatic trypsin and lipase, because they were little correlated with trypsin and lipase activities. Only secretin seemed to be involved in the regulation of pancreatic α-amylase because they negatively correlated with each other. CCK, GIP seemed to be involved in the regulation of pancreatic lactase because they were negatively correlated with each other (but we didn't find reports at present), because lactase always be deemed to be secreted by small intestine mucosa. But in the present study considerable lactase activity was observed.

CONCLUSION

pH was acidic in abomasum (relatively lower in fundic gland region) and alkaline and stable in pancreas. α-Amylase, lactase, lipase, trypsinase and chymotrypsin activities were observed in pancreas at birth, and changed little with increasing age. The concentrations of gastrin, GIP, CCK, and secretin in plasma tended to decreasing with increasing age, and PP tended to increase. The correlation between the concentration of pancreatic polypeptide and other regulatory peptide concentrations seemed to be negative. Gastrin, GIP, CCK, and secretin may play a positive regulatory function to chymotrypsin activity in abomasum, while pancreatic polypeptide had a negative regulatory function. The tested plasma regulatory peptides were insignificantly correlated with pepsin and pregastric esterase activities in abomasum. The plasma regulatory peptides correlated with chymotrypsin activity in pancreas positively or negatively; PP positively regulates α-amylase activity in pancreas; GIP and CCK negatively regulate the lactase activity in pancreas.

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