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The Effects of Tibetan Ewe Fed the Diets with Different Energy Levels on Ruminal Parameters

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Abstract: The study was evaluated the effects of dietary energy levels on ruminal fluid parameters in Tibetan ewes. Tibetan ewes with similar weight, age, pregnant period and after delivering were assigned to three dietary treatments (Digestion Energy (DE) levels: 13.00, 13.50, 14.00 MJ kg^{-1}) and pasturing group as control. After 60 days of experimental treatments, 14 sheep were selected rationally and ruminal fluid were collected to determine the content of Volatile Fatty Acid (VFA), pH, the concentrate of ammonia Nitrogen (NH_3-N), urea N, protein N and total N of ruminal fluid. The results showed that concentration of TVFAs in ruminal fluid with higher DE than that of control group ($p<0.05$), meanwhile, TVFA concentration differed at every treatment group ($p<0.05$). Acetic/propionic, acetic, propionic, delphinic acid of treatments significantly differed with control groups ($p<0.05$) but no difference between each treatment group, butyric and pentanoic acid were not difference among all groups ($p<0.05$). Supplemental concentration might improve total N and protein N while different energy level did not affect total N significantly ($p>0.05$). Compared to pasturing group, the ewes' supplementations with different energy levels did not significantly affect pH and the relative abundance of total N, NH_3-N, urea N and protein N ($p>0.05$).

Key words: Tibetan sheep, ruminal fluid, ruminal fermentation, level, effect

INTRODUCTION

Tibetan sheep has been recognized as an important source of income for pastoral nationalities staying in the remotest cold desert area of Qinghai-Tibetan Plateau. The latitudes and longitudes of the experimental area are 37°20'N and 100°14'E, respectively with the altitude range of 3200~3800 m above mean sea level and the prevailing temperature range is always under subzero to -37°C, annual temperature is -0.6°C. Cold seasons is long and warm seasons is short, annual rainfall is 371.0 mm, evaporation, relative humidity and annual sunshine are 1607.4 mm, 53.0% and 3036.8 h, respectively solar radiation is strong. Experimental area are the typical alpine dry grasslands, major vegetation types are *Kobersia capillifolia*, *Achnatherum splendens*, *Elymus dahuricus*, *Stipa purpurea* and *Poa crvmophila* followed by *Agropyron cristatum*, *Levmus secalinus*, *Festuca sinensis*, *Festuca rubra* and so on. The special climatic conditions and extremely harsh natural environment results in grass growing during a short period (4 months) hay grass period up to 8 months during hay grass period due to the serious shortage of grass nutrient in the traditional grazing conditions, the reproductive performance of Tibetan ewes were affected, ewes estrus rate and pregnant rate are low and birth weight and survival rate of lambs are low (Hou *et al*., 2012). Supplement feed of ewes may change ewe body condition to improve ewe reproductive performance during pregnancy and lactation period. And also good nutrition is closely related to normal rumen digestion and metabolism. Rumen of ruminant is a huge fermentation tank with a large of various rumen microorganisms regulating nutrients metabolism. Many nutritional and non-nutritional factors affect the form of rumen fermentation. The experiment was adding different energy level feed supplementation to observe varieties of rumen metabolic parameters at ewes during lactation period, provide the basic data for improving the effect of feeding lactating ewes.

MATERIALS AND METHODS

Animals and experimental design: The 40 Tibetan ewes with similar weight, age, pregnant period and after delivering were assigned to three dietary treatments (Digestion Energy (DE) levels A: 13.00 MJ kg^{-1}, B: 13.50 MJ kg^{-1}, C: 14.00 MJ kg^{-1}) and D group is control

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which grazing without supplemental feed. The experiment is over a period of 60 days, the lambs weaned at 60 days of age, after 60 days of experimental treatments, 14 sheep were selected rationally and ruminal fluid of the animals were collected before they were fed at morning.

Management and diet: The ewes of treatment groups (A, B and C) were fed 0.1 kg supplemental feed at 8:30 and pasturing at 9:30-16:30 and fed 0.15 kg experiment diet at 16:30, the control group was pastured for 11 h during 7:00-18:00. All ewes access to water twice at morning and evening. Supplementation containing (as-fed basis) as the following Table 1 and forage nutrition in Spring grassland is as Table 2.

Ruminal fluid collection and pre-treatment: The ruminal fluid were collected from sheep before morning meal and filtered with four layers gauzes, PH value was tested. ruminal fluid was add to 2 drops 10% $HgCl_2$ inactivating enzyme. Ruminal fluid for each sheep was transferred to 3 bottles and freezed at -20°C. Prepared for determining total nitrogen (total N), ammonia N (NH_3-N), urea nitrogen (urea N), Protein N and Volatile Fatty Acids (VFAs).

