Journal of Animal and Veterinary Advances 11 (12): 2129-2137, 2012
ISSN: 1680-5593

Influences of Total Mixed Diets with Different Concentrate-Roughage Ratio on pH and Activity of Digestive Enzymes in Alimentary Canal of Fattening Lambs at Tibetan Plateau

[1, 2]Jian-Bin Liu, [1]Fa-Di Li, [3]Fan Wang, [2]Xia Lang, [2]Jian Guo, [2]Xiao-Ping Sun, [2]Yao-Jing Yue, [2]Rui-Lin Feng and [2]Bo-Hui Yang
[1]Faculty of Animal Science and Technology, Gansu Agricultural University, 730070 Lanzhou, China
[2]Lanzhou Institute of Husbandry and Pharmaceutical Sciences of Chinese Academy of Agricultural Sciences, 730050 Lanzhou, China
[3]Lanzhou Veterinary Research Institute of Chinese Academy of Agricultural Sciences, China Agricultural Veterinarian Biology Science and Technology Co. Ltd., 730046 Lanzhou, China

Abstract: This study was conducted to investigate the effects of concentrate-roughage ratio in diets on concentration of VFA in rumen liquid, pH and digestive enzyme activities in different sections of alimentary canal. The 48 weaned male lambs of Ganjia sheep weighing (23.18±2.16) kg at the age of 3-5 months were divided similarly into three groups which each group included 16 lambs. Lambs were fed diets with a concentrate-roughage ratio of 40:60 (A), 50:50 (B) and 60:40 (C). Digestible energy and crude protein levels were 0.8, 0.9 and 1.0 times the NRC-recommended levels during the 60 days experimental period. About 12 lambs from each group were slaughtered for sampling at the end of the regular feeding period. The results showed that concentration of Total VFA (TVFA) in rumen liquid of lambs from Group C was obviously higher than those from Group A and B ($p<0.05$); the pH of rumen liquid, homogenates of mucosa and contents in posterior segment-jejunum and of contents in ileum were affected significantly by the concentrate-roughage ratio of diets ($p<0.05$); there were non-uniform pattern ranked of activities of various digestive enzyme along small intestine with concentrate-roughage ratio of the diets; the highest activities of chymotrypsin in content and those of lipase in mucosa homogenate and content occurred in the middle piece-jejunum, activity of trypsinase in contents of posterior segment-jejunum was the highest and the highest activities of α-amylase were noted in content of duodenum and posterior segment-jejunum. It was concluded that still acetic acid pattern of rumen fermentation was kept in lambs fed total mixed diet with higher concentrate-roughage ratio; the pH of contents in rumen and abomasums of mucosa and contents in posterior segment section of small intestine were affected by the concentrate-roughage ratio of the diets; there was also higher activity of α-amylase in duodenum.

Key words: Fattening lamb, concentrate-roughage ratio, nutritional level, pH of alimentary canal, digestive enzymes

INTRODUCTION

In China, sheep breeding has always played an important role mainly in less developed, pastoral and mountain areas where the production systems are interrelated with local traditions and native breeds. The Ganjia is one of the sturdy and meat type breed of sheep which is a new turf-type Tibetan sheep population and which also provide green organic mutton for the 28th Olympic Games. The animals were from the Ganjia town of Xiahe county of Gansu province of China which is on the Northwest slope of the East Qilian mountains at the border of Gansu and Qinghai (102°31'E, 35°12'N). The area is cold alpine pastoral range and belongs to sub-alpine meadow and mountain rangeland. The altitude is from 2,500-3,800 m even upto 2,950 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 2.6°C, the highest is 10.8°C in July and lowest is -9.4°C in January. Annual sunshine hours are 2,270 h. The absolute frost-free period is 60-88 days. Mean annual rainfall is 325.1 mm, annual evaporation is 1,120 mm, mean annual relative humidity is 40-80%. The soil type is Kastanozem, mountain Phaeozem and

Corresponding Author: Fa-Di Li, Faculty of Animal Science and Technology, Gansu Agricultural University, 730070 Lanzhou, China

Cinnamon soil. 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.

Two significant constituents of animal diets are protein and energy and each plays a vital role in animal growth, production and reproduction (Fazaeli *et al*., 2006; Nassier, 2010; Enyenihi *et al*., 2009; Erener *et al*., 2010; Hlophe and Moyo, 2011; Misieng *et al*., 2011; Khan, 2012; Ofuya, 2006). Feeding diets with different concentrate-roughage ratio of protein and energy in intake feed has benefits for feed lot performance in steers (Sultan *et al*., 1991). Enzose (corn dextrose), maize bran and maize gluten are all products of the maize industry. All are also potential sources of protein and energy for ruminants (Nisa *et al*., 2004; Khan *et al*., 2004; Javaid *et al*., 2008) and are generally used to fatten the animals. Supplementation of the typical diet of concentrate-roughage ratio not only enhances the protein and energy contents of the diet but also results in the increased nutritional worth of corncobs (Nisa *et al*., 2004). Digestive enzymes also play an important role in herbivore digestive processes particularly because protein components dominate in the bodies of their prey. Several digestive enzymes are used to digest heterogeneous food items; the enzymes are secreted from salivary glands leading into the mastax from gastric glands leading into the stomach and from secretion cells of the stomach (Kleinow *et al*., 1991; Kleinow and Rohrig, 1995; Yu and Cui, 1997). Trypsinase, chymotrypsin, α-amylase and lipase, all assumed to have a digestive role have been successfully isolated (Herold and Meadow, 1970; Wurdak, 1987; Kleinow, 1993; Hara *et al*., 1997). However even these enzymes are somewhat susceptible to digestive proteases and/or low pH values. In addition, concentrate diets are generally provided because DMI and VFA production are higher than with diets based only on roughage. On the other hand, concentrate diets may cause a rapid accumulation of the fermentation end products accompanied by a decrease in pH (Beharka *et al*., 1998), a decrease in rumen motility (Nocek, 1997; Owen *et al*., 1998) and a decrease in VFA absorption (Hinders and Owen, 1965). Therefore, digestive enzyme research requires more sophisticated technical and regulatory investigation in different sections of the alimentary canal of fattening lambs on Tibetan plateau.

