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Effects of Dietary Protein Sources on the Rumen Microorganisms and Fermentation of Goats

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Abstract: The objectives of this study were to investigate how rumen fermentation, microbial community and Microbial Protein (MCP) yields changed with dietary protein. Experiments were conducted using four goats fitted with rumen cannula in a 4×4 Latin square design. Experimental diets were divided into 4 groups according to their nitrogen source, which was feather meal (A), corn gluten meal (B), soybean meal (C) and fish meal (D), respectively. The results showed that the mean pH value of group A and C were high, the reverse was true for group B and D ($p<0.05$); the change patterns of pH with time differed from each other although, the mean pH value of group A and C (B and D) seemed to be similar. Concentration of NH_3-N ranged between 6.77-21.67 mg/100 mL, the lowest average NH_3-N concentration (11.08 mg/100 mL) was observed in feather meal supplemental diet (A), while, the highest peak occurred in soybean meal supplemental diet (C) (15.04 mg/100 mL). No significant difference was detected in VFA concentrations among groups, except for valeric acid. Yields of microbial protein also varied with diets; microbial protein of the group C and D were comparatively higher than that of the group A and B ($p<0.05$); while, bacterial protein yields of group C was significantly higher than that of other 3 groups, protozoa to bacteria ratio was also lowest in group C. Further genetic fingerprint analysis revealed that microbial profile was modified by dietary protein within bacteria or protozoa community. It was concluded that rumen fermentation, microbial profile and rumen microbial protein could be modified properly by dietary protein.

Key words: Dietary protein, rumen fermentation, microbe, community

INTRODUCTION

Intestinal supply of amino acids in ruminants mainly arises from two sources: dietary protein, which escapes ruminal degradation and microbial cells, which flow out of the reticulo-rumen. Utilization of protein sources resistant to ruminal degradation has often been studied as a strategy for increasing postruminal protein supply to the host (Stern *et al*., 1983; Santos *et al*., 1984). Great differences can exist in various feed protein in the contents of ammonia-N, peptides, free AA, amides, nucleotides and other nitrogenous compounds (Givens and Rulquin, 2004; Yan and Agnew, 2004), as well as the degradability of feed protein. This, in turn affects the microbial community (Cone *et al*., 1999), such as, the protozoa to bacteria ratio, the profiles of protozoal or bacterial community, etc. and then might affect rumen inner circumstance and metabolism of nutriment subsequently (Ipharraguerre and Clark, 2005; Yu, 2005) and influence the efficiency of dietary protein utilization and the performance of ruminant animals, resultantly (Bach *et al*., 2005). A number of dietary variables clearly influence on ruminal nitrogen metabolism (Ørskov and Shand, 1997; De Boever *et al*., 2004; Ikhimioya *et al*., 2005). Effects of altering intact protein contents supply due to dietary protein source and structure changes on microbial fermentation, especially protozoa and bacterial community composition is unclear. In this study, total mixed ration offered with four different sources of protein with different structure and degradability, were used, adopting to 4×4 Latin square design, this study was designed to evaluate the effects of four various intact protein sources on the fermentation and protein synthesis of ruminal microbes and to demonstrate the characteristics of rumen micro-ecosystem using a fingerprint technique, single-strand conformation polymorphism. This study also hopes to offer some reference for improving the protein utilization of ruminant animals.

MATERIALS AND METHODS

Animals and diets: This experiment was conducted at the Experimental farm of Yangzhou University, Yangzhou, China and was approved by the Yangzhou University

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Table 1: Composition and nutrient level of the experimental diet

Ingredient (g kg^{-1})	A Feather meal	B Corn gluten meal	C Soybean meal	D Fish meal
Corn	254.70	240.00	209.40	237.30
Protein supplement	45.30	60.00	90.60	62.70
Urea	3.40	3.40	3.40	3.40
Dicalcium phosphate	3.60	3.60	3.60	3.60
Salt	3.40	3.40	3.40	3.40
Premix[1]	2.60	2.60	2.60	2.60
Straw	687.00	687.00	687.00	687.00
Total	1000.00	1000.00	1000.00	1000.00
Nutrient level[2]				
Metabolism energy (MJ kg^{-1})	8.38	6.72	7.59	7.14
Dry matter (%)	91.10	89.67	89.80	90.70
Crude Protein (CP) (DM% basis)	10.89	10.88	10.65	10.96
Ca (%)	0.43	0.48	0.46	0.53
P (%)	0.28	0.27	0.25	0.31
Ca/P	1.54	1.78	1.84	1.71
(SC)[3] Structure Carbohydrate (%)	43.40	43.69	43.51	43.26
(NSC)[3] Non-Structure Carbohydrate (%)	35.87	35.43	34.71	34.50
SC/NSC	1.21	1.23	1.25	1.25