Laboratory analysis: VFA content of ruminal fluid was analyzed by gas chromatography (Agilent 6890N). The pH was measured by Sartorius PB-10 acidity meter. The NH_3-N concentrate was determined by Colorimetric Method (Feng and Gao, 2010). Urea N was tested by Diacetyl Monoxime Method, total N of ruminal fluid was determined by Semimicro-Kjeldahl Method. The crude protein was determined by Kjeldahl determination (Method 984.13).

Table 1: The composition and nutritional level of the experiment diets

Diets	A	B	C
Ingredients (%)			
Corn	57.00	55.00	57.00
Wheat meal	4.30	4.30	4.30
Rape seed cake	18.70	18.70	15.20
Concentrated feeds	20.00	20.00	20.00
Rape oil	0.00	2.00	3.50
Nutritional level			
CP (%)	15.62	15.69	15.59
DE (MJ kg^{-1})	13.01	13.50	14.00
Ca (%)	0.62	0.61	0.55
P (%)	0.55	0.53	0.46
Lys (%)	0.55	0.58	0.68
Met (%)	0.28	0.28	0.26
EE (%)	12.27	14.00	15.57

Table 2: Forage nutrition levels of grazing land (DM)

Diets	Values
CP (%)	4.94
DE (MJ kg^{-1})	7.68
Metabolizable Energy (ME) (MJ kg^{-1})	6.86
NDF (%)	68.08
ADF (%)	42.47

Statistical analysis: The data were analyzed by Univariate procedure of General Linear Model (SPSS 16.0). Tukey post hoc tests were used to determine any significant differences between groups.

RESULTS AND DISCUSSION

NH_3-N content of ruminal fluid: Total N of treatment groups were increased compared to control group without supplement diet. Total N of group A and B was significant higher than that of group D ($p<0.05$), Rumen NH_3-N and Urea N did not differ ($p>0.05$) between the ewes in the four treatments (average±SEM, 6.9±0.170) (Table 3). Protein N of treatments were higher than that control group ($p<0.05$) and they did not differ among supplement diet groups.

The pH and VFAs of ruminal fluid: Rumen pH of treatments were lower than control group ($p<0.05$) while there were no difference among treatment groups that ewes were supplemented ($p>0.05$). Table 4 reports concentration of TVFAs in ruminal fluid that treatments were higher than that of control group meanwhile, TVFA concentration differed at every treatment group ($p<0.05$), especially that of group C was highest, compare to group A and B. In contrast, acetic/propionic, acetic, propionic, delphinic acid of treatments significantly differed with control group ($p<0.05$) but no difference among treatment groups: butyric and pentanoic acid were not difference among all groups ($p<0.05$).

Traditionally, the pattern of the Tibetan sheep is that all sheep always be grazing all of the year at grassland without any concentrate supplement. And nutrition condition of ewes is important for estrus, ovulation, pregnancy and lactating (Robinson, 1990). But to date, just a few of Tibetan sheep were complemented and little information has been published on the effect of different energy level diet on Tibetan ewe ruminal fluid.

The NH_3-N level of ruminal fluid may reflect the protein content of diets and the extent of protein degradation in rumen, the supply of energy and synthesis of microbial protein and so on (McCarthy *et al*., 1989). The change range of ruminal fluid's N-NH_3 was 10~50 mg/100 mL and it achieved peak after feeding 1.0~1.5 h. The ruminal fluid's N-NH_3 was an important intermediate links of N metabolism in rumen because it

Table 3: Concentration of nitrogen fractions in rumen fluid mg/100 mL

Treatments	Total N	NH_3-N	Urea N	Protein N
A	56.590^{a}	32.780	3.000	20.810^{a}
B	52.310^{ab}	32.700	2.200	17.410^{a}
C	58.050^{a}	31.740	1.860	24.440^{a}
D	45.510^{b}	33.850	1.750	9.920^{b}
SEM	1.660	0.340	0.200	1.750
p-values	0.019	0.190	0.123	0.007