This study is part of a project that aims to determine digestive enzymes and pH value of weaned male lambs of Ganjia sheep slaughtered at one factorial treatment, in which the lambs had been fed on the diets with concentrate-roughage ratios of 40:60, 50:50 and 60:40 during 60 days experimental period. During this time, digestive enzymes and pH value of different sections of the alimentary canal of mucosa and contents were investigated. The investigation was done on the relationship of the concentrate-roughage ratio to digestive enzymes and pH value as discovered from cultures developed under controlled conditions and at sufficient densities.

MATERIALS AND METHODS

Experiment design and animals: The study was adapted to one factorial design of 48 lambs which were divided into three groups with each group containing 16 lambs. Weaned male lambs of Ganjia sheep at the age of 3-5 months fed on diets with three different concentrate-roughage ratios of 40:60 (A), 50:50 (B) and 60:40 (C). Digestible energy and crude protein levels were at 0.8, 0.9 and 1.0 times the NRC recommended levels. Experimental treatment consists of three phases: a transition period of 10 days, a preliminary trial period of 20 days and a regular experimental period of 60 days.

Experimental diet composition and preparation: The experimental diet composition was designed based on levels recommended by the NRC for fattening lambs with a body weight of 30 kg and average daily intake weight of 295 g. Nutritional requirements were expressed in Digestible Energy (DE) and CP. The diet included feedstuffs of Dry Matter (DM), Crude Protein (CP), Calcium (Ca) and Phosphorus (P). Experimental diets were elaborated granular pattern test diets according to feed formulation of experimental design (particle diameter: 5 mm and particle length: 10 mm). The nutritional composition and content of feed used during the fattening period is shown in Table 1.

Table 1: Composition and nutrient levels of diets (dry matter-based)

Ingredients	A	B	C
Corn	8.42	25.74	42.38
Soybean meal	9.60	10.50	12.10
Rapeseed meal	5.00	5.60	6.50
Cottonseed meal	5.50	6.60	7.00
Alfalfa meal	16.50	11.30	8.00
Wheat bran	7.92	4.40	1.00
Wheat straw	18.00	14.50	10.00
Corn straw	28.40	20.60	12.00
Limestone powder	0.10	0.20	0.46
Salt	0.26	0.26	0.26
Premix*	0.30	0.30	0.30
Total	100.00	100.00	100.00
Nutrient level			
DE (MJ kg^{-1})	10.86	11.30	11.92
CP (%)	11.72	12.40	13.32
CF (%)	18.65	14.98	11.52
Ca (g kg^{-1})	0.53	0.56	0.57
P (g kg^{-1})	0.29	0.28	0.27
Calcium-phosphorus ratio	1.96	2.00	1.97
Crude protein-digestible energy ratio (g MJ^{-1})	11.18	11.20	11.24
Concentrate-roughage ratio	40:60	50:50	60:40

*Provided elements (mg) and vitamins (IU) per kilogram diet: S 200; Zn 40; Mn 40; Fe 25; Cu 8; I 0.3; Se 0.2; Co 0.1; VA 940; VE 20

Feeding and management of lambs: Climate-suited semi-open pen conditions housed the lambs during the fattening period and the stocking density was 0.7 m^2 per lamb. The diet of B was fed to lambs in the transition period and in the preliminary trial period because it is closest to local, large-scale fattening lamb diets.

Before the fattening period began, 1 day of dietary adaptation was applied to guard against parasites in the adaptation period. During the fattening period, lambs were weighed individually on a daily basis and daily feed consumption was recorded. Daily the ram lambs were fed diets of A-C at 07:00, 12:00, 17:00 and 22:00 h, lambs were fed the same food amount regularly, 4 times daily during the experimental period. All lambs had free access to water.

Samples: Twelve selected lambs from each group were slaughtered for sampling at the end of the regular feeding period. Lambs were transported to the research laboratory and final weights were obtained and recorded which 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.

Rumen, reticulum, pancreas, duodenum, jejunum of anterior segment, middle piece and posterior segment and ileum were cut off, 100-200 g abomasal and small intestinal 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 and small intestinal 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.

pH and digestive enzymes analyses: pH levels in the pancreas, abomasums and small intestinal mucosa were measured with a portable pH meter. The homogenate of abomasal mucosa was analyzed for chymosin activity using the method described by Arima. Pepsin activity were determined by spectrophotometry using bovine haemoglobin and olive oil as substrates. The activities of pancreatic trypsin and chymotrypsin were determined by spectrophotometry using benzoyl-Larginine-p-nitroanilide and N-glutaryl-L-phenylalanine-pnitroanilide, respectively as substrates. Lipase activity was determined by spectrophotometry using olive oil as a substrate. The α-amylase was measured according to determination with methods of enzyme activity of lipase was measured according to principles and methods of biochemical experiments (Li, 1994).

Statistical analyses: All statistical analyses were performed using the SPSS 12.0 statistical package. The differences among groups were tested by analysis of variance with one factorial and Tukey test. Values were considered to be significant at $p<0.05$.

