Alfalfa Hay Supplementary Intake is Advancing Effect To Rumen Fermentation and Microbial Function of Gansu Alpine Fine Wool Sheep During Cold Season

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Abstract

Bodyweight loss and rumen microbial dysfunction of grazing sheep was a challenge for the sheep production industry during cold season, which were considered to correlated with under-roughage-feeding. The objective of our research was to assess the role of Alfalfa hay supplementary intake in roughage in ewes' rumen fermentation and microbial function. 120 ewes were allocated randomly into 4 groups, and were fed with different level of alfalfa hay, respectively. Individual ewes' bodyweight, blood biochemical indexes and rumen microbial characteristics were analyzed after the end of feeding trials. The results showed that alfalfa hay supplementary could significantly enhance sheep body weight, nitrogen components (Total-N, Soluble protein-N and Ammonia-N), blood biochemical indices (LDH, BUN and CHO) and ruminal volatile fatty acids ($P < 0.05$). Meantime, alfalfa hay supplementary increased the richness and diversity of ruminal fluid microbiota, and decreased ruminal fluid microbiota beta-diversity. The ruminal fluid microbiota of alfalfa hay supplementary feeding showed low immune pathway and high carbohydrate metabolism pathway. Overall, the study suggested that there was an increasing tendency of alfalfa-hay-supplementary group in 30% Yellow Maize Silage + 70% Alfalfa Hay roughage in body weight, ruminal fermentation and microbial function, which improved GS performance through developing hay supplementary system during cold season.

Introduction

The Gansu alpine fine-wool sheep (GS) is the first cultivated breed of plateau type fine wool-sheep in Sunan plateau area, which is breed by means of crossbreeding and selective improvement from Xinjiang and Caucasus fine-wool sheep as male parents, and Tibetan sheep and Mongolian sheep as female parents(zhou WJ et al.2017). The Sunan plateau (Zhangye, Gansu) is known for its extreme harsh conditions,the characteristics is high altitude, low air oxygen and short forage growing season. GS grazed grassland all year round with traditional grazing management in Sunan plateau, however, long-lasting cold season and decline in nutritive value of pasture making it difficult to meet nutritional requirement for sheep, resulting in growth retardation, hypoimmunity and higher mortality rate(Deng KD et al.2018). During cold season (winter and early spring), there is a shortage of high-quality grass hay for GS, and it always struggle to maintain the normal physiological function. Previous researches demonstrated that there are positive effects of hay supplementary on sheep productive and reproductive performance, which can reduce body weight-lost, feeding costs, and increase economic efficiency(Tulu A et al.2018;Huang JQ et al.2017). Therefore, there is a big potential to improve GS performance through developing hay supplementary system in cold season.

Alfalfa has the advantages of rich nutrition and high quality, which is widely used in livestock production, and it is also praised as "the king of forage". Alfalfa hay is a good roughage resources for ruminants, which could improve nutrient digestibility, meat quality and production performance(Wang C et al.2019). There is great significance to promote animal health and improve animal production performance. Alfalfa was widely planted in Gansu Province, and Alfalfa hay, as high-quality roughage, had been well received in animal husbandry in Sunan plateau area. The diet nutritional value was affected by the feed intake,

animal digestibility rate(Liu JH et al.2016). Many studies show that adding appropriate alfalfa hay to roughage could improve diet digestion, however, with the elevated proportion, the digestibility has a downward trend, which was resulting in a negative effect(Babiker EE et al.2016). Rumen was an important organ of nutrition digestion, absorption and metabolism, also a main barrier against harmful substances. The research showed that ruminal epithelium barriers was easy to be damaged by the influence of external factors, which leaded to microflora dysfunction, decrease the production performance(Fustini M et al.2016). Dietary interventions can improve production traits via changing gastrointestinal microflora abundance. The rumen microbiota is help to the host, including nutrient absorption, metabolism and developing the immune defense systems maintenance(Sun HZ et al.2016). Distinct microorganism (bacteria, archaea, fungi and protozoa) lived in the rumen with a symbiotic relationship of ruminant herbivore. These microbes could supply vitamins, protein and carbonhydtrate for host by secreting lytic enzymes available for the microbial fermentation. Additionally, rumen microorganisms improved both host organic immunity and resistance to invading pathogens(Cui ZH et al.2020).

The compositions of microbial communities of the ruminants were highly responsive to change in diet type, physiological status, and management strategy. Although several microbiome studies performed on ovine rumen, the adaption of rumen microbiome to changes in roughage was rarely reported. It is speculated that the feed rich in cellulose and hemicellulose is digested in rumen, and the nutrients are absorbed into organism through epithelium to provide energy for ruminants(Ozbayram EG et al.2017). Sheep could exhibit high dry matter, fiber digestion and efficient protein utilization when limiting by energy intake. Dietary protein and energy are often concomitantly limited in grazing GS during cold season. We questioned whether Alfalfa hay digestive rate would favor GS when offered the maintenance energy and protein intakes. Here, different levels of alfalfa hay were offered GS to simulate the supplement dietary intakes in the cold season to determine GS rumen biochemical indexes and microflora population. Collectively, our results shed new light on the effect of alfalfa hay supplement dietary on GS feeding.