Table 4: Concentration of VFAs in rumen fluid

Treatments	pH	TVFA (mmol L^{-1})	Acetic/propionic	Molar ratios of all kinds of acid (mol moL^{-1}) (%) Acetic	Propionic	Butyric	Pentanoic acid	Delphinic acid
A	6.860[a]	47.570[c]	4.600[b]	70.880[b]	15.680[a]	11.950	0.490	1.000[a]
B	6.300[a]	59.890[b]	4.230[b]	71.280[b]	17.150[a]	10.350	0.820	0.620[b]
C	6.320[a]	67.290[a]	3.660[b]	67.390[b]	18.440[a]	13.050	0.540	0.590[b]
D	7.230[b]	34.180[d]	6.910[a]	78.060[a]	11.380[b]	9.090	0.390	1.080[a]
SEM	0.140	3.510	0.370	1.310	0.810	0.620	0.070	0.070
p-values	0.012	0.000	0.001	0.016	0.001	0.093	0.088	0.008

might be used by ruminal microbe and absorbed by ruminal wall (Harrison *et al*., 1975; Han and Chen, 1988). Protein in the diet may increase the supply of products from proteolysis and that this may increase efficiency of microbial protein synthesis and microbial protein flow to the small intestine (Cecava *et al*., 1991). Material containing N of diet entering into rumen of ruminant animal is degraded into peptides and amino acids and eventually deaminated into ammonia N meanwhile at energy enough condition, ruminal microorganisms may utilize free amino acids and nitrogen synthesizing microbial nitrogen in the rumen. The distribution of N within bacterial cells changes with rate of fermentation (Bach *et al*., 2005) so the concentrations of total N, NH_3-N, urea N and protein N content were important to elevate fermentation and degradation of material containing N and express microbial protein synthesis. This experiment showed that supplement feed might increase the concentration of total N compared with pasturing group. Forage of the pasturing group had lower quality and the content of total N supplemental diet may increase intake N on the other hand, supplement diet may increase protein N but there were no difference of protein N content among different energy level supplementation. So, supplying enough energy may increase microbial protein synthesis.

The pH of ruminal fluid is an important parameter to reflect ruminal environment and it's normal range is fluctuating from 5.0-7.5. In general, it achieves the minimum level after feeding 2~6 h (Han and Chen, 1988). Bach *et al*. (2005) report that ruminal protein degradation is affected by pH and the predominant species of microbial population. Ruminal proteolytic activity decreases as pH decreases with high-forage dairy cattle-type rations but not in high-concentrate beef-type rations. The VFAs were the main products of carbohydrate fermentation in rumen and they were the main energy sources and raw materials for milk fat and body fat synthesis. In general, the ratio of acetic acid, propionic acid and butyric acid to TVFAs was 50~65, 18~25 and 12~20%, respectively. The content and ratio of individual acid were impacted by a lot of ingredients, especially feeds and feeding condition (Sauvant *et al*., 1999; Commun *et al*., 2009). Na *et al*. (2004) reports adding 10 g day^{-1} yeast culture to the diet of sheep, after 30 days, daily gain increased by 11.1% and meanwhile it might stable pH. This experiment showed that pH of grazing group was higher than that of supplementation groups in Tibetan ewes. Fiber material that ruminants intake such as soluble carbohydrates and cellulose, hemicellulose and pectin are degraded into monosaccharides by all enzymes secreted by rumen microorganisms these monosaccharides are rapidly degraded to VFA-acetic acid, propionic acid, butyric acid and other lower fatty acids by the intracellular enzymes of microorganisms. The amount and the molar ratio of VFAs produced by the rumen are related to the diet type and energy levels of diet, specially, the change of the molar ratio of acetic acid and propionic acid is most evident, therefore, acetic acid and propionic acid ratio is often used to compare feeds and estimation of the relative nutritional value.

In this experiment, the concentration of TVFAs in ruminal fluid at supplemental feed groups were higher than that of grazing group so, it indicated that rumen microbial proliferation was increased with supplement feed added and more carbohydrates were fully degraded to produce more VFA. Molar ratio of acetic/propionic and acetic in ruminal fluid of group D ewe were higher than those of other groups and the molar ratio of butyric was significantly lower than the other three groups and also indicated that the diet of sheep based on grazing forage and roughage, the type of rumen fermentation tends to Acetic type fermentation while feeding concentrate supplementation might improve rumen fermentation, the fermentation type were changed from acetic type to propionic type, the molar ratio of propionic acid molar ratio was increased and acetic was reduced and improved energy efficiency, reduced molar ratio of acetic/propionic.

CONCLUSION

This study shows for the first time that dietary energy levels affect ruminal fluid parameters in Tibetan ewes, supplemental concentration might improve total N and protein N while different energy level did not affect total N significantly. Compared to pasturing group, the supplementations of ewes with different energy levels did not significantly affect pH and the relative abundance of total N, NH_3-N, urea N and protein N. It showed that rumen fermentation was improved after feeding concentrate supplement and more carbohydrates fully depredated to produce more VFA.

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