RESULTS

pH and VFA concentration of rumen liquid: The pH value in rumen liquid of lambs from Group C was obviously smaller than those from Group A and B ($p<0.05$) and concentration of Total VFA (TVFA) in rumen liquid of lambs from Group C was obviously higher than those from Group A and B ($p<0.05$) which accorded with testing results of pH value. Among other indicators of treatments was no significant difference ($p>0.05$) (Table 2).

pH and activity of pepsin in abomasum mucosa and contents: The pH and activities of pepsin were observed in the abomasum mucosa and contents (Table 3). pH value in the abomasums of mucosa and contents was not significantly affected by nutrient levels of the concentrate-roughage ratio of diets ($p>0.05$) however which was similar with pH value of trends in rumen liquid. The activity of pepsin of treatments in abomasum mucosa and contents was no significant difference ($p>0.05$) but the activity of pepsin in abomasum contents of lambs from Group A was obviously higher than those from Group C ($p = 0.097$).

Table 2: pH and VFA concentration of rumen liquid

			Molar ratio of acids				
Treatments	pH	TVFA (mmol·L^{-1})	Acetic	Propionic	Butyric	Isovalerate+valerate	Acetic/Propionic
A	6.16±0.18^a	158.84±72.13^b	0.65±0.03^a	0.18±0.02^a	0.15±0.02^a	0.02±0.01^a	3.55±0.36^a
B	6.01±0.30^a	140.12±26.96^b	0.64±0.03^a	0.19±0.01^a	0.14±0.03^a	0.02±0.01^a	3.46±0.76^a
C	5.72±0.11^b	246.32±56.86^a	0.62±0.01^a	0.19±0.02^a	0.17±0.04^a	0.03±0.02^a	3.36±0.82^a

Means with different lowercase letter superscripts within the same column differ significantly ($p<0.05$)

pH and activity of digestive enzymes in pancreas: The pH value and activity of digestive enzymes (trypsinase, chymotrypsin, α-amylase and lipase) in pancreas was not significantly affected by nutrient levels of the concentrate-roughage ratio of diets (p>0.05) (Table 4).

Activity of trypsinase and chymotrypsin in small intestinal contents: With the exception of the duodenum and ileum, there was a significant increase in trypsinase activity toward the distal jejunum. Trypsinase and chymotrypsin activity in the duodenum contents showed that activity in C was significantly higher than that of B (p<0.05) and activity of trypsinase showed a higher tendency than that of A (p = 0.081). Other treatments of these two enzyme activities showed no significant difference in the intestines (p>0.05). However, results showed a higher tendency of trypsinase activity in middle piece-jejunum of contents of lambs from Group C (p = 0.099) and showed a lower tendency of trypsinase activity in the middle piece-jejunum and posterior segment-jejunum of contents of lambs from Group B (p = 0.063, p = 0.060).

The activity of trypsinase of contents was significant higher in the posterior segment-jejunum than in that of the anterior segment-jejunum, middle piece-jejunum, duodenum and ileum (p<0.05) and it was significant higher in the middle piece-jejunum and ileum than in that of the anterior segment-jejunum (p<0.05) and it was a higher tendency in duodenum than in that of anterior segment-jejunum (p = 0.069). The activity of chymotrypsin in the middle piece-jejunum of contents was significant higher than in that of the duodenum, anterior segment-jejunum, posterior segment-jejunum and ileum (p<0.05) and the activity of chymotrypsin in the posterior segment-jejunum of contents was significant higher than in that of the anterior segment-jejunum (p<0.05) (Table 5).

Activity of α-amylase and lipase in small intestine: The activity of α-amylase of lambs from Group B and C in ileum contents was obviously lower than those from A (p<0.05) and the activity of lipase of from Group C in duodenal contents was significantly higher than those from A and B (p<0.05). Lipase activity of lambs from Group B in the middle piece-jejunum of contents (p = 0.061) and C in the posterior segment-jejunum of mucosa (p = 0.071) showed a lower tendency.

The activity of α-amylase was significantly different in the duodenum contents, middle piece-jejunum, posterior segment-jejunum and in the ileum and anterior segment-jejunum (p<0.05). Lipase activity of the contents of the anterior segment-jejunum and middle piece-jejunum showed a significant difference from that of the duodenum, posterior segment-jejunum and ileum (p<0.05). Lipase activity in the ileum was also more significant than in the duodenum and posterior segment-jejunum (p<0.05). Similarly, α-amylase activity of mucosa decreased in the distal intestine. Its activity was powerful in the proximal intestine. The α-amylase activity in duodenum mucosa, anterior segment-jejunum, middle piece-jejunum and the activity of the posterior segment-jejunum and ileum showd a significant difference (p<0.05). The difference of activity in the posterior segment-jejunum mucosa and the

Table 3: pH and activity of pepsin in abomasum mucosa and contents

	pH		Activity of pepsin in abomasums ($U \cdot mg^{-1}$ protein)	
Treatments	Mucosa	Content	Mucosa	Content
A	5.16±0.42^a	3.46±0.34^a	0.042±0.01^a	0.034±0.02^a
B	5.02±0.41^a	3.47±0.66^a	0.046±0.02^a	0.048±0.03^a
C	4.87±0.22^a	3.28±0.20^a	0.046±0.02^a	0.022±0.01^a

Table 4: pH and activity of digestive enzymes in pancreas

Treatments	pH	Trypsinase	Chymotrypsin	α-amylase	Lipase
A	6.73±0.09^a	0.35±0.03^a	0.10±0.02^a	0.57±0.02^a	5.56±0.63^a
B	6.79±0.08^a	0.33±0.04^a	0.10±0.02^a	0.58±0.04^a	6.59±0.45^a
C	6.77±0.10^a	0.33±0.04^a	0.09±0.01^a	0.57±0.02^a	6.79±0.65^a