Material And Methods

Animals and Experimental Design

The study was carried out in Yugur Autonomous County of Sunan, Gansu Province, China, which is situated at Qilian Mountain of northeastern Qinghai-Tibetan Plateau. This area is over 3 000 m above the sea level and has a dry cold climate.

Gansu alpine fine wool ewes (aged 1.5-yr-old and 28.71 ± 1.22 kg) were housed at the Sunan herdsmen's professional cooperatives, Zhangye state, Gansu province, China. During the experimental period, all sheep were fed in intensively sheep-shed in supplementary feeding on different level hay, and the chemical composition of concentrate feed was the same in all groups. 120 ewes were allotted randomly into four groups (30 ewes/group), which were as follows: 100% Yellow Maize Silage roughage group (A),

70% Yellow Maize Silage + 30% Alfalfa Hay roughage group (B), 30% Yellow Maize Silage + 70% Alfalfa Hay roughage group (C) and 100% Alfalfa Hay roughage group (D). The dietary of each sheep consisted of 0.2 kg concentrate and 1.0 kg roughage (DM). Alfalfa hay were chopped into 1–2 cm length. The ingredients composition and nutritional level of Dietary were listed in Table 1. The experiment lasted for 100 days from 16th November 2017 to 23rd March 2018, where the first 10 days was adjustment period, followed by 90 days of data collection period.

Table 1
Dietary and Proximate Nutrition Content(DM)

Maize	50	50	50	50
Soybean Meal	6.5	6.5	6.5	6.5
Cottonseed meal	3	3	3	3
Rapeseed meal	3.5	3.5	3.5	3.5
wheat bran	3	3	3	3
Premix [b]	4	4	4	4
Yellow Maize Silage	30	21	9	0
Alfalfa Hay	0	9	21	30
Nutritional level				
ME(MJ/kg) [a]	11.45	11.45	11.45	11.45
CP(%DM)	14.08	14.89	15.98	16.79

[a] Metabolizable energy (ME) was the calculated value, and others were the measured values.

[b] The premix provided the following per kg of dietary: VA 12000IU, VD 20 00IU, VE 30IU, Cu 12mg, Fe 64mg, Mn 56mg, Zn 60mg, I 1.2mg, Se 0.4mg, Co 0.4mg.

Bodyweight, Blood biochemical indices and Rumen fermentation measurement

The GS were weighed at 1 day and 90 days of sample collection period by using platform scale before feeding in the morning.

Jugular blood (10 mL) was collected into heparinized vacutainer tubes and centrifuged at 2 500 rpm for 15 min. Plasma samples were stored at -20°C for analysis of blood urea-N (BUN) concentration. Total protein (TP), Albumin (Alb), alanine aminotransferase (ALT), Aspartate aminotransferase (AST), triglyceride (TG), cholesterol (CHO), glucose (GLU), lactate dehydrogenase (LDH) was measured colorimetrically with auto analyzer (Aeroset, Abbot Toshiba, Japan) used commercially TaKaRa kits.

The stomach tube was inserted into the rumen to collect rumen fluid. Approximately 50–100 ml of rumen fluid was collected from each sheep, strained through 4 layers of cheesecloth and then the pH was determined immediately. The rumen fluid samples were aliquoted into 10mL sterile tubes and then transferred to a -80°C ultracold storage freezer in the lab for further analysis. Ruminal TVFA, including acetic acid, propionic acid, butyric acid, analyzed by gas chromatography (TRACE GC 1300, Thermo Scientific, USA) using a capillary column (AT-FFAP: 30 m). Ammonia-N, soluble protein-N and urea-N were determined by colorimetry (GENESYS 10S Vis, Thermo Scientific, USA): ammonia-N assay according to Hristov (2001); soluble protein-N assay according to Oosta (1978); and urea-N assay according to Marsh (1957).

Rumen microbial characteristics

The collected rumen fluid sampling was filtered with the 4-layer gauze. The liquid was filtered with a mixing fibroid membrane (aperture 0.45 μm) and filter membranes were stored at -80°C for the extraction of DNA with the Magnetic Universal Genomic DNA Kit (TIANGEN, Beijing, China), following operational guidelines. TBS-380 (Turner BioSystems, USA) was employed to determine con-centration of extracted DNA while NanoDrop2000 (Thermo Fisher Scientific, USA) was used for assessing the purity of extracted DNA.

16S rRNA gene sequencing detection was outsourced to BIOMARKER (Beijing, China). Illumina HiSeq 2500 sequencing of 16S rRNA gene was performed to characterize microbial diversity and community composition. The V3-V4 hypervariable region of the microbial 16S rRNA gene were amplified by PCR according to primers forward (5'-CCTACGGGNGGCWGCAG) and reverse (5'-GGACTACHVGGGTATCTAAT). The cycling protocol was 95°C for 2 min; 35 cycles at 95°C for 2 min; 72°C for 30 s; and 72°C for 5 min.

