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Effects of Different Diets on Digestive Function of Bull Calves

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Abstract: In the world wide, it is very common that calves feed Total Mixed Rations (TMR). In this study, researchers want to evaluate TMR feed plus early weaned whether it is one kind of burden to the calves' digestive function. Thirty nine Holstein bull calves were randomly assigned to 3 groups: Early-Weaned (EW), Low Quantity Milk (LQM) and High Quantity Milk (HQM) to evaluate the effect of diets on the calves' grow and rumen function. The calves were slaughtered at the 0, 7, 30, 60 and 90 days in each group. The activity of protease among the three groups increased with calves' growth and activity in EW and LQW did not differ was lower than that in HQM. pH of ruminal fluid in HQW was lower than that in EW and LQM at the 30 and 60 days but did not differ at 7 and 90 days. The calves that were fed without solid feed before 60 days had stunted growth of ruminal papillae length (white colour). This study suggests that it could prevent the ruminal function from formation that calves only intake liquid food before the 60 days. Calves feed TMR and early weaned may stimulated the development of rumen digestion function.

Key words: Bull calves, diets, digestive function, protease, ruminal fluid

INTRODUCTION

The fine treatment is a major concern for calves' feeders which could strengthen calves' digestive function, improve calves development and growth and decrease breeding cost. Researchers already know that calves need colostrum to obtain IgG (Duse *et al*., 2013; Pithua *et al*., 2013). Many studies about calves weaned at early time indicated that growth was better for HQM than LQM and EW but the rumen is smaller for HQM (Songjia, 2002; Hopkins, 1997; Arthington and Kalmbacher, 2003). Bailey *et al*. (2013) study's showed that efficiency of early weaned beef calves is not improved by restricting feed intake during 84 days growing phase. However, the EW Method should be adopted because they are more profitable and practical than the other two. Calves undergo 4 feeding processes: single liquid feed, the liquid feed primarily, the solid feed auxiliary, solid feed primarily, liquid feed auxiliary and sole solid feed. Malmuthuge *et al*. (2013) suggested that solid feed consumption may alter gut microbiome and host mucosal functions during weaning transition. These feeding processes result in the ability of a calf to transit from a monogastric animal to a ruminant. Therefore, it is important to understand the factors responsible for cellular biology and physiological changes, maturation of the rumen tissues and establishing rumen function and digestion kinetics in the calves.

The developments of calves' four stomachs are mainly included the absorption, metabolism, morphology and weight. Development of rumen is represented by the change of anatomic structure such as rumen epithelium tissue. Solid feed could stimulate the development of fore-stomach increase volume and weight and promote growth of rumen papillae as well as the absorption and utilization of volatile fatty acids. The VFA which stimulates the rumen epithelia development mainly roots in fermentation of diet carbohydrates. The lambs infused of physiological concentrations of VFA have longer rumen papillae but there are no differences in width or number of papillae per square centimetre across treatments (Lane and Jesse, 1997). The total amount of VFA in rumen fluid of early weaned calves is greater than those of late weaned (Cheng and Wangen, 1997). The less solid feed intaked could decrease the VFA concentration

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in rumen and suppress the growth of rumen wall tissues and papillae (Brownlee, 1956). In addition, more liquid feed could depress secreted amount of saliva and decrease rumen buffering capacity. The calves, installed with permanent fistula in rumen, fed different diets show the absorbed amount to organic acid of calves fed milk is less than those fed dry feed and starters (Sutton *et al*., 1963). It is hypothesized that the growth of rumen papillae is probably a result of the metabolism of certain compounds by the rumen wall and/or their effect on blood flow in the rumen and sodium butyrate>sodium propionate>sodium acetate (Sander *et al*., 1959).

Particle size of concentrate feed can influence ruminal development as well. The 70% of the end product fermented from coarse fibrous feed in rumen is acetic acid whereas greater percentage of product fermented from higher concentration of feed is propionic acid. More feed intake would result in uncompleted papillae keratinisation and short papillae. Epithelial absorption to acetic acid in rumen raises with the week age increased while calves are fed ground or cut short dry hay. Addition of controlled particle size of hay to diets appears to favorably alter rumen environment increase dry feed intake, improve feed efficiency and stimulate the rumen development (Coverdale *et al*., 2004). Furthermore, non-degradable materials such polyethylene could produce enough physical stimulation to rumen wall. Polyurethane cubes don't affect growth or morphological characteristics of either ruminal or jejunal epithelia (Rompala *et al*., 1990).