Means with different lowercase letter superscripts within the same column differ significantly (p<0.05)

Table 5: Activity of trypsinase and chymotrypsin in small intestinal contents ($U \cdot mg^{-1}$ protein)

		Jejunum			
Treatments	Duodenum	Anterior segment	Middle piece	Posterior segment	Ileum
Activity of trypsinase					
A	0.251±0.125^{ab}	0.142±0.042^a	0.268±0.092^a	0.503±0.102^a	0.408±0.113^a
B	0.190±0.072^b	0.187±0.045^a	0.232±0.16 4^a	0.496±0.226^a	0.224±0.120^a
C	0.351±0.076^a	0.237±0.217^a	0.352±0.050^a	0.504±0.150^a	0.377±0.165^a
Average	0.264±0.112^{BC}	0.189±0.082^C	0.284±0.102^B	0.501±0.170^A	0.336±0.15^B
Activity of chymotrypsin					
A	0.021±0.006^{ab}	0.022±0.006^a	0.040±0.016^a	0.027±0.006^a	0.021±0.005^a
B	0.017±0.006^b	0.017±0.003^a	0.037±0.012^a	0.023±0.005^a	0.024±0.007^a
C	0.025±0.004^a	0.017±0.004^a	0.043±0.015^a	0.026±0.006^a	0.026±0.004^a
Average	0.021±0.005^{BC}	0.019±0.005^C	0.040±0.014^A	0.025±0.005^B	0.024±0.005^{BC}

$^{a, b}$Means with different lowercase letter superscripts within the same column differ significantly (p<0.05); $^{A-C}$Means with different capital letter superscripts within the same row differ significantly (p<0.01)

Table 6: Activity of α-amylase and lipase in small intestine ($U \cdot mg^{-1}$ protein)

Activity	Treatments		A	B	C	Average
α-amylase		Duodenum	0.180 ± 0.035^a	0.173 ± 0.021^a	0.192 ± 0.056^a	0.182 ± 0.034^A
	Mucosa	Jejunum				
		Anterior segment	0.170 ± 0.036^a	0.176 ± 0.036^a	0.138 ± 0.042^a	0.161 ± 0.040^A
		Middle piece	0.147 ± 0.043^a	0.151 ± 0.032^a	0.160 ± 0.042^a	0.153 ± 0.041^A
		Posterior segment	0.109 ± 0.032^a	0.107 ± 0.012^a	0.112 ± 0.021^a	0.109 ± 0.026^B
		Ileum	0.086 ± 0.019^a	0.077 ± 0.016^a	0.085 ± 0.023^a	0.083 ± 0.020^C
		Duodenum	0.668 ± 0.136^a	0.697 ± 0.158^a	0.798 ± 0.154^a	0.721 ± 0.114^A
	Content	Jejunum				
		Anterior segment	0.579 ± 0.145^a	0.608 ± 0.116^a	0.482 ± 0.218^a	0.556 ± 0.126^B
		Middle piece	0.692 ± 0.038^a	0.564 ± 0.249^a	0.726 ± 0.328^a	0.661 ± 0.211^A
		Posterior segment	0.982 ± 0.132^a	0.772 ± 0.579^a	0.697 ± 0.324^a	0.817 ± 0.308^A
		Ileum	0.652 ± 0.068^a	0.368 ± 0.241^b	0.356 ± 0.118^b	0.348 ± 0.126^B
Lipase						
		Duodenum	3.780 ± 0.582^a	3.943 ± 0.916^a	3.928 ± 0.814^a	3.884 ± 0.791^A
	Mucosa	Jejunum				
		Anterior segment	4.108 ± 0.712^a	4.082 ± 0.686^a	3.968 ± 0.948^a	4.053 ± 0.781^A
		Middle piece	3.770 ± 1.224^a	3.879 ± 0.804^a	4.319 ± 0.216^a	3.989 ± 0.723^A
		Posterior segment	1.992 ± 0.241^a	2.498 ± 0.582^a	2.665 ± 0.501^a	2.385 ± 0.462^B
		Ileum	2.260 ± 0.330^a	1.948 ± 0.186^a	2.412 ± 0.619^a	2.207 ± 0.366^B
		Duodenum	7.968 ± 1.116^b	8.264 ± 1.430^b	10.818 ± 1.126^a	9.017 ± 1.761^B
	Content	Jejunum				
		Anterior segment	11.418 ± 2.718^a	11.330 ± 3.013^a	12.192 ± 2.660^a	11.647 ± 2.667^A
		Middle piece	13.160 ± 2.612^a	11.158 ± 2.219^a	14.892 ± 3.026^a	13.070 ± 2.916^A
		Posterior segment	7.162 ± 3.250^a	8.618 ± 1.662^a	9.231 ± 2.586^a	8.337 ± 2.708^B
		Ileum	4.752 ± 2.173^a	6.508 ± 1.345^a	6.559 ± 2.112^a	5.940 ± 1.950^C