The 16S rRNA analysis was performed by R software (version 3.1.2), QIIME software (version 1.9.1) and UPARSE software. According to the UPARSE pipeline, multiplexed reads were clustered into operational taxonomic units (OTUs) based on 97% sequence identity. The 16S rRNA gene sequence was classified by RDP Classifier (version 2.2). Present figures were conducted using R package.

Statistical analysis

The results of the analysis were given as mean ± SD and determined differences were shown according to level of hay supplementary. Duncan post hoc test was used to determine any significant differences between groups. Differences were considered as significant at $P < 0.05$ and as a trend at $P < 0.1$.

Results

Bodyweight and blood biochemical indexes

The initial weights, final weights of the GS (kg) as well as daily body weight gain (g/day) of different alfalfa hay supplementary levels were presented in Table 2. Our results showed that the final weights and daily body weight gain of alfalfa-hay-supplementary groups (B, C and D group) were significantly higher

than that of alfalfa-hay-free group (A group) ($P < 0.05$). There were no obvious differences among alfalfa-hay-supplementary groups in final weights ($P > 0.05$), and there was an increasing tendency of 30% Yellow Maize Silage + 70% Alfalfa Hay roughage group (C) in final weights, and daily body weight gain.

Table 2
Effect of Alfalfa Hay Supplementary on Body Weight

Items	A	B	C	D
Initial Weight (Kg)	28.03 ± 0.72	28.26 ± 0.55	27.97 ± 0.95	28.31 ± 0.45
Final Weight (Kg)	33.57 ± 0.77^{a}	34.83 ± 0.59^{b}	35.08 ± 0.84^{b}	34.67 ± 0.64^{b}
Average daily gain (g/d)	61.56 ± 4.43^{c}	73.00 ± 4.95^{a}	78.89 ± 4.03^{a}	70.37 ± 3.25^{b}

The blood biochemical indices obtained of different alfalfa hay supplementary levels were summarized in Tables 3. The BUN Value was above normal range, and others were within the normal range. LDH, BUN and CHO value of mixed-roughage groups (B and C group) were significantly greater than single-roughage groups (A and D group, $P < 0.05$), and there were no significant differences in TP, Alb, AST, ALT, TG and GLU value among all groups ($P > 0.05$).

Table 3
Alfalfa Hay Supplementary on Blood biochemical indices

Items	A	B	C	D	Normal Range
TP g/L	62.25 ± 0.75	63.25 ± 1.42	65.67 ± 1.55	63.28 ± 1.18	47.30-78.03
Alb g/L	32.33 ± 0.33	32.25 ± 0.48	32.75 ± 0.48	32.42 ± 0.51	15.48–38.11
AST U/L	130.25 ± 4.31	132.67 ± 4.06	133.48 ± 3.08	129.81 ± 4.11	106.5-142.5
ALT U/L	30.25 ± 1.85	30.33 ± 1.88	30.85 ± 2.14	30.18 ± 1.59	19.5–34.8
BUN mmol/L ↑	7.21 ± 0.15^{b}	7.41 ± 0.24^{a}	7.45 ± 0.06^{a}	7.25 ± 0.17^{b}	2.9–7.3
TG mmol/L	0.23 ± 0.02	0.24 ± 0.05	0.26 ± 0.01	0.02 ± 0.01	0.14–1.09
CHO mmol/L	2.62 ± 0.02^{b}	2.92 ± 0.05^{a}	2.93 ± 0.04^{a}	2.54 ± 0.06^{b}	2.0-3.4
LDH mmol/L	286.00 ± 5.72^{b}	304.75 ± 8.16^{a}	303.00 ± 9.51^{a}	289.37 ± 7.98^{b}	233.3-341.5
GLU mmol/L	2.22 ± 0.27	2.30 ± 0.08	2.31 ± 0.22	2.31 ± 0.17	1.5–2.5

Rumen fermentation

The ruminal pH and Nitrogen composition of different alfalfa hay supplementary levels were listed in Table 4, Total-N and Soluble protein-N of alfalfa-hay-supplementary groups (B, C and D group) were significantly higher than that of alfalfa-hay-free group (A group) ($P < 0.05$), Ammonia-N of mixed-roughage groups (B and C group) were significantly greater than single-roughage groups (A and D group, $P < 0.05$), there was no significant difference in ruminal pH and Urea-N among all groups ($P > 0.05$). The ruminal Volatile Fatty Acids (VFAs) of different alfalfa hay supplementary levels were listed in Table 5, TVFA, Butyrate and Acetate/ Propionate of alfalfa-hay-supplementary groups (B, C and D group) were significantly higher than that of alfalfa-hay-free group (A group, $P < 0.05$), Propionate of mixed-roughage groups (B and C group) were significantly greater than single-roughage groups (A and D group, $P < 0.05$), there was no significant difference in Acetate between 4 groups ($P > 0.05$).