Diets have the same ingredients but different particle sizes also influence abrasive values and papillae development. Normal growth of fore-stomach is suppressed, rumen tissue is thin and growth of papillae is retarded when calves are fed only pasty feed (Greenwood *et al*., 1997). Calves fed starter with 33% Whole Corn (WC) have higher rumen pH and lower rumen VFA concentration than calves fed Dry-Rolled (DRC), roasted-rolled (RC) or Steam-Flaked (SFC) corn. Papillae length and rumen wall thickness at 4 weeks are significantly greater in calves fed starter with SFC (Lesmeister and Heinrichs, 2004). It showed that the type of processed corn incorporated into calf starter can influence rumen parameters in neonatal calves. The different VFA concentrations in rumen were detected when calves were fed with TMR (tester group), concentrated and coarse feed (control group) separately. The 3 h after intake began, propionic acid concentration of tester group was higher than that of control group ($p<0.05$) butyric acid concentration of tester group was lower than that of control group ($p<0.05$) no difference of acetic acid concentration between two groups (Li *et al*., 2003). Granular concentrate feed can increase the amount of feed intake of ruminants, promote the function of abomasum and change the fermentation type in rumen and increase the ratio of acetic acid and propionic acid (Li *et al*., 2003). As researchers know the digestive function of calves' was not well developed. TMR contained so much silage which is acid feed. In this study, researchers want to evaluate the premature TMR feed whether it is one kind of burden to the calves' digestive function.

MATERIALS AND METHODS

Design: The trial adopted single factor complete random design. Thirty nine Holstein bull calves were divided into three groups that were Early Weaned (EW), Low Quantity Milk (LQM) and High Quantity Milk (HQM), single pen and bred, drinking water freely. Experimental period was 90 days from calves' birth to 90 days age.

Breed treatments (EW group): The calves was fed with milk at 118 kg (90 days) and weaned at 30 days. They were separately given starter at 3 days, hay at 7 days until 30 days. TMR was given from 3-90 days and the quantity of ration was not restrained.

LQM group: Milk offered 213.5 kg (90 days) and weaned at 60 days. Calves offered starter at 8 day and hay at 10 days until 30 days. TMR given was the same as EW.

HQM group: Milk offered 370 kg, only given milk without the solid feed before the 60 days. TMR was given from 6-90 days. The ingredient and nutritional content of TMR was as in Table 1.

Table 1: Ingredient and nutritional content of TMR (on air dry basis %)

Ingredient of TMR	Values
Feeds	
Concentrated feed (%)	-
Corn	48
Soybean meal	19
Wheat bran	10
Cotton meal	5
DDGS	5
Corn's embryo meal	5
Fish meal	2
Monocalcium phosphate	2.5
Salt	1
Dicalcium phosphate	1.5
Premix feed	1
TMR (%)	-
Forage	22.2
Corn silage	55.6
Concentrated feed	22.2
Nutritional content (Items)	
Dry matter (%)	56.40
Crude protein (%)	9.49
Crude fat (%)	9.26
Crude fiber (%)	17.62
Ca	0.9847
P	0.5976
NE_L (MJ/kg)	12.90

Compound feeds were composed of concentrates, silage and leymus chinensis, the ratio was 1:2.5:1 (fresh sample weight). The main ingredients of premix were Fe, Cu, Mn, Zn, I, Co, Se, vitamins and antibiotics

Sampling: The slaughter method was vein-bloodletting after calves bred for 1 h. Three calves were slaughtered at 0 day. At 7, 30, 60, 90 days three calves were slaughtered in each group. A total of 100-150 mL ruminal fluid and 100~150 mL abomasal fluid were separately collected. The samples were put in sampling bags, rapidly frozen at liquid nitrogen within 3~5 min and shifted in refrigerator for storing. Activity of protease and cellulose in rumen fluid, pH value of rumen, chymosin (Arima *et al*., 1967) in the abomasums content were determined. For HE stain sample, rumen tissue was collected (Shen, 2000) and put in wide mouthed bottles with Boin's stationary liquid. The main equipments were followed: DELTA320 precise pH calculate (Zhen Yuan Industry Limited Corporation), 723A spectrophotometer (ShangHai JingKe), oscillator (DK-600B electrothermal, ShangHai SenXin Experiment Equipment Limited Corporation), electronic balance.

Statistical analyses: All statistical analyses were done using SAS for Window, Release 8.2 (ANOVA) and Duncan Method.