Table 7: pH of mucosa and contents in small intestine

Treatments	Organs	A	B	C	Average
Mucosa	Duodenum	6.62 ± 0.08^a	6.48 ± 0.09^b	6.53 ± 0.06^{ab}	6.54 ± 0.10^B
	Jejunum				
	Anterior segment	6.65 ± 0.12^a	6.59 ± 0.08^a	6.61 ± 0.21^a	6.62 ± 0.12^B
	Middle piece	6.94 ± 0.28^a	6.70 ± 0.32^a	7.08 ± 0.10^a	6.91 ± 0.23^A
	Posterior segment	7.35 ± 0.14^a	7.30 ± 0.16^a	7.03 ± 0.14^b	7.23 ± 0.20^A
	Ileum	7.24 ± 0.26^a	6.96 ± 0.22^a	7.00 ± 0.22^a	7.07 ± 0.22^A
Content	Duodenum	6.24 ± 0.16^a	6.08 ± 0.26^a	6.12 ± 0.18^a	6.15 ± 0.24^C
	Jejunum				
	Anterior segment	6.46 ± 0.16^a	6.38 ± 0.18^a	6.42 ± 0.28^a	6.42 ± 0.20^B
	Middle piece	6.98 ± 0.32^a	7.08 ± 0.40^a	7.12 ± 0.16^a	7.06 ± 0.26^A
	Posterior segment	7.58 ± 0.21^a	7.32 ± 0.16^b	7.20 ± 0.22^b	7.37 ± 0.19^A
	Ileum	7.56 ± 0.12^a	7.33 ± 0.10^b	7.16 ± 0.14^c	7.35 ± 0.13^A

Means with different lowercase letter superscripts within the same row differ significantly ($p<0.05$); Means with different capital letter superscripts within the same column differ significantly ($p<0.05$)

ileum was also significant ($p<0.05$). The activity of lipase in duodenum mucosa, anterior segment-jejunum and middle piece-jejunum was significantly different than that in the posterior segment-jejunum and ileum ($p<0.05$) (Table 6).

pH in small intestinal mucosa and contents: Table 7 shows the pH in the small intestinal mucosa and contents. In the duodenum of mucosa, pH value in the lambs from Group A was significant higher than those B ($p<0.05$). Both showed a higher tendency ($p = 0.063$). The mean pH was affected significantly by the concentrate-roughage ratio of diets with homogenates of mucosa in the posterior segment-jejunum which was more significant in the lambs from Group A and B than those C ($p<0.05$) and in the lambs from group A showed a higher tendency than those B and C ($p = 0.066$). In the posterior segment-jejunum and ileum, the pH value of contents decreased as the concentrate-roughage ratio increased. The posterior segment-jejunum contents pH value was affected more ($p<0.05$) in the lambs from Group A than in those of B and C ($p<0.05$). The pH value gradually increased from the proximal small intestine to the distal small intestine in each section of small intestine mucosa and contents as shown by a comparison of average values. This also, showed that pH had a neutral tendency in the middle-jejunum. Behind-jejunum mucosa and ileum contents showed slightly alkaline pH levels and these pH levels were significantly higher than those of the other intestines ($p<0.05$).

There were non-uniform patterns of activity of the above stated digestive enzymes along the small intestine corresponding to the concentrate-roughage ratios of the diets. The highest activities of chymotrypsin and lipase in mucosa homogenate and contents occurred in the middle piece-jejunum. The highest activity of trypsinase was shown in the posterior segment-jejunum contents and the highest activities of α-amylase were noted in the duodenum and posterior segment-jejunum contents.

DISCUSSION

Effects of concentrate-roughage ration in diets on ruminal metabolism of parameters: pH value of rumen liquid reflect a comprehensive index on rumen fermentation conditions which depend on dietary construction, nutrition level and time after feeding (Murph and Kennell, 1987; Han and Chen, 1988; Ga *et al*., 2002). pH value (6.5) is the best suitable for digestion on cellulose and dry matter in rumen reported by Hoover and Stokes (1991). pH value of ruminal contents were <6.3 (Tan *et al*., 1999), 6.2 and 6.1 (Chen, 2003) reported by different references which inhibit cellulolytic bacteria in order to reproduction. The concentrated feeding stuff contain affluent digestible carbohydrates in order to rapid fermentation of acid production in the rumen which is much smaller than coarse fodder of diameter so that ruminant frequency, time and reduced saliva into the rumen lead to rapid decline in pH value (Zhou and Li, 2003). This experimental result showed that pH value in rumen liquid of lambs from Group C was obviously smaller than those from Group A and B ($p<0.05$) which is consistent with pH value of variation with rumen liquid on feeding diets of beef cattle with different concentrate-roughage ration (75.6, 55.6 and 9.4%) reported by Zhou and Li (2003).

The tests proved that TVFA concentration of rumen liquid increased and acetic acid of molar ratio decreased and propionic acid of molar ratio increased with concentrated feeding stuff of proportion increased in diets which is consistent with this experimental result. However, molar proportions of acetic acid and propionic acid were ranged from 0.62-0.65 and from 0.18-0.19, respectively the acetic/propionic ratios varied ranging from 3.36-3.55 which was still kept acetic acid pattern of rumen fermentation in lambs fed total mixed diet with higher concentrate-roughage ratio. It was also concluded that ruminal environment is relatively stable, digestible carbohydrate be diluted by the affluent fiber diets and highly concentrated feeding stuff of rapid fermentation could eliminated inhibition on the activity of cellulolytic bacteria, acetic acid fermentation was dominant which were affected significantly by the concentrate-roughage ratio of diets. The total mixed concentrated feeding stuff is not this feature when it pressed into pellets. It was reported that typically high propionic acid pattern of rumen fermentation was kept in fattening lambs fed the total mixed diet, molar proportions of acetic acid and propionic acid were ranged from 0.42-0.43 and from 0.37-0.43 (Hao *et al*., 2002).