Table 4
Alfalfa Hay Supplementary on ruminal pH and nitrogenous components

Items	pH	Ammonia-N	Urea-N	Total-N	Soluble protein-N
A	6.41 ± 0.07	20.79 ± 0.15^{b}	1.09 ± 0.05	125.31 ± 0.12^{b}	102.40 ± 0.05^{b}
B	6.45 ± 0.83	23.77 ± 0.48^{a}	1.17 ± 0.09	131.94 ± 0.33^{a}	108.21 ± 0.25^{a}
C	6.44 ± 0.11	24.24 ± 0.70^{a}	1.15 ± 0.04	134.29 ± 0.56^{a}	112.65 ± 0.92^{a}
D	6.43 ± 0.58	21.23 ± 0.71^{b}	1.11 ± 0.03	129.54 ± 1.23^{a}	106.37 ± 0.73^{a}

Table 5
Effect of level of hay intake on ruminal VFAs

Items	TVFA(mmol/L)	Acetate(%)	Propionate(%)	Butyrate(%)	Acetate/ Propionate
A	48.71 ± 3.16^{b}	27.84 ± 2.28	12.93 ± 0.62^{c}	4.80 ± 0.26^{b}	2.15 ± 0.08^{a}
B	57.84 ± 2.40^{a}	27.23 ± 4.03	20.30 ± 0.74^{a}	7.59 ± 0.50^{a}	1.39 ± 0.11^{c}
C	59.49 ± 3.28^{a}	29.90 ± 2.49	21.39 ± 0.91^{a}	7.34 ± 0.78^{a}	1.34 ± 0.12^{c}
D	55.97 ± 1.96^{a}	28.46 ± 3.15	17.29 ± 0.54^{b}	7.64 ± 0.38^{a}	1.64 ± 0.13^{b}

Rumen microbial community and function

After filtering out low-quality reads and chimeras of Illumina sequenced reads, we obtained a total of 996 032 clean tags high-quality sequences. The number of sequences for 24 sample ranged from 887 134 to 1 011 715. The Good's coverage was in the range of 0.9818 to 0.9867. The remaining high-quality

sequences were clustered into OTUs according to 97% similarity level by UPARSE software. All detected OTUs were 24 432and the averaged value of OTUs for each sample was 687, which mapped to 21 phyla, 41 classes, 89 orders, 147 families, 297 genera and 324 species (Fig. 1). The top 10 relative ruminal fluid microbiota abundances at the genus level are presented in Fig. 2. *Ruminococcus*, *Ruminococcaceae* and *Prevotella* were observed as the predominant phyla in ruminal fluid microbiota, followed by *Christensenellaceae*, *Quinella*, *Rikenellaceae* and *Butyrivibrio*. The relative abundances of *Ruminococcus*, *Christensenellaceae*, *Ruminococcaceae* and *Prevotella* accounted for more than 1% of the total microbiome. For 30% Yellow Maize Silage + 70% Alfalfa Hay roughage group (C), the relative abundance of beneficial microorganisms was significantly increased ($P < 0.05$) compared with other groups ($P < 0.05$). *Ruminococcus* and *Quinella* of mixed-roughage groups (B and C group) were significantly greater than single-roughage groups (A and D group, $P < 0.05$).

The alpha diversity was estimated through the diversity index (Shannon and Simpson) and richness estimate (Chao1 and ACE). As can be seen in Table 6, the richness estimate (Simpson, ACE and Chao1) increased significantly for mixed-roughage groups (B and C group) compared to single-roughage groups (A and D group), whereas the Shannon indices were significantly lower than mixed-roughage groups (B and C group, $P < 0.05$). 30% Yellow Maize Silage + 70% Alfalfa Hay roughage group (C) almost showed significant enhancement in the alpha-diversity of ruminal fluid microflora than other groups, this indicated that 70% Alfalfa Hay roughage feeding increased the richness and diversity of ruminal fluid microbiota. Furthermore, the differences in the microbial beta-diversity between the different feeding groups were evaluated based on binary jaccard distances (Fig. 3). The mixed-roughage groups (B and C group) showed lower ruminal fluid microbiota beta-diversity, which indicated their higher microbiota stability and lower dispersion. Principal co-ordinates analysis (PCoA) using the weighted unifrac similarity method revealed that the PC1 and PC2 explained 49.12% and 12.10% of the variation between the samples, respectively, it indicated that ruminal fluid samples from different groups formed different clusters in the ordination space (Fig. 4).