[1]The composition of premix: $FeSO_4 \cdot 7H_2O$ 170 g kg^{-1}; $CuSO_4 \cdot 5H_2O$ 70 g kg^{-1}; $MnSO_4 \cdot 5H_2O$ 290 g kg^{-1}; $ZnSO_4 \cdot 7H_2O$ 240 g kg^{-1}; $CoCl_2 \cdot 6H_2O$ 510 mg kg^{-1}; KI 220 mg kg^{-1}; Na_2SeO_3 130 mg kg^{-1}; VA1, 620, 000 IU kg^{-1}; VD3 324, 000 IU kg^{-1}; VE 540 IU kg^{-1}; VK3 150 mg kg^{-1}; VB1 60 mg kg^{-1}; VB2 450 mg kg^{-1}; VB12 0.9 mg kg^{-1}; VB5 1, 050 mg kg^{-1}; Pantothenic acid calcium: 750 mg kg^{-1}; Folacin 15 mg kg^{-1}; [2]Analyzed value; [3]SC = NDF-NDFN; NSC = 100-(NDF+CP+EE+ASH-NDFN)

Animal Care Committee. Four yearlings Xuhuai goat wethers with an initial Body Weight (BW) of 26.2±1.6 kg and fitted with rumen cannulas were used. The diet formulas used were showed in Table 1 and divided into A (Feather meal), B (Corn gluten meal), C (Soybean meal) and D (Fish meal) groups according to their nitrogen source. Amounts of energy offered were calculated to meet energy requirements recommended by National Research Council (NRC, 1981). The goats were fed experimental diet at 1.2 times their Metabolizable Energy (ME) requirement for Maintenance (1.2 M). The animals were given in equal meals per day at 8:00 and 20:00; they also had free access to clean drinking water.

Sampling and procedures: The animals were randomly assigned to four dietary treatments in a 4×4 Latin square design. Each experimental period consisted of a 14 day preliminary period and then followed by 7 days for sampling. Approximately 10 mL digesta mixture was taken from rumen at an interval of 2 h on the first day (8:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00, 22:00, 0:00, 2:00, 4:00 and 6:00, respectively) by using stomach tube. After collection, pH value and ammonia nitrogen concentration was tested immediately. Approximately, 100 mL mixture was obtained from rumen during the followed consecutive 6 days, the sampling time was set to 8:00, 11:00, 14:00, 17:00, 20:00, 23:00, 02:00 and 05:00, respectively for each day, respectively. After fully mixing, approximately, 600 mL of well-mixed liquid effluent was stored at -20°C for microbial isolation and the coming analysis.

Rumen fermentation parameters: Ruminal pH value was measured immediately with a standardized laboratory pH meter (pHS-3C, Huanyu Comp., Jingtan, China) at each sampling time point. Ammonia N concentration was determined by the colorimetric spectrophotometric method (Chaney and Marbach, 1962). The method of protein degradability, *in vitro* culture was described by Tilly and Terry (1963).

Samples of strained ruminal fluid were acidified with 2 mL 6N HCl for analyses of Volatile Fatty Acids (VFA), the concentration of VFA were determined with Gas Chromatography (GC) by the method proposed by Dehority *et al*. (1967), a series of standard solutions were prepared (Acetate, 57.27 mmol mL^{-1}; Propionate, 53.63 mmol mL^{-1}, Isobutyric acid, 3.29 mmol mL^{-1}, Butyrate 17.57 mmol mL^{-1}, Isovaleric acid 3.67 mmol mL^{-1}, Valeric acid 4.61 mmol mL^{-1}) and the concentration of internal standard (Crotonic acid) solution added was 10 mM.

Microbial separation and microbial protein: Microbe was isolated from rumen samples using a modification method based on the works described by Wang *et al*. (2008). For protozoa fraction, the whole sample was diluted in 0.85% of equality volume saline solution and incubated for 1 h in a shaking water bath (125 r min^{-1}) at 39°C, whisking continuously and then squeezed through four layers of surgical gauze. The mixture was then centrifuged at 750× g for 5 min at 4°C and pellet thus obtained as protozoa, subsequently washed twice by saline solution and re-suspended using the same solution in a volume equivalent to the original. For bacteria fraction, the suspension was collected from the above centrifugation and further centrifuged at 22, 000× g for 15 min at 4°C to harvest bacteria, followed by the same steps as protozoa. Microbial true protein was determined by a Trichloroacetic Acid (TCA) precipitation method.