Effects of concentrate-roughage ration in diets on abomasum and small intestinal contents of pH: The results showed that pH values fell within a normal range (3.28-3.47) in the abomasum contents while pH values fell in a higher than normal value range (4.87-5.16) in the abomasums mucosa. The status of rumen fermentation and secretion of gastric juice in the abomasum was affected. This is an important factor in the production of Volatile Fatty Acid (VFA) from the proventriculus to abomasums and promotes secretion of digestive juice in the abomasum. Some researchers found that higher nutrient level diets were fed to animals to increase the production of VFA in rumen and to promote a corresponding VFA increase in the stomach and intestinal tract. There, it stimulates chemical receptors of abomasums, promotes secretion of gastric juice and releases more hydrochloric acid. In this experiment, the pH values of the abomasum mucosa and contents in lambs from Group C were lower than those from Group A and B while levels were similar in lambs from Group A and B. The pH values in the duodenum contents and abomasum mucosa and contents decreased as the concentrate-roughage ratio increased.

Some interesting differences were also observed in the small intestinal contents: pH values in the distal ileum decreased significantly as the concentrate-roughage ratio increased (Zhang *et al*., 2000; Zhou and Li, 2003). Some researchers showed that the optimum pH levels for digestive enzyme activity are as follows: bovine pancreatic lipase: 6.5-7.5 (Dong, 2001), pancreatic α-amylase: 6.8-7.0 and pancreatic lipase: 7.0-7.5 (Liu *et al*., 2004). The pH values gradually increased from the proximal to the distal small intestinal contents with increasing acidic chyme from abomasum into the small intestine being neutralized by bicarbonate in small intestinal pancreatic juice, bile and intestinal fluid. However, the chyme is more acidic in the small intestine than in the abomasum and the concentration of bicarbonate is lower in pancreatic juice where neutralization is slow. The posterior three-quarters segment of jejunum of posterior usually displayed alkaline pH levels, a consequence of protection from both pepsin and low pH-mediated inactivation. Conjugation also provided significant protection against the proteolytic component of pancreatin. In this study, the pH values were similar to other studies on pH values in small intestinal contents (Dong, 2001). Furthermore, pH values were mediosilicic in middle-jejunum contents because of the alkalescency in posterior segment-jejunum and ileum contents.

Effects of concentrate-roughage ration in diets on activity of digestive enzymes of abomasums and pancreas and small intestinal mucosa and contents: Many enzymes used as digestive aids exhibit at best moderate stability when incubated under gastrointestinal conditions. The results of this study essentially support the hypothesis that small herbivorous animals have intestines that function as Plug-Flow Reactors (PFRs). The activities of trypsinase, chymotrypsin, α-amylase and lipase decreased or increased in the small intestinal mucosa and contents. The activities of α-amylase and lipase remained more or less the same, concomitant with regional pH concentrations along the intestines. Although, digestive enzyme activities have been measured along the digestive tracts of numerous herbivorous animals (Chakrabarti *et al*., 1995; Smoot and Findlay, 2000; Logothetis *et al*., 2001; Gawlicka and Horn, 2005; Skea *et al*., 2007), few have considered these activities in the context of chemical reactor models. The α-amylase and trypsin activities are known to decrease distally in the intestine of several cyprinid taxa (Bitterlich, 1985; Das and Tripathiy, 1991; Chakrabarti *et al*., 1995). Given that α-amylase and trypsin are pancreatic in origin and therefore secreted in the proximal intestine, it makes sense that the activities of these enzymes decrease in the hindgut as their substrates are diminished and the enzymes themselves are broken down or reabsorbed (Hofer, 1982; Clements and Raubenheimer, 2006). Lipase activity decreases distally in the guts of several cyprinids (Das and Tripathi, 1991; Chakrabarti *et al*., 1995), most likely for the same reason as this enzyme is also secreted by the small intestinal mucosa and contents.

Trypsinase and chymotrypsin activities were expected to increase in the mid and distal intestine of the herbivorous animals as the concentrate-roughage ratio increased. However, two patterns of trypsinase and chymotrypsin activity along the digestive tract emerged in this study. First, trypsinase activities increased moving distally from the anterior segment-jejunum to the posterior segment-jejunum with the exception of the duodenum and ileum in small intestinal contents. Chymotrypsin activities displayed a wave tendency along the digestive tract. Second, trypsinase and chymotrypsin activities were relatively similar throughout the gut in the different concentrate-roughage ratios. Sister-taxa similarity in digestive enzyme activities has been observed in lambs (Gorrill *et al*., 1968). Some interesting differences were observed in the digestive enzyme activity and luminal nutrient concentration patterns along the intestines.

The α-amylase and lipase activities generally decreased in the distal intestines of the herbivores. The α-amylase was noticeably lower in the posterior segment-jejunum and ileum. α-amylase activity is shown to be higher in herbivorous than in carnivorous animals (Fernandez *et al*., 2001; German *et al*., 2004; Horn *et al*., 2006).

Studies of cattle and sheep have shown that trypsin activity is apparent only up to the proximal jejunum because the velocity of acidic chime into the small intestine slows and intestinal enzymes activities are postponed. Proteolytic activity in calf beef and lamb was significantly higher in the first two-thirds of the small intestine segments than in the final third of the small intestine segment (Gorrill *et al*., 1968).

CONCLUSION

In this study, chymotrypsin activity of small intestinal contents and mucosa and lipase activity of small intestinal contents all displayed the highest values in the middle piece-jejunum and trypsinase activity in the small intestinal contents was highest in the posterior segment-jejunum. Similar results were observed by numerous researchers who have measured trypsin activity of calf: trypsinase activity was highest in the behind-jejunum contents, α-amylase activity was highest in the middle-jejunum and behind-jejunum contents and lipase activity was highest in the middle piece-jejunum contents (Dong, 2001). These results are in agreement with those of Zhang *et al*. (2005) who studied the development of pH and digestive enzymes in lamb small intestines as concentrate-roughage ratio increased (Zhang *et al*., 2005).