Table 6
Alpha diversity of ruminal fluid samples

Items	A	B	C	D
Shannon	4.13 ± 0.28^{b}	4.79 ± 0.15^{a}	4.73 ± 0.21^{a}	4.71 ± 0.29^{a}
Simpson	0.064 ± 0.019^{a}	0.026 ± 0.003^{b}	0.027 ± 0.08^{b}	0.032 ± 0.007^{b}
ACE	878.87 ± 3.47^{a}	734.79 ± 6.29^{b}	758.33 ± 9.99^{b}	747.25 ± 5.35^{b}
Chao1	873.69 ± 15.07^{a}	749.93 ± 17.54^{b}	702.43 ± 17.47^{b}	747.99 ± 16.33^{b}
Coverage/%	98.67	98.18	98.33	98.36

Microbial community structure

We performed LEfSe analyses to identify the significance of different taxa (relative abundance > 1%) among 4 groups, and LDA results from the LEfSe analysis were showed in Fig. 5. In A group, we found that *Bacilli*, *Lactobacillales*, *Firmicutes*, *Streptococcaceae* and *Streptococcus* were significantly abundant taxa. In B group, *Gammaproteobacteria*, *Escherichia_Shigella*, *Negativicutes*, *Selenomonadales*, *Veillonellaceae* and *Prevotellaceae* were significantly abundant taxa. In C group, *Buchnera*, *Alphaproteobacteria*, *Rickettsiales*, *Rickettsiaceae* and *Rickettsia* were significantly abundant taxa. In D group, *Prevotella_7*, *Bacteroidales*, *Bacteroidia*, *Bacteroidetes*, *Megasphaera* and *Pseudomonadales* were significantly abundant taxa. To investigate the functional capacity of the ruminal fluid microbiota communities, PICRUSt was used to further analysis the KEGG pathway compositions (Fig. 6). The results showed that the second level KEGG pathway of Amino acid transport and metabolism, Carbohydrate transport and metabolism, Cell cycle control, Cell motility, Cytoskeleton, Energy production and metabolism were enriched.

Discussion

Rumen is an important part of the digestion, absorption, and metabolism of nutrients, and acts as an important barrier against the harmful substances, thereby, playing an important role in the sheep health(Sun XZ et al.2020). The ruminant need intake 30%-70% dietary roughage to maintain normal rumen function and gastrointestinal microflora environment. Many researchers have reported that additional hay feeds can elicit a response in terms of reduced weight loss, increased growth and production in grazing sheep farming system(Scocco P et al.2016). The fine-wool ewes supplemented with concentrate feed or hay lost less weight over winter than the hay supplementary feed systems have been widely used to balance nutrition of ruminants fed low quality forage-based diets on confinedness(He B et al.2019). In our studies, 30% Yellow Maize Silage + 70% Alfalfa hay supplementary intake could be useful to increase sheep serum chemistry values, it was in accordance with earlier reports for grazing sheep with supplemented hay in cold season.

Modern hay supplementary intake pattern changed the feedstuff residing time in sheep rumen and improved the amount of carbohydrate and microbial fermentation level(Belanche A et al.2019;Hristov AN et al.2001;Oosta GM et al.1978). Basically, Ruminal pH depends mainly on the balance of VFAs production and buffer secretion, which are used to reflect rumen natural activities status(Marsh WH et al.1957). Our research showed that the pH value of GS was between 6.41–6.45, which was within the optimal range. It indicated that our sheep dietary could maintain the stability of rumen environment, and ensure the degradation of roughage via rumen microorganisms. Ammonia-N is the most important N source for microbial protein synthesis in the rumen(Zhou XL et al.2018;Wishart H et al.2018). Ammonia-N, Total-N and Soluble protein-N were observed greater in alfalfa-hay-supplementary groups (B, C and D group) than alfalfa-hay-free group (A group), indicating the larger N resources for microbial growth in Alfalfa hay supplementary intake. In the present study, a greater efficiency of urea recycling was observed

in 30% Yellow Maize Silage + 70% Alfalfa Hay roughage group (C), which helps these animals to survive under poor dietary condition of the Sunan Plateau.

As far as we know, VFAs originated from carbohydrates via hydrolysis by ruminal microbes. The observed negative relationship between ruminal pH and VFAs concentration in the present study had been reported in several publications(Guo W et al.2020). The greater ruminal TVFA in alfalfa-hay-supplementary groups (B, C and D group) than alfalfa-hay-free group (A group) implied that there was more energy absorption for alfalfa-hay-supplementary than alfalfa-hay-free when consuming with the same energy intakes. The changed tendencies in molar proportions of propionate and butyrate reflected the shift in VFA-producing pathways and microbial populations(Huang XD et al.2020). The predominant ruminal microbes would be fibrolytic microbes(Zhang ZG et al.2016). The decreased molar proportion of propionate and butyrate with a decrease first and then increase in alfalfa hay intake most likely resulted from the stimulated activity of fibrolytic microbes for TVFAs incorporation in microbial protein synthesis(Colmenero JJ,Broderick GA.2006). Serum BUN level is associated with protein uptake, the alfalfa hay was rich in protein, according to our results, BUN activities not only increased in mixed-roughage groups (B and C group), but also its level increased above the normal range during cold season. The increased BUN levels prove the high protein uptake in mixed-roughage groups (B and C group) than single-roughage groups supplementary feed groups. There is evidence that CHO deficiencies are common in grazing animals. In previous study, CHO levels were found below 2.0 mmol/L in grazing GS, and increased CHO activities in present study can be depend on alfalfa hay supplementary intake, which increased Cholesterol Oxidase, then compensation of the decreased CHO levels. LDH is an important enzyme in glycolysis, taking together our results associated with carbohydrate metabolism that LDH of mixed-roughage groups (B and C group) were significantly greater than single-roughage groups (A and D group),the reasons for this phenomenon that mixed-roughage supplementary in sheep dietary might be increased carbohydrate metabolism.