RESULTS AND DISCUSSION

The protein enzyme activity in rumen fluid of the three groups increased from 7-30 days (Table 2). It continued to increase slowly in EW group and LQM group from 30-90 days but in HQM group it decreased from 30-60 days and then increased from 60-90 days. From 0-90 days, the change of cellulase activity in EW and LQM group appeared to increase. The cellulose activity was not examined for HQM from 0-60 days. During the period of 60-90 days when the solid feed was taken, the enzyme activity increased. Chymosin activity in abomasum of the three groups reduced after 7 days especially in EW. It cannot be detected in three groups at 90 days.

The difference in protease activity was not remarkable ($p>0.05$) between the three groups at 7 days, so were cellulase and chymosin (Table 2). The protease activity did not differ at 30 days. At 60 days, the preotease activity was similar between EW and LQM ($p>0.05$) and both higher than HQM ($p<0.05$). At 90 days, it also did not differ ($p>0.05$) among the groups. The cellulase activity was not significantly different ($p>0.05$) between EW and LQM at 30 and 60 days and not detected in HQM. There was no remarkable difference among groups ($p>0.05$) at 90 days. The difference of chymosin activity was significant ($p<0.05$) among the groups at the 30 and 60 days, EW<LQM<HQM. It was not detected in EW and LQM at 90 days and low in HQM.

No significant difference was observed for pH of calves' rumen at 7 days ($p>0.05$). At 30 and 60 days, pH in HQM was lower than that in EW and LQW ($p<0.05$) but the difference was not significant between EW and LQM ($p>0.05$). It also did not significantly differ ($p>0.05$) among groups at the 90 days (Table 3).

No significant difference was observed in the length of calves' rumen papillae at the 7 day ($p>0.05$). At 30, 60 and 90 days, the length in HQM were significantly lower than those in EW and LQW ($p<0.05$) but the difference was not significant between EW and LQM ($p>0.05$). The colour of rumen papillae was taken as the primary index for measuring the growth of rumen. The colour of mature papillae was brown, on the contrary was white (Table 4).

Effects of different diets on morphological of calves' ruminal papillae (Fig. 1-26). Although, rumen development is a natural process which grows along with bull calves, numerous researchers have conducted the research of bull calves digestion physiology to point out that the development of rumen is influenced by diets and metabolic products, especially solid feed (Anderson *et al*., 1987). The growth of bull calves appears well if the only liquid feed is taken up to 219~270 days but the rumen volume is small (Roy, 1980b). It is not true that the more

Table 2: Effects of different forms diet on main enzyme activity in calve's stomach (day, U/mL, SU/mL)

Age (days)	Variables	Treatments EW	LQM	HQM	p-value
7	Protease activity ($U\ mL^{-1}$)	1.96±0.50	1.83±0.35	1.76±0.50	0.95
	Cellulase activity ($U\ mL^{-1}$)	0	0	0	-
	Chymosin activity ($SU\ mL^{-1}$)	3976±722.51	4031±616.34	4024±520.07	0.99
30	Protease activity ($U\ mL^{-1}$)	15.27±0.17	14.58±1.36	11.89±0.07	0.46
	Cellulase activity ($U\ mL^{-1}$)	1.37±0.08	1.34±0.07	0	-
	Chymosin activity ($SU\ mL^{-1}$)	1147±141.30	2134±162.82	2758±113.21	0.0006
60	Protease activity ($U\ mL^{-1}$)	18.44±1.79	16.35±1.41	11.27±1.04	0.31
	Cellulase activity ($U\ mL^{-1}$)	1.48±0.09	1.39±0.08	0	-
	Chymosin activity ($SU\ mL^{-1}$)	561±64.66	987±82.86	1046±142.53	0.029
90	Protease activity ($U\ mL^{-1}$)	19.48±1.58	19.63±1.60	16.74±1.44	0.38
	Cellulase activity ($U\ mL^{-1}$)	3.48±0.16	3.88±0.06	3.47±0.18	0.15
	Chymosin activity ($SU\ mL^{-1}$)	0	0	114±13.58	-

[a]Values are estimates±SE; [b]Value at the 0 day was minimum did not participate comparison. The activity of protease and cellulase was not determined, the chymosin activity was 2372±182.44

Table 3: Effects of different forms diet on pH in calves' rumen

Age (days)	Treatments EW	LQM	HQM	p-values
7	5.12±0.14	5.23±0.32	5.21±0.26	0.94
30	6.12±0.01	6.14±0.03	5.94±0.06	0.02
60	6.17±0.11	6.35±0.12	5.77±0.08	0.02
90	6.25±0.27	6.74±0.14	6.46±0.36	0.48