PCR-SSCP, SSCP fingerprint analysis and statistical analysis

DNA extraction: DNA was extracted from samples as described by Zhou *et al.* (1996) with slight modifications, using 2% CTAB (100 mmol L^{-1} Tris-CL; 50 mmol L^{-1} EDTA; 2% CTAB); 2% SDS and Proteinase K (working concentration: 100 μg mL^{-1}). The sample allowed to thaw at 55°C for 2 h firstly and then frozen at -20°C for 2 h to disrupt cell wall. Then, the DNA was extracted by phenol and chloroform. The molecular size of DNA was checked using 0.7% agarose gels and the quantity and quality of DNA samples were assessed by spectrophotometry. Polymerase chain reaction: Bacterial V3 primer used was F338: 5'CCT ACG GGA GGC AGC AG 3'(S- D-B-338-a-S-17) and R518 5' ATT ACC GCG GCT GCT GG 3' (S-D-B-518-b-A-17) according to Muyzer *et al.* (1993). Polymerase chain reactions were performed on automated PCR system 9600 (Applied Bio-systems, USA), ready-to-use Kit was used as instructed by user' guide. The 50 μL PCR reaction mixture contained DNA template 1 μL, 1 μL of each primer, 25 μL PCR master, 22 μL distilled H_2O. PCR amplification was then done at 33 cycles consisting of 94°C for 50 sec, 54°C for 40 sec, 72°C for 45 sec and final extension 72°C for 5 min. The size of DNA fragment by PCR was checked using 2% agarose gel. Protozoal ITS1 primer was designed based on the rDNA sequences of protozoa from Genebank as follows, F1738: 5' AAC AAG GTT TCC GTA GGT 3' and R1951: 5' ACT TCG CTG CGT TCT TCA 3'. The 50 μL PCR reaction mixture contained DNA template 1 μL, 1 μL of each primer, 25 μL PCR master and 22 μL dH_2O. The PCR amplification using condition: initial heating 94°C for 3 min, 35 cycles consisting of 94°C for 50 sec, 52°C for 45 sec, 72°C for 45 sec and final extension 72°C for 5 min. The size of DNA fragment was checked using 2% agarose gel.

Single-Strand-Conformation Polymorphism (SSCP): Before analysis by SSCP, 10 μL of denaturing loading buffer (95% formamide, 10 mM NaOH, 0.025% bromophenol blue and 0.025% xylene cyanol) was added to 10 μL PCR productions. The mixture was heated in a boiling water bath for 10 min and cooled on ice for another 10 min. SSCP analysis was carried out as follows: A gel of 12% concentration, 16 cm length and 10.5 cm width and 1 mm thickness was prepared. Electrophoresis was performed at 4°C at 150 V for 30 min, then 80 V for 16 h in a running buffer of 1× TBE. For community analysis, the gels were digitized to tagged image file format. The lanes were normalized, only bands with intensity of 2% or more of the total intensity per lane were considered in statistical analysis. In order to compare the similarity degree of DNA fingerprint profile between samples, Jaccard index (Sj) was calculated using the equation presented bellow Wang *et al.* (2008):

$$Sj = j/(a+b-j)$$

where:

j = Number of bands common to each sample
a = Number of bands unique to first sample
b = Number of bands unique to second sample

The resulting coefficient can be multiplied by 100 and expressed as a percent. For example, a coefficient of 0.60 can be expressed as 60% similar or 40% dissimilar. Practically speaking, a score of 0.60 means that searching in only one of the 2 samples would result in overlooking 40% of the unique sources.

Statistical analysis: Data manipulation was being done in Excel and statistical analysis was carried out using the software SPSS for Windows (v 11.5). The difference between treatments means was measured by least significant difference.

RESULTS

Ruminal pH-value and NH_3-N concentration: Ruminal pH-value range throughout the sampling period was maintained between 5.60 and 6.80 and the mean pH value of group A and C were high, the reverse was true for group B and D ($p<0.05$) as presented in Table 2 and Fig. 1. The daily variation patterns of pH value were clearly illustrated by Fig. 1. It is worth mentioning that pH value decreased after feeding 1-2 h and dropped to their lowest value at 4 or 6 h and then increased slowly until the next time's feeding. It was however noted that slight fluctuations of pH occurred in groups A, with comparisons to those exhibited by the groups B, C and D and fluctuation at the high value zone.