Ruminant rumen encompass a large number of complex and diverse microorganisms, which are critical for the body metabolism and the homeostasis of ruminal barrier by regulating the transformation of nutrients' uptake(Yang XX et al.2019;Xue D et al.2017). A number of studies have shown that lacking hay supplementary intake ruminant had impaired ruminal barrier function, which enhanced the transferring of toxic metabolic products from the rumen into blood circulation via ruminal microflora dysfunction and microbial translocation, causing an inflammatory response and tissue dysfunction(Li C et al.2017;Rahman MK et al.2018;Sunny NE et al.2006). Therefore, the dynamic equilibrium of the structure and function of rumen microbiota contributes to explore underlying mechanism of sheep rumen digestive function in GS alfalfa hay supplementary feeding system(Ji SK et al.2017). The 16S rRNA microbial sequencing technology is an important method to explore the functions and activities of the ruminant's gastrointestinal microbiota(Xue D et al.2017). Ruminant gastrointestinal microorganisms adjusted their own protein synthesis and gene expression to assist in the digestion and synthesis of nutrients(Zhang LH et al.2019;Cao DX et al.2019;Wang JB et a.2015;Ma L et al.2019). The hay supplementary feeding has been shown to be the important factors that affect the gastrointestinal microorganisms(Kim M et al.2019;Mamiko K et al.2017).Our results showed that Ruminococcus were the dominant microbiota in

alfalfa hay supplementary intake trials. The abundance of Ruminococcus changed, which led to the changes of fiber digestive ability and the formation of short chain fatty acids (SCFAs) in different alfalfa hay supplementary trials. It is well-known that the SCFAs provides energy, enhance digestion, and regulates the metabolism in ruminal epithelial cells by binding to G protein–coupled receptors of SCFAs. Meanwhile, the ruminal epithelial cells proliferated and differentiated by inhibiting the histone deacetylase. The expression of low immune pathway and high carbohydrate metabolism pathway genes appeared to be distinguishing features of the alfalfa-hay-supplementary groups rumen microorganisms. Our study suggests that the 30% Yellow Maize Silage + 70% Alfalfa Hay roughage group (C) had significant effects on the microbial diversity and Energy production and metabolism of GS rumen, and this research may help to explain the better adaptability of Gansu fine-wool sheep to the harsh plateau environment.

Declarations

Author contribution All authors have contributed substantially to the work and approved the fnal manuscript.

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Data Availability All data and materials used to support the findings of this study have been included in this publication.

Ethical approval This study was approved by the Institutional Animal Care and Use Committee of Gansu Agriculture University (Protocol 20170407-6). All research involving animals was conducted according to either the Canadian Council on Animal Care (CCAC) guidelines or the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press).

Conflict of Interest the Authors declare no conflict of interest.