[a]Values are estimates±SE. [b]Value at the 0 day was minimum did not participate comparison. The pH was 4.15±0.50

Table 4: Effects of different diets on length and colour of calves' rumen papillae (μm)

Age (days)	Treatment	Length	Colour
0	-	104±46	Ivory
7	EW	107±51[a]	Ivory
	LQM	129±56[a]	Ivory
	HQM	112±48[a]	Ivory
30	EW	150±53[a]	Brown
	LQM	156±58[a]	Brown
	HQM	96±41[b]	Ivory
60	EW	261±53[a]	Brown
	LQM	254±49[a]	Brown
	HQM	109±61[b]	Ivory
90	EW	490±124[a]	Brown
	LQM	458±137[a]	Brown
	HQM	246±78[b]	Brown

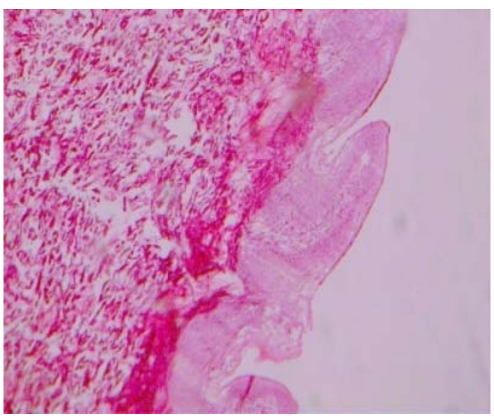

Fig. 1: Rumen papillae 0 day (0x100)

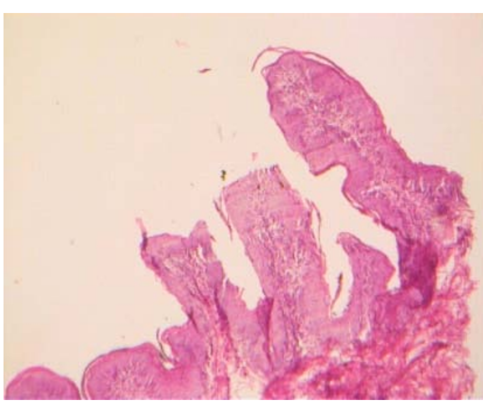

Fig. 2: Rumen papillae 7 days (EW~7x100)

solid feed taken, the better growth for calves weaned at 5 weeks ages. In the calves taking 0.45 kg concentrate feed per day and pick hay freely, the volume of rumen and reticulum take up approximately 87% of complex stomach at 12 weeks. If the quantity of concentrate feed is increased to 2.3 kg per day, the proportion of the rumen

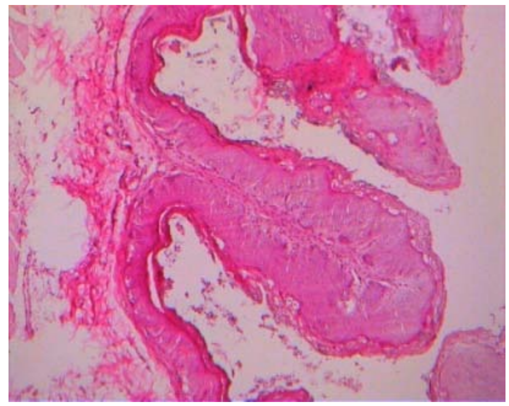

Fig. 3: Rumen papillae 30 days (EW~30x100)

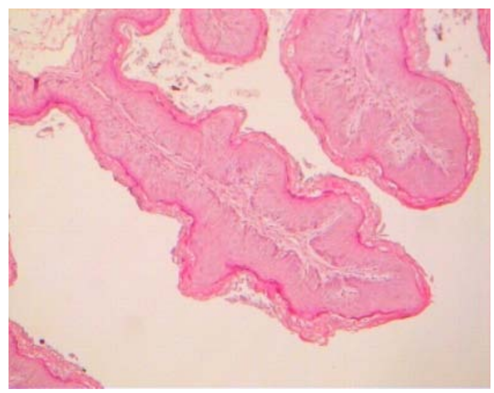

Fig. 4: Rumen papillae 60 days (EW~60x100)