As Fig. 2 shown, the continuous recording of NH_3-N concentration ranged between 6.77-21.67 mg/100 mL. It was observed that group A, interpreted the lowest average NH_3-N concentration (11.08 mg/100 mL), while, group C, demonstrated the highest peak (15.04 mg/100 mL). As Fig. 2 shows NH_3-N concentration of all groups increased in the post-feeding period for 2-4 h and after reaching a maximum at 2 h, excepting group B (4 h); then gradually decreased to reach a minimum 4 or 6 h later, after which it rose again to its previous level. It was note that group D reached its minimum at 6 h after feeding, which was the earliest among 4 groups. The variation patterns of NH_3-N concentration after the second meal were similar to those observed for the first feed.

Table 2: Effects of different dietary protein on fermentation and microbial protein in the rumen

Items	A Feather meal	B Corn gluten meal	C Soybean meal	D Fish meal	Mean	SEM	p-value
Bacterial protein (mg mL^{-1})	5.5312±0.2730^{A}	5.8509±0.1400^{A}	7.7086±0.3412^{C}	6.9389±0.1736^{B}	6.5074±0.9228	0.1734	0.000
Protozoal protein (mg mL^{-1})	5.2348±0.2461^{A}	5.5106±0.1731^{A}	6.2652±0.2832^{B}	6.4963±0.1289^{B}	5.8767±0.5707	0.1530	0.000
Microbial protein (mg mL^{-1})	10.7659±0.5149^{A}	11.3615±0.3111^{A}	13.9737±0.6243^{B}	13.4352±0.3000^{B}	12.3841±1.4539	0.3255	0.000
Protozoa/Bacteria ratio (P/B)	0.94.66±1.2073^{B}	94.17±0.8935^{B}	81.27±0.1763^{A}	93.63±0.7108^{B}	90.93±5.8212	0.5900	0.000
pH value	6.27±0.22^{B}	6.20±0.41^{A}	6.27±0.32^{B}	6.19±0.31^{A}	6.23±0.32	0.0289	0.000
NH_3-N concentration (mg/100 mL)	11.08±2.04^{A}	12.06±2.18^{A}	15.04±2.62^{B}	14.28±3.40^{B}	13.12±3.05	0.5447	0.027
Protein degradability	0.339±0.26^{AB}	0.333±0.11^{A}	0.449±0.14^{C}	0.383±0.40^{ABC}	0.376±0.53	0.0255	0.031

Microbe: The sum of protozoa and bacteria; Protozoa to Bacteria ratio (P/B): The ratio of protozoal protein to bacterial protein. The same superscript within The same row indicated that the means were not significantly different ($p>0.05$); Neighbor letters indicated that the means were significantly different ($p<0.05$); Parted letters indicated that the means were extremely different ($p<0.01$)

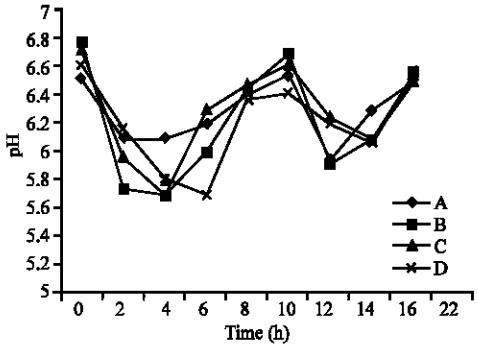

Fig. 1: Change pattern of pH with time

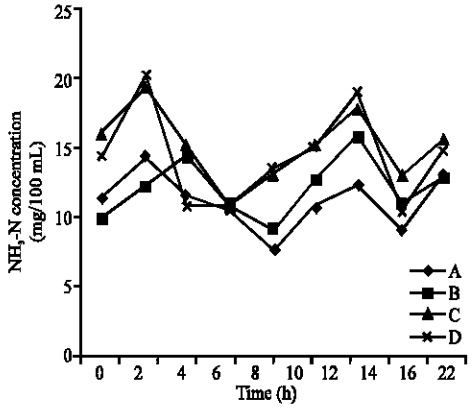

Fig. 2: Change pattern of NH_3-N concentration with time

Volatile Fatty Acid (VFA): The pre-and-postprandial variations total VFA (volatile fatty acid) concentration and main proportion of acetate, propionate, isobutyric acid, butyrate, isovaleric acid, valeric acid were given in Table 3. Table 3 shows the total VFA concentration in the rumen fluid ranged from 74.88-86.34 mM. The concentration of most VFA profiles and total VFA were not affected by dietary protein and time interval, except for a variable valeric acid proportion with dietary protein source ($p<0.05$). The valeric acid proportion of group B was higher than that of group C and D and no valeric acid was detected in group A. Interestingly, no isobutyric acid and isovaleric acid were detected in all the four groups of experiments. Thus, the VFA in the effluent mainly consisted of acetate, propionate and butyrate, accounting for >97% of total VFA and other VFAs was detected at low concentrations (Table 3). Additionally, the acetate to propionate ratio varied significantly with treatment of groups, the lowest ratio was found in group D.