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Figures

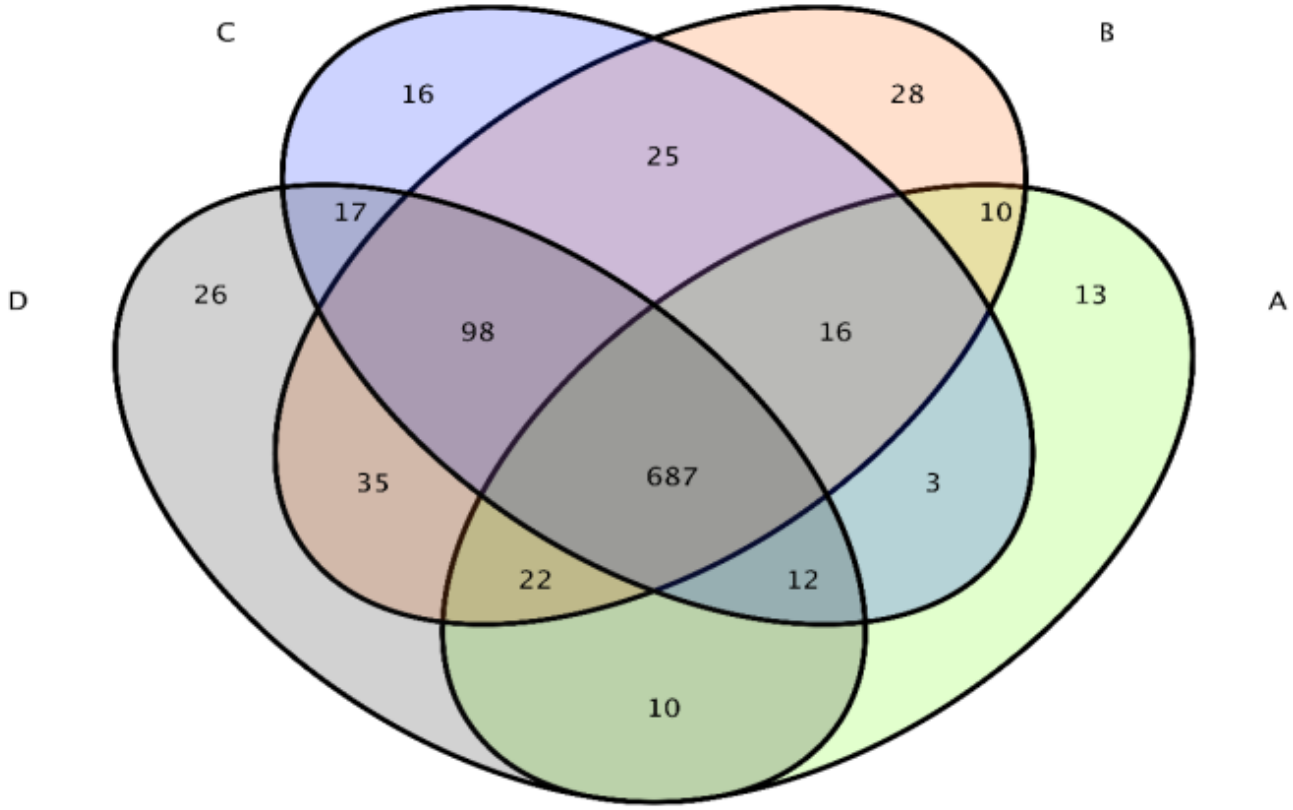

Figure 1

Ruminal fluid samples microbiota Venn diagram

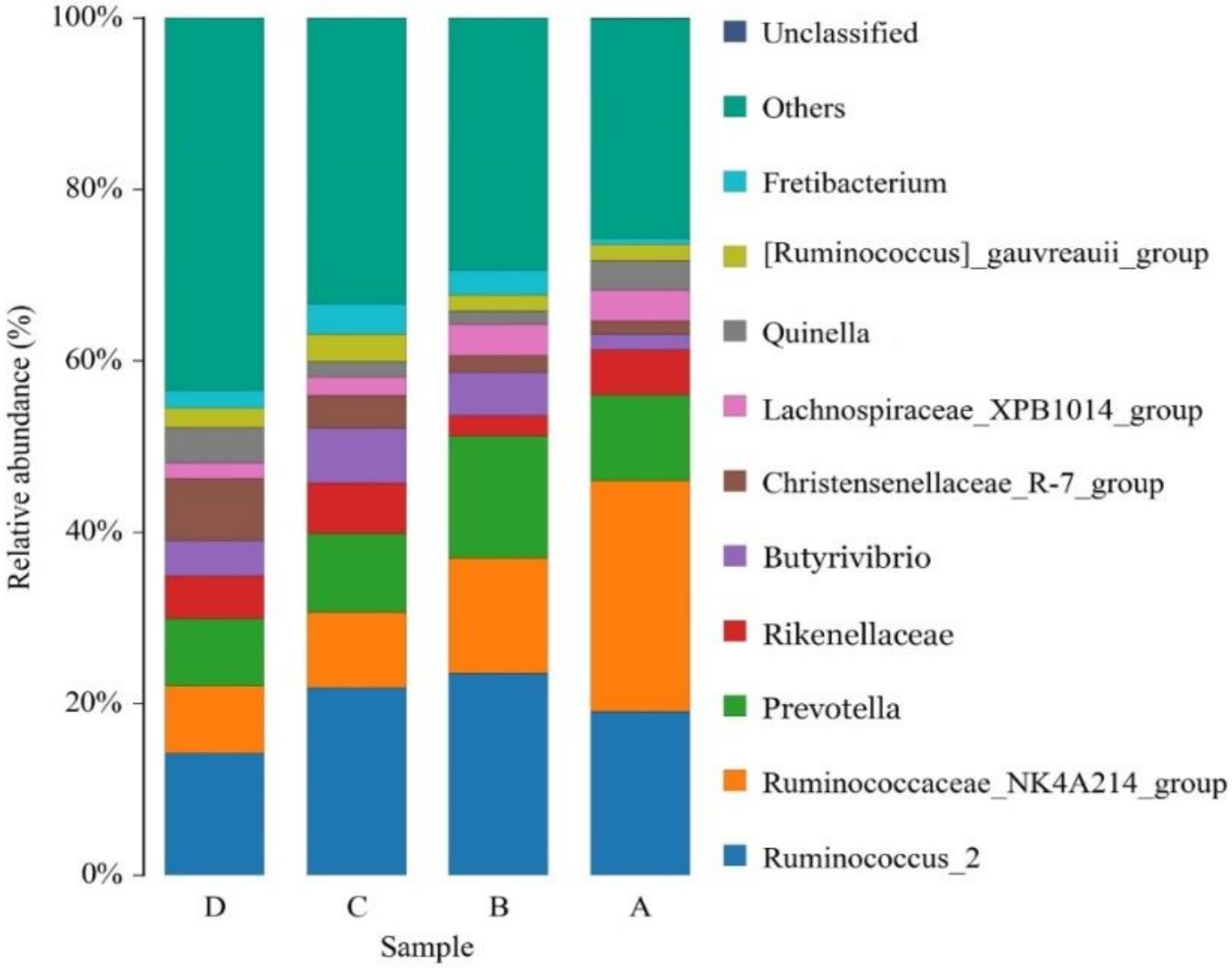