ACKNOWLEDGEMENTS

This research was supported by the Special Fund for the Chinese Agricultural Research System (CARS-39), the National Key Technology R&D Program (2011BAD28B05), the Gansu Key Technology R&D Program (201104NKCA083) and the Central Level, Scientific Research Institutes for Basic R&D Special Fund Business (1610322012006).

REFERENCES

Beharka, A.A., T.G. Nagaraja, J.L. Morrill, J.A. Kennedy and R.D. Klemm, 1998. Effects of form of the diet on anatomical, microbial and fermentative development of the rumen of neonatal calves. J. Dairy Sci., 81: 1946-1955.

Bitterlich, G., 1985. Digestive enzyme pattern of two stomachless filter feeders, silver carp, Hypophthalmichthys molitrix Val. and bighead carp, Aristichthys nobilis Rich. J. Fish Biol., 27: 103-112.

Chakrabarti, I., M.A. Gani, K.K. Chaki, R. Sur and K.K. Misra, 1995. Digestive enzymes in 11 freshwater teleost fish species in relation to food habit and niche segregation. Comparative Biochem. Physiol. Part A Physiol., 112: 167-177.

Chen, J., 2003. Animal Physiology. 4th Edn., Beijing Agriculture Press, Beijing, China.

Clements, K.D. and D. Raubenheimer, 2006. Feeding and Nutrition. In: The Physiology of Fishes, Evans, D.H., (Ed.). CRC Press, Boca Raton, pp: 47-82.

Das, K.M. and S.D. Tripathi, 1991. Studies on the digestive enzymes of grass carp, *Ctenopharyngodon idella* (Val.). Aquaculture, 92: 21-32.

Dong, L.R., 2001. Studies on development of pancreas and small intestine of digestive enzymes from birth to six month old. Shanxi Agricultural University, Shanxi, China.

Enyenihi, G.E., A.B.I. Udedibie, M.J. Akpan, O.L. Obasi and I.P. Solomon, 2009. Effects of 5 h wetting of sun-dried cassava tuber meal on the hydrocyanide content and dietary value of the meal for laying hens. Asian J. Anim. Vet. Adv., 4: 326-331.

Erener, G., A. Altop, N. Ocak, H.M. Aksoy, S. Cankaya and E. Ozturk, 2010. Influence of black cumin seed (*Nigella sativa* L.) and seed extract on broilers performance and total coliform bacteria count. Asian J. Anim. Vet. Adv., 5: 128-135.

Fazaeli, H., A. Azizi and M. Amile, 2006. Nutritive value index of treated wheat straw with *Pleurotus* fungi fed to sheep. Pak. J. Biol. Sci., 9: 2444-2449.

Fernandez, I., F.J. Moyano, M. Diaz and T. Martinez, 2001. Characterization of α-amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleostei). J. Exp. Marine Biol. Ecol., 262: 1-12.

Ga, E.D., Z.L. Qi, R.H. Zhang, H.J. Chen and F.R. Zhao, 2002. Influences of different processing with corn on pH and NH3-N and concentration of total VFA in rumen liquid of sheep. Heilongj. J. Anim. Sci. Vet. Med., 9: 18-20.

Gawlicka, A. and M.H. Horn, 2005. Storage and absorption in the digestive system of carnivorous and herbivorous prickleback fishes (Teleostei: Stichaeidae): Ontogenetic, dietary and phylogenetic effects. Physiol. Biochem. Zool., 78: 879-892.

German, D.P., M.H. Horn and A. Gawlicka, 2004. Digestive enzyme activities in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): Ontogenetic, dietary and phylogenetic effects. Physiol. Biochem. Zool., 77: 789-804.

Gorrill, A.D., D.J. Schingoethe and J.W. Thomas, 1968. Priteolytic activity and *in vitro* enzyme stability in small intestinal contents from ruminants and nonruminants at different ages. J. Nutr., 96: 342-348.

Han, Z.K. and J. Chen, 1988. Digestion and metabolism on ruminant of rumen. Beijing Science Press, Beijing, China.

Hao, Z.L., T.F. Guo, Y.G. Sun, F.D. Li and S.L. Wei *et al*., 2002. Metabolic parameters of rumen fluid from lambs fed on complete diet pellets with different combinations. J. Gansu Agric. Univ., 37: 145-152.

Hara, K., H. Pangkey, K. Osatomi, K. Yatsuda, A. Hagiwara, K. Tachibana and T. Ishihara, 1997. Some properties of b-1, 3-glucan hydrolyzing enzymes from the rotifer Brachionus plicatilis. Hydrobiol., 358: 89-94.

Herold, R.C. and N.D. Meadow, 1970. Age-related changes in ultrastructure and histochemistry of rotiferan organs. J. Ultrastructure, 33: 203-218.

Hinders, R.G. and F.G. Owen, 1965. Relation of ruminal parakeratosis development to volatile fatty acid absorption. J. Dairy Sci., 48: 1069-1073.

Hlophe, S.N. and N.A.G. Moyo, 2011. The effect of different plant diets on the growth performance, gastric evacuation rate and carcass composition of *Tilapia rendalli*. Asian J. Anim. Vet. Adv., 6: 1001-1009.

Hofer, R., 1982. Protein digestion and proteolytic activity in the digestive tract of an omnivorous cyprinid. Comp. Biochem. Physiol., 72: 55-63.

Hoover, W.H. and S.R. Stokes, 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. J. Dairy Sci., 74: 3630-3644.