The pattern of acetate, propionate, butyrate, valeric acid were quit similar to that of TVFA production at different sampling time, they increased rapidly after feeding and reached the highest peak at 2 h or 4 h postprandial and then began to decrease gradually till the second meal (Fig. 3-8). The proportion of VFA profiles did not considerably different among treatments at each sampling time, excepting for valeric acid. The valeric acid was significantly lower for group B than for group C and D during the whole day.

Microbial production and Protozoa to Bacteria ratio (P/B): Table 2 and Fig. 9 show that microbial production was wide differences among groups (A, B, C, D) and microbial yields of the group C and D were comparatively higher than that of the group A and B ($p<0.05$). It was obviously noted that the microbial yield of group C and D seemed to be more similar, while, bacterial protein yield of group C was significantly higher than that of group D and higher than that of other 3 groups as well. From Table 2, it was also observed that the protozoa to bacteria (P/B) ratio were greatly variable with treatments, the lowest P/B ratio was found in group C ($p<0.05$).

SSCP fingerprint analysis: The results of photometry showed that OD260/OD280 of DNA ranged from 1.68-1.91; results of agarose gel whose concentration was 0.7 and 2% showed that the sizes of the extracted DNA were >20 kb and the sizes of PCR amplified DNA fragments were about 0.2 kb, respectively.

For the bacterial SSCP fingerprint (Fig. 10), there was a total of 18 clear bands and 11, 13, 13 and 12 bands were detected in group A, B, C and D, respectively and the fingerprint characteristic of each group differed from each other. The 1st, 5th and 16th peculiar band were found in group A and D, but not in group B and C; while, the 2nd peculiar band was found in group B, the 7 and 17th

Table 3: The VFAs production in the rumen under different dietary protein (mmol/L)

Items	A Feather meal	B Corn gluten meal	C Soybean meal	D Fish meal	F	p-value
0 h	78.33±5.98	79.63±7.65	81.49±8.54	80.54±7.89	0.127	0.942
2 h	82.25±6.63	82.11±6.95	87.01±7.53	86.34±7.35	0.536	0.666
4 h	79.54±6.87	83.52±7.81	82.74±8.79	84.42±7.94	0.290	0.832
6 h	82.04±6.65	82.03±8.20	84.32±9.33	83.39±8.28	0.076	0.972
8 h	78.36±6.05	79.74±7.81	83.30±10.13	80.97±8.03	0.265	0.849
12 h	76.95±5.88	79.00±8.16	80.32±8.94	79.73±7.33	0.147	0.929
14 h	81.76±5.96	82.18±7.76	83.77±8.50	84.78±7.27	0.081	0.969
16 h	82.96±6.40	84.56±8.26	84.33±8.96	86.30±7.79	0.120	0.947
22 h	74.88±6.53	77.53±8.41	82.00±9.18	80.83±8.73	0.609	0.622
Average	79.67±6.16	81.15±7.27	83.25±8.03	83.03±7.31	0.130	0.941
VFAs concentration						
Acetate	51.93±2.58	52.63±3.74	51.14±3.86	50.77±4.32	0.225	0.877
Propionate	15.46±1.82	15.43±2.43	16.93±2.22	17.75±1.76	1.121	0.379
Butyrate	12.29±1.99	11.92±1.73	13.11±2.62	12.63±1.64	0.253	0.857
Valeric acid	-	1.16 ± 0.01^{A}	2.07 ± 0.03^{B}	1.88 ± 0.02^{B}	19.767	0.001
Acetate/propionate	3.36 ± 0.23^{A}	3.41 ± 0.30^{A}	3.02 ± 0.22^{A}	2.86 ± 0.09^{B}	5.457	0.013
VFAs percentage (%)						
Acetate	65.18	64.87	61.41	61.16	-	-
Propionate	19.39	19.01	20.34	21.37	-	-
Butyrate	15.43	14.69	15.76	15.21	-	-
Valeric acid	-	1.43	2.49	2.26	-	-