Figure 2

Percent of community abundance of microorganism

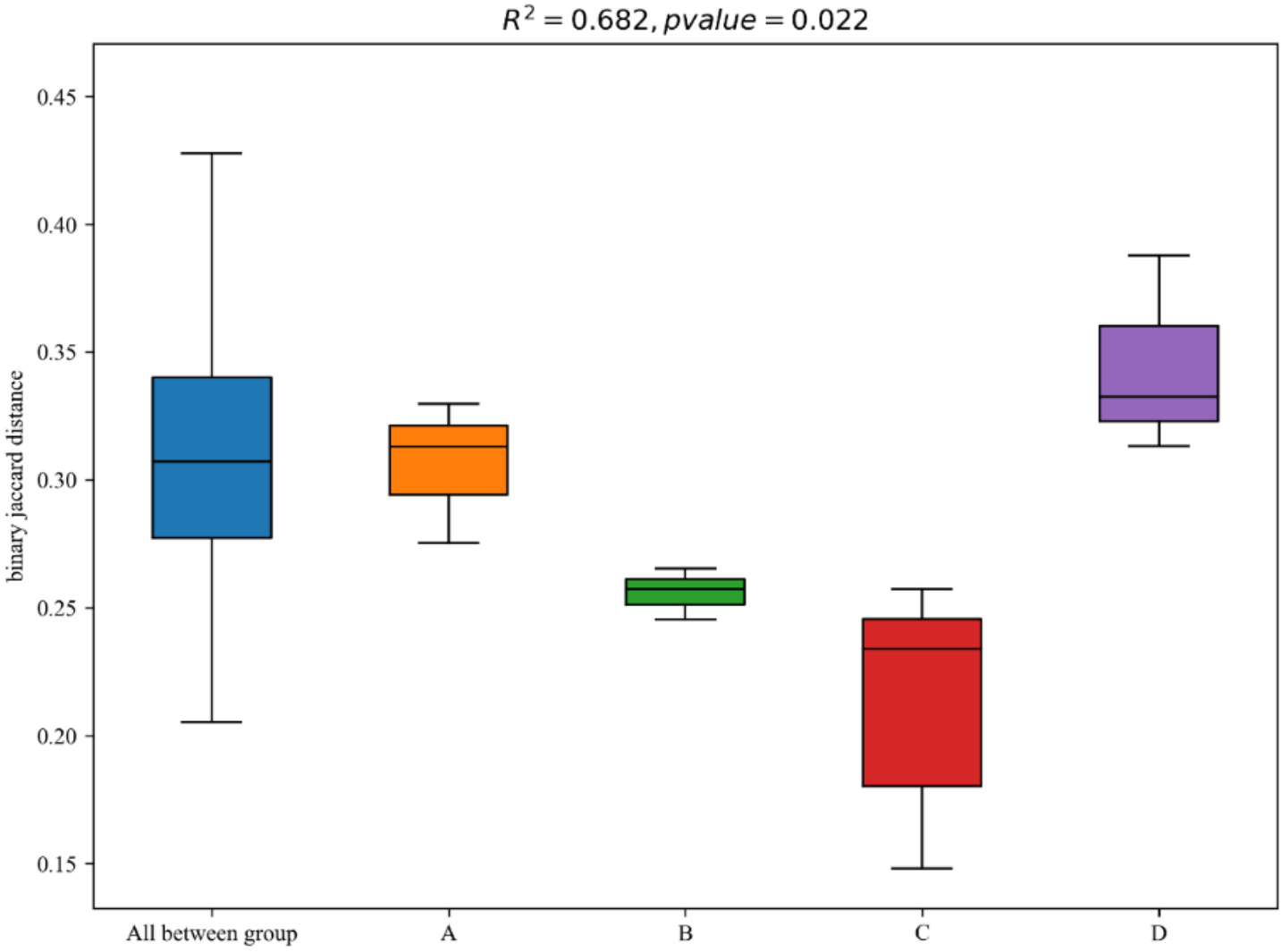

Figure 3

Ruminal fluid samples microbiota Beta-diversity Analysis