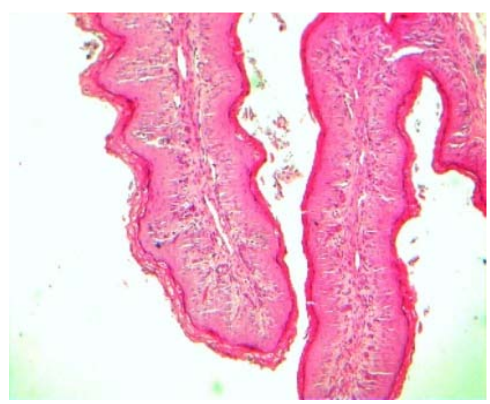

Fig. 5: Rumen papillae 90 days (EW~90x100)

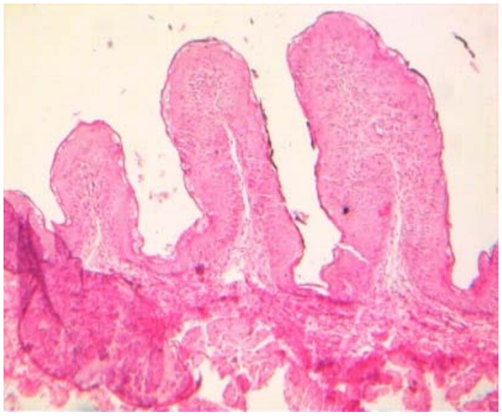

Fig. 6: Rumen papillae 7 days (LQM~7x100)

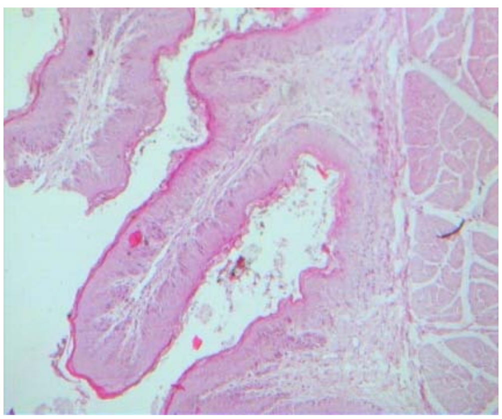

Fig. 7: Rumen papillae 30 days (LQM~30x100)

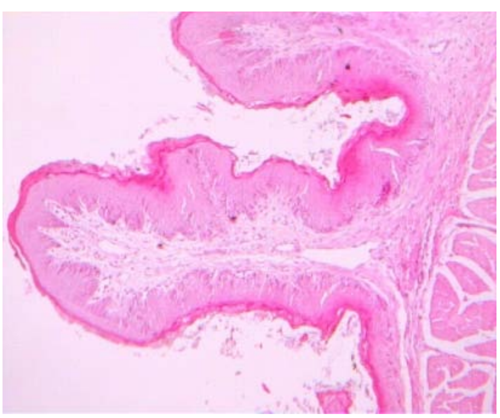

Fig. 8: Rumen papillae 60 days (LQM~60x100)

and reticulum falls to 84% (Roy, 1980a, b). In order to promote calves grow better, studies about calves' alimentary organs growth characteristic are necessary by using the reasonable raising method to stimulate the rumen development.

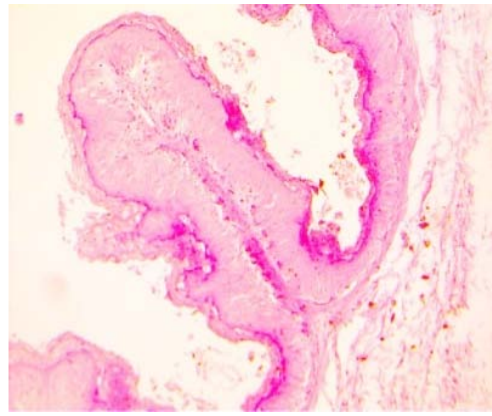

Fig. 9: Rumen papillae 90 days (LQM~90x100)

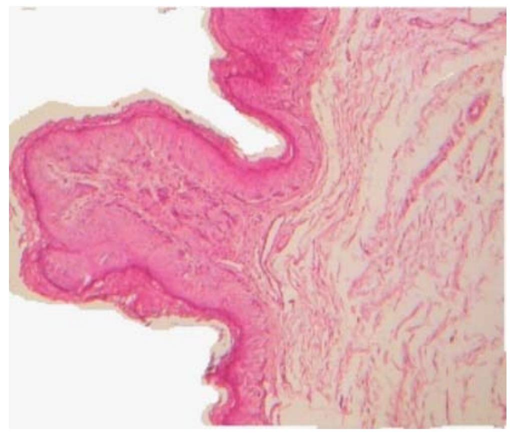

Fig. 10: Rumen papillae 7 days (HQM~7x100)