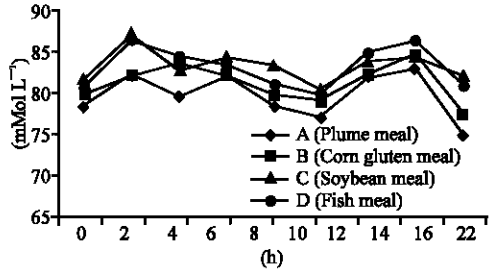

Fig. 3: Change pattern of TVFA in rumen with time

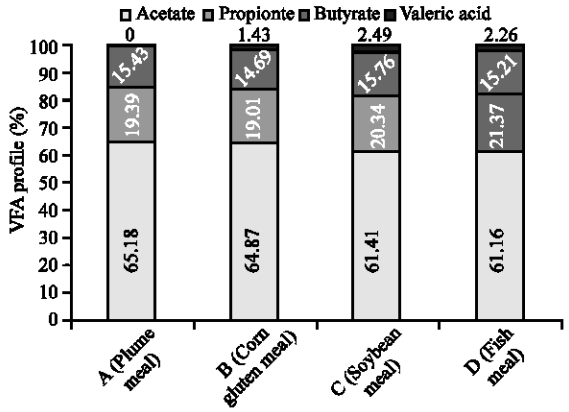

Fig. 4: The profiles of VFAs in rumen

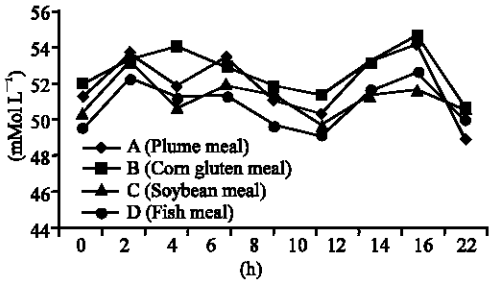

Fig. 5: Change pattern of acetate with time

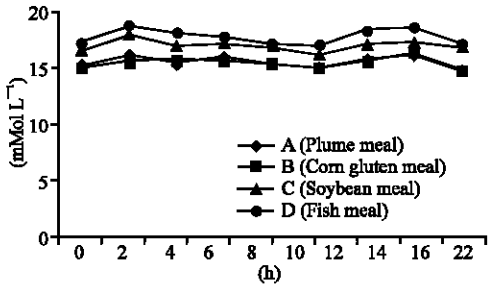

Fig. 6: Change pattern of propionate with time

peculiar band were found in group C and so forth. It was also observed that the grey degree of some bands were different between groups, for the instance, the 16th band of D was brighter than that of A, while, the 18th band of C was more intense than that of B. From Table 4, it was clearly that band-based similarity coefficients between any two gel band patterns were widely different. So, the results indicated that bacterial composition was modified by dietary protein.

For the protozoal SSCP fingerprint (Fig. 10), there were 20 clear bands in total. About 13, 12, 12 and 12 bands were detected in group A, B, C and D, respectively and the characteristics of SSCP fingerprint in each group differed from each other. For examples, the 7th and 13th peculiar band were found in group A, B and C, but not in group D; the other way round, the 1st, 4th and 5th peculiar band emerged in group D. The similarity coefficient between SSCP band patterns were also