Horn, M.H., A.K. Gawlicka, D.P. German, E.A. Logothetis, J.W. Cavanagh and K.S. Boyle, 2006. Structure and function of the stomachless digestive system in three related species of New World silverside fishes (Atherinopsidae) representing herbivory, omnivory and carnivory. Marine Biol., 149: 1237-1245.

Javaid, A., S.M. Mahr-un-Nisa and M.A. Shahzad, 2008. Ruminal characteristics, blood pH, blood urea nitrogen and nitrogen balance in Nili-Ravi buffalo (Bubalus bubalis) bulls fed diets containing various levels of ruminally degradable protein. Asian-Australasian J. Anim. Sci., 21: 51-59.

Khan, M.A., M. Sarwar, M. Nisa and M.S. Khan, 2004. Feeding value of urea treated corncobs ensiled with or without enzose (corn dextrose) for lactating crossbred cows. Asian-Australasian J. Anim. Sci., 17: 1093-1097.

Khan, T.H., 2012. Soy diet diminish oxidative injure and early promotional events induced by CCl4 in rat liver. Int. J. Pharmacol., 8: 30-38.

Kleinow, W. and A. Rohrig, 1995. Enzyme activity measurements on isolated organs of Brachionus plicatilis (Rotifera). Hydrobiol., 314: 171-174.

Kleinow, W., 1993. Chemical studies on Brachionus plicatilis: Hydrolytic enzymes, integument proteins and composition of trophi. Hydrobiol., 256: 1-12.

Kleinow, W., H. Wratil, K. Kuhle and B. Esch, 1991. Electron microscope studies of the digestive tract of Brachionus plicatilis (Rotifera). Zoomorphol., 111: 67-80.

Li, J.W., 1994. Principles and Methods of Biochemical Experiments. Beijing University Press, Beijing, China.

Liu, Y.Q., B.S. Wang and Y.J. Zhang, 2004. Influences of different diets on activity of digestive enzymes in small intestine of Small-tail Han sheep. China Herbivores, 24: 131-134.

Logothetis, E.A., M.H. Horn and K.A. Dickson, 2001. Gut morphology and function in Atherinops affnis (Teleostei: Atherinopsidae), a stomachless omnivore feeding on macroalgae. J. Fish Biol., 59: 1298-1312.

Misieng, J.D., M.S. Kamarudin and M. Musa, 2011. Optimum dietary protein requirement of Malaysian mahseer (*Tor tambroides*) fingerling. Pak. J. Biol. Sci., 14: 232-235.

Murph, Y.J.J. and Y.J.J. Kennell, 1987. Effect of protein concentration and protein source on the degradability of dry matter and protein *in situ*. J. Dairy Sci., 70: 1841-1849.

Nassier, O.A., 2010. Protein undernutrition in tumor-bearing mice, response and toxicity to paclitaxel. Int. J. Pharmacol., 6: 296-300.

Nisa, M., M. Sarwar and M.A. Khan, 2004. Nutritive value of urea treated wheat straw ensiled with or without corn steep liquor for lactating Nili-Ravi buffaloes. Asian-Australasian J. Anim. Sci., 17: 825-829.

Nocek, J.E., 1997. Bovine acidosis: Implications on laminitis. J. Dairy Sci., 80: 1005-1028.

Ofuya, Z.M., 2006. The effect of cowpea oligosaccharides on gas production in adult rats. Asian J. Plant Sci., 5: 590-597.

Owen, F.N., D.S. Secrist, W.J. Hill and D.R. Gill, 1998. Acidosis in cattle: A review. J. Anim. Sci., 76: 275-286.

Skea, G., D. Mountfort and K.D. Clements, 2007. Contrasting digestive strategies in four New Zealand herbivorous fishes as rerected by carbohydrase activity profiles. Comp. Biochem. Physiol. B Biochem. Mol. Biol., 146: 63-70.

Smoot, J.C. and R.H. Findlay, 2000. Digestive enzyme and gut surfactant activity of detrivorous gizzard shad (Dorosoma cepedianum). Canadian J. Fish. Aquatic Sci., 57: 1113-1119.

Sultan, J.I., F.L. Fluharty and S.C. Loerch, 1991. Effects of energy level, protein level and protein source on steer feedlot performance. Pak. J. Agric. Sci., 28: 350-354.

Tan, Z.L., D.Y. Lu, M. Hu, W.Y. Niu, C.Y. Han, X.P. Ren and S.L. Lin, 1999. Effects of different proportions of dietary structural and nonstructural carbohydrates on flow and digestibility of fibre in different digestive sites for sheep fed a wheat straw diet. Acta Zoonutrimenta Sinica, 11: 29-38.

Wurdak, E.S., 1987. Ultrastructure and histochemistry of the stomach of Asplanchna sieboldi. Hydrobiol., 147: 361-371.

Yu, J.P. and S.J. Cui, 1997. Ultrastructure of the rotifer Brachionus plicatilis. Hydrobiol., 358: 95-103.

Zhang, Y.J., Y.Q. Liu and Y.L. Feng, 2000. Influences of diets on pH and trypsin activity in small intestine in beef cattle. China Herbivores, 20: 8-9.

Zhang, Y.J., Y.Q. Liu, H.X. Sun, E.Y. Zeng, B.S. Wang and Y.Z. Liu, 2005. Study on the development of digesta pH and main digestive enzymes of small intestine in lamb. Chinese J. Anim. Vet. Sci., 36: 149-152.

Zhou, S. and S.M. Li, 2003. Influences of different concentrate-roughage level on rumen liquid and pH of small intestine in beef cattle. Feed Industry, 24: 27-29.