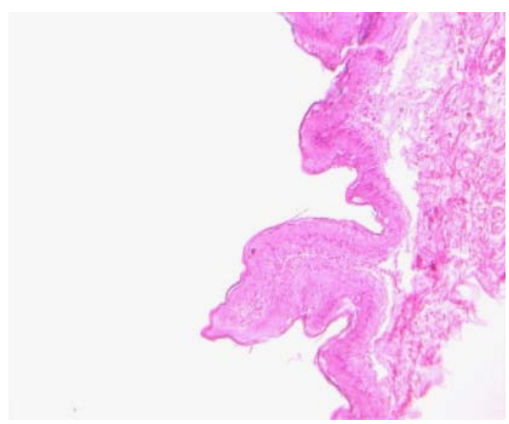

Fig. 11: Rumen papillae 30 days (HQM~30x100)

Abomasum is the unique glandular stomach for ruminant which secrets digestive enzyme. The digestion of nutrition mainly relies on chymosin secreted by abomasums for neonatal calves. Rumen plays more

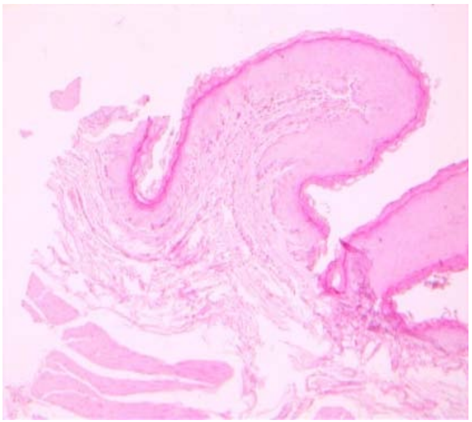

Fig. 12: Rumen papillae 60 days (HQM~60x100)

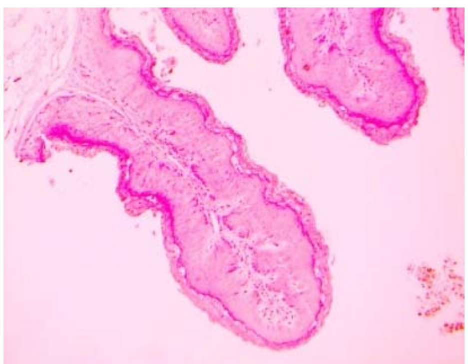

Fig. 13: Rumen papillae 90 days (HQM~90x100)

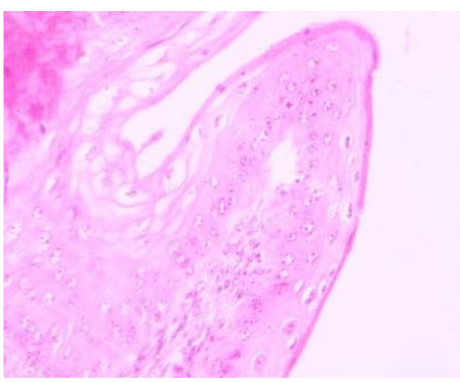

Fig. 14: Rumen papillae 0 day (0x400)

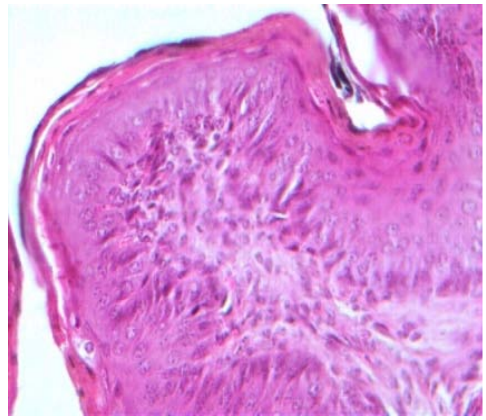

Fig. 15: Rumen papillae 7 days (EW~7x400)

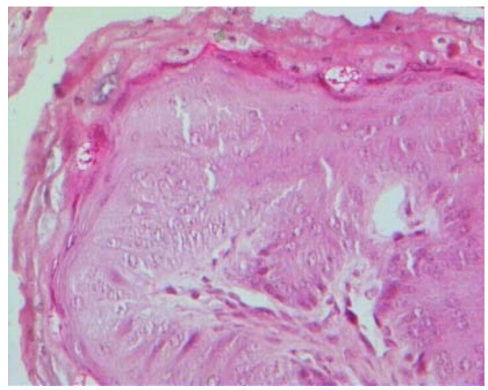

Fig.16 Rumen papillae 30 days (EW~30x400)