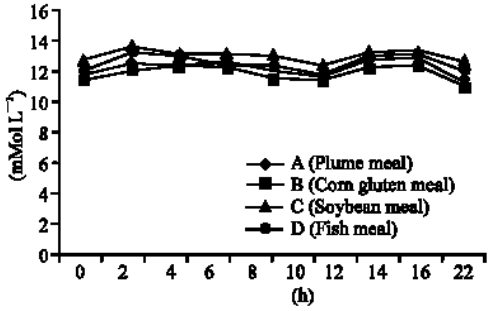

Fig. 7: Change pattern of butyrate with time

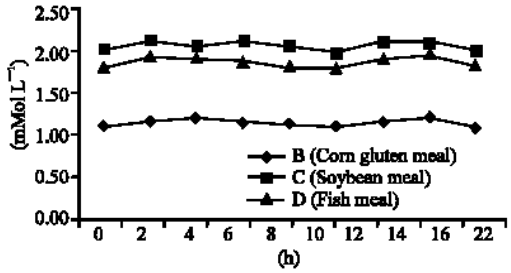

Fig. 8: Change pattern of valeric acid with time

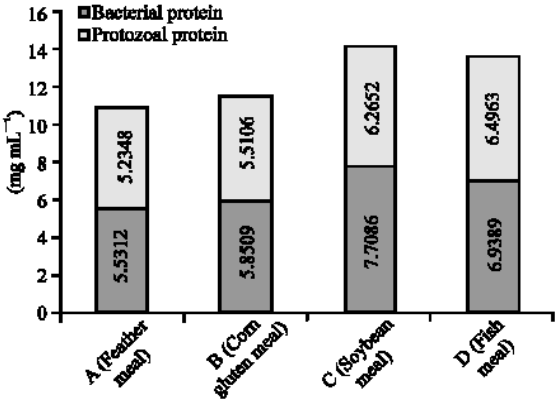

Fig. 9: The biomass of bacteria and protozoa in rumen

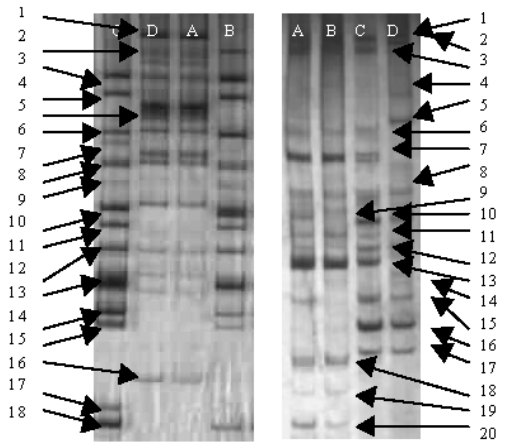

Fig. 10: The results of SSCP test in microbes

Table 4: Matrix of kindred index of microbes

Coefficients	B	C	D
Bacteria			
A	0.333	0.263	0.917
B	-	0.733	0.389
C	-	-	0.563
Protozoa			
A	0.733	0.667	0.471
B	-	0.600	0.263
C	-	-	0.412

different (Table 4). Took an example, there was a higher similarity between groups A and B (0.733), compared to similarity relation between A and C (0.667). Furthermore, the grey degree of certain bands was different between groups, such as the 13th band. In a word, protozoal profiles were subjected to dietary protein.

DISCUSSION

Rumen pH: Rumen fluid pH normally has maintained stable range between 6 and 7, otherwise may be affected by saliva, diet and ruminal degradation products etc. Meanwhile, rumen pH also has profound effects on the microbial flora and the rumen fermentation. So, rumen pH value is a colligation index reflecting microbial activity and fermentation condition in the rumen. NRC (1991) suggested that if pH dropped to about 6.20, microbial populations became markedly depressed due to acid intolerance. And micro-organisms thrived in rumen above pH 5.70, which suitable for microbial growth. In our study, pH-responsive behavior had also been characterized with relatively great fluctuation ranged between 5.60 and 6.80; they were however, all within the desired range for microbe's growth. The mean pH of group soybean meal and feather meal, corn gluten meal and fish meal seemed to be similar, whereas the change patterns of pH value of group soybean and feather meal, corn gluten meal and fish meal were quite different. So, for objective evaluation of rumen pH, it is necessary to combine the mean value and the dynamics of pH. For instance, a large extent of decline was found in soybean meal, compared with feather meal possessed the same pH means, the most probable reasons was estimated as follows. Soybean meal provided an excess metabolizable protein mass, according to the Cornell Net Carbohydrate and Protein System (CNCPS), compared with other sources of protein. Thus, it was easy to degrade within the rumen NRC (2001), Rumen-Degraded Protein (RDP) was important to increase overall microbial growth, subsequently simulated the fermentation of carbohydrate, corresponding with the faster pH decline; While, feather meal contained low concentrations of RDP and inadequate for the amount needed by microbial growth and fermentation and showed that a slight drop of the pH resultantly. In addition, these pH results also agreed well with the TVFA production of current study.

NH_3-N concentration: NH_3-N concentration is an indicator of the protein degradation and the ammonia usage by rumen microbes. In current study, the change range of NH_3-N concentration was from 6.77-21.67 mg/100 mL in the ruminal fluid, with all of them falling within the range suitable for microbial growth (6-30 mg/100 mL) (Devant *et al*., 2000). The mean NH_3-N concentration of group soybean and fish meal were higher than that of group plume and corn meal and indicated that dietary protein in the latter two groups were easy to degrade in rumen, attributing to the differences in structure and degradability of different protein sources (NRC, 2001). This was also in agreement with the degradation results of protein in present research. Moreover, corn meal came to the top point at 6 h, little later than other 3 groups; the probable reason was estimated as follows. The corn gluten meal contained less soluble proportion than other proteins; the degradation rate of digestible true protein was also slower than that of other proteins. So, corn gluten meal showed extremely slow release of ammonia. Thus, the corn gluten meal might reach its peak value of NH_3-N concentration late. However, NH_3-N concentration of fish meal decreased sharply after its peak and reaching its lowest value firstly. The main reason was that the degradation rate of true protein fraction in fish meal was comparatively slow and couldn't match to the speed of ammonia utilization by microbes. This consequently, made a more rapid decline in NH_3-N concentration.

Diets might influence NH_3-N concentration through manipulating microbial flora (Ivan *et al*., 2000), because great differences might exist between microbial species in deaminase activity. Wallace *et al.* (1996) considered that deaminase activity of M. elsdenll, P. Ruminicola were much lower than that of Peptostreptococcus, C. aminophilum; E. caudatum and E. simplex ciliate had high deaminase activity (Forsberg *et al*., 1984; Lockwood *et al*., 1988). The other way round, Isotiicha ciliate could lower the concentration of NH_3-N in the rumen. The higher NH_3-N concentration was observed in soybean meal supplemented treatments, but lower in fish meal in current study. The probable reason behind this might be that soybean meal group had big populations of Peptostreptococcus and Entodinium, or small populations of M. elsdenll and Isotiicha, compared to fish meal group. Furthermore, in current research, the structural diversity of both the bacteria and the protozoa was monitored by SSCP, single-strand conformation polymorphism, showed that the community composition of microbe, including bacteril and protozoa, were modified by dietary protein and certain bands emerged as dominant bands and peculiar bands under given protein. But what species they were, was still not very clear before conducting further investigation in this research. If the bands were sequenced, the species could be identified according to the sequencing information.

Volatile fatty acids: Volatile Fatty Acids (VFA's) are produced in large amounts through ruminal fermentation from carbohydrate and are of paramount importance in that they provide >70% of the ruminant's energy supply (Takagi and Block, 1991). VFA mixture consist of a large percentage of acetic, proprionic and butyric acids, accounting for 95% and a small percentage of isobutyric acid, isovaleric acid and valeric acid. It is possible to improve energy utilization efficiency, through controlling diet, because VFA profile is associated with diet (Wan *et al*., 2003; Wang *et al*., 2005). Han *et al.* (2002) reported that rumen low-degradability protein or rumen bypass protein supplement could increase the amounts of utilizable protein in small intestine and this would increase the disposition of dietary protein into body stores and promote gluconeogenesis of glucogenic amino acid, led to increased NADPH production, resultantly improve energy conversion efficiency of roughage diet with the main end-product of VFA being acetate. Tan and Lu (2000) reported that a fish meal based supplement increased the percentage of proprionic acid and dynamic degradability of straw cellulose. Current research also found a lower actate to proprionic ratio in fish meal group, agreeing with Tan and Lu (2000). Differences in the fermentation pattern of the rumen contents could be reflected in the VFA profile (Martyx and Demeyer, 1973). In this research, the concentrations of total VFA and most of VFA profiles were little difference across groups, this meant that VFA did not alerted greatly by dietary protein.

Synthesis of MCP: Many experiments showed that synthesis of microbial protein was affected by dietary protein (Tigemeyer *et al*., 1989; Cecava *et al*., 1991; Ludden and Cecava, 1995). Ipharraguerre and Clark (2005) suggested that the degradability of dietary protein might modulate the MCP outflow, resulted in the change of animal performance. *In vitro* experiment by Li (2001) revealed that bacterial protein yields of soybean meal and fish meal were higher than that of feather meal. Similarity, the *in vivo* experimental results described by Wang *et al.* (1999) showed that blood meal decreased the microbial nitrogen flow into the duodenum of sheep and host productivity, the authors attributed to the structure of blood protein, which containing low content of degraded portion and resultantly leading to low efficiency of MCP synthesis in rumen. This research revealed that microbial protein of soybean meal and fish meal was much higher than that of the feather meal and corn gluten meal,

conforming to previous reports described above. Furthermore, the bacterial protein yield of soybean meal was significantly higher than that of fish meal, whereas, the MCP yields of soybean meal and fish meal were similar. The variations in synthesis of microbial protein might be explained by the special structure of different feed proteins and the specific metabolic characteristics of different microbes. For example, protozoa had a remarkable ability to degrade particle protein, including bacteria, fungi, or feed undegraded protein particle etc., while, bacteria was liable to degrade soluble protein. Just coincidently, the rumen degradability of soybean meal was higher than that of fish meal, more suitable for bacteria growth and led to a low P/B ratio in soybean meal.

CONCLUSION

The dietary protein had great effects on rumen fermentation pattern; nevertheless it did not strongly affect the VFAs. Dietary protein has a great impact on microbial protein synthesis and microbial community structure. Further sequencing experiments of SSCP band sequences would help to identify the main species within bacterial and protozoal community.

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