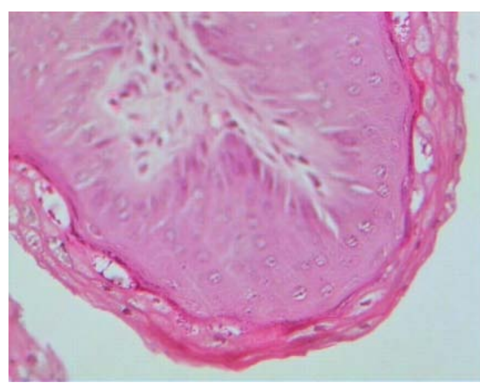

Fig. 17: Rumen papillae 60 days (EW~60x400)

important roles in nutrition absorption for growth because microorganisms secrete huge amounts of digestive enzyme in rumen. Protease and chymosin secreted from abomasum have highly activity in the neonatal calves. The trial results showed that the activity of protease and cellulase in rumen of EW and LQM increased with the age increasing. This finding was similar to the report of Li. Activity of protease in rumen fluid of HQM was significantly lower than that of EW and LQM at 30 and 60 days. Activity of cellulase in rumen was not detected from the 0-60 days; the difference of pH in rumen between EW and LQM was not significant at the different ages

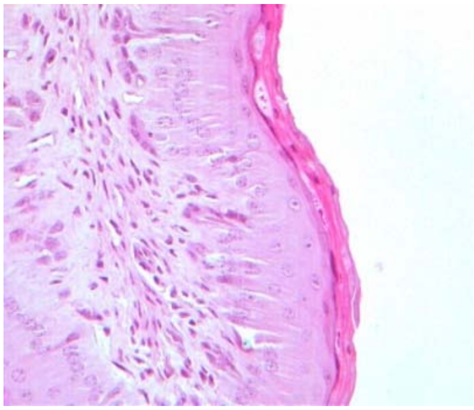

Fig. 18: Rumen papillae 90 days (EW~90x400)

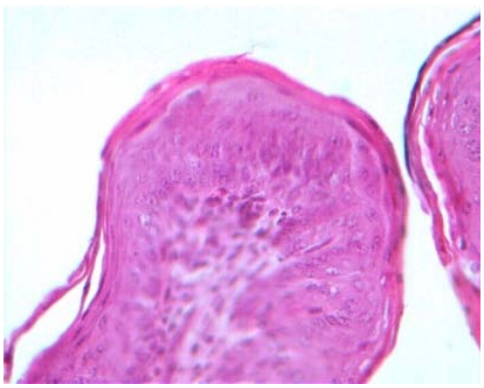

Fig. 19: Rumen papillae 7 days (LQM~7x400)

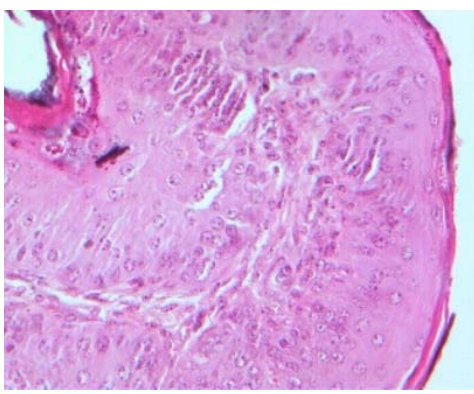

Fig. 20: Rumen papillae 30 days (LQM~30x400)

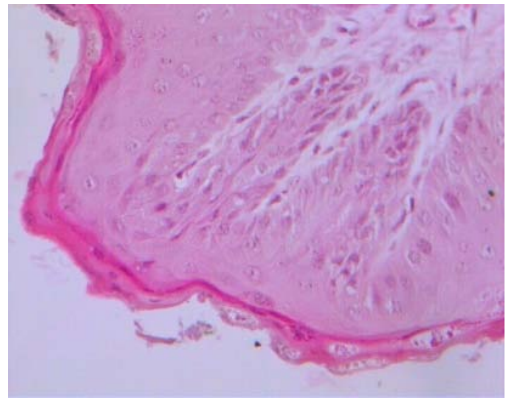

Fig. 21: Rumen papillae 60 days (LQM~60x400)

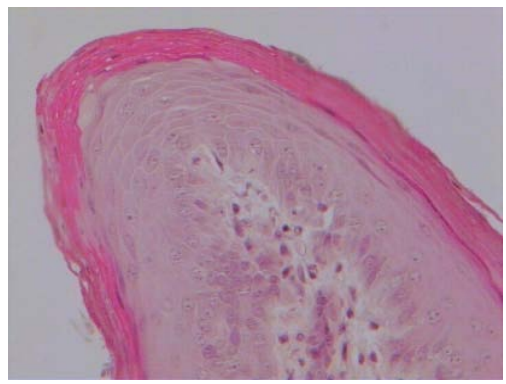

Fig. 22: Rumen papillae 90 days (LQM~90x400)

Fig. 23: Rumen papillae 7 days (HQM~7x400)

because calves weaned at 30 and 60 days were earlier fed solid feed. pH in HQM was significantly lower than that in EW and LQM at 30 and 60 days ($p<0.05$). These indicated that rumen buffering system could not be perfectly consummated and saliva can not fully secreted if calves only taking the liquid feed before the 60 days. The difference in pH was not significant ($p>0.05$) among the three groups because the calves took the same solid food from the 60-90 days. The shift tendency of rumen for EW and LQM was the same that the papillae appearance of 7 days was similar with that of 0 day with temper rolling linguoid. Reductus increased

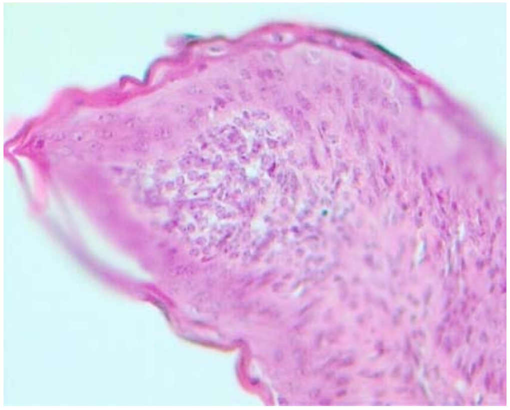

Fig. 24: Rumen papillae 30 days (HQM~30x400)

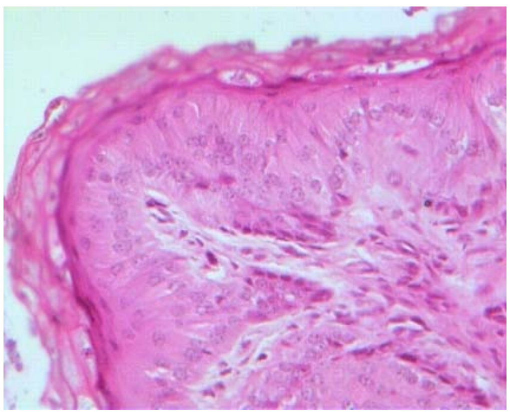

Fig. 25: Rumen papillae 60 days (HQM~60x400)

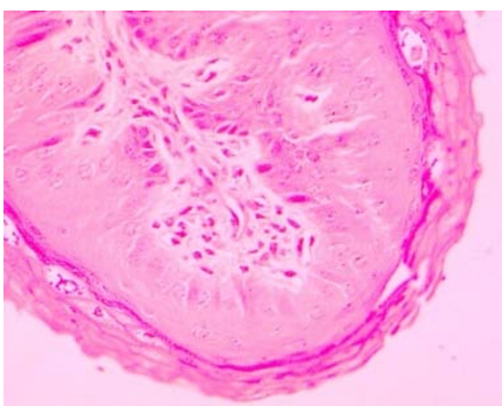

Fig. 26: Rumen papillae 90 days (HQM ~90x400)

after 30 days and the length increased. In HQM, papillae appeared linguoid drape at 7, 30, 60 days; unobvious drapes at 90 days. Thus calves should be fed TMR feed at the right moment.

CONCLUSION

Along with the calves' growth, cellulase and protease activity enhanced the secretion of rumen's microorganisms. The diet has more influence on cellulase and protease activity in the rumen's contents than pH value and chymosin activity. Calves fed without solid feed before 60 days resulted in stunted growth of rumen papillae length, white papillae and disadvantage of nutrient absorption. It can hinder the formation of rumen digestion function if only feed the liquid food was provided to calves before 60 days. Calves feed TMR and early weaned could made cellulase and protease activities enhanced in the rumen and establish stronger rumen digestion function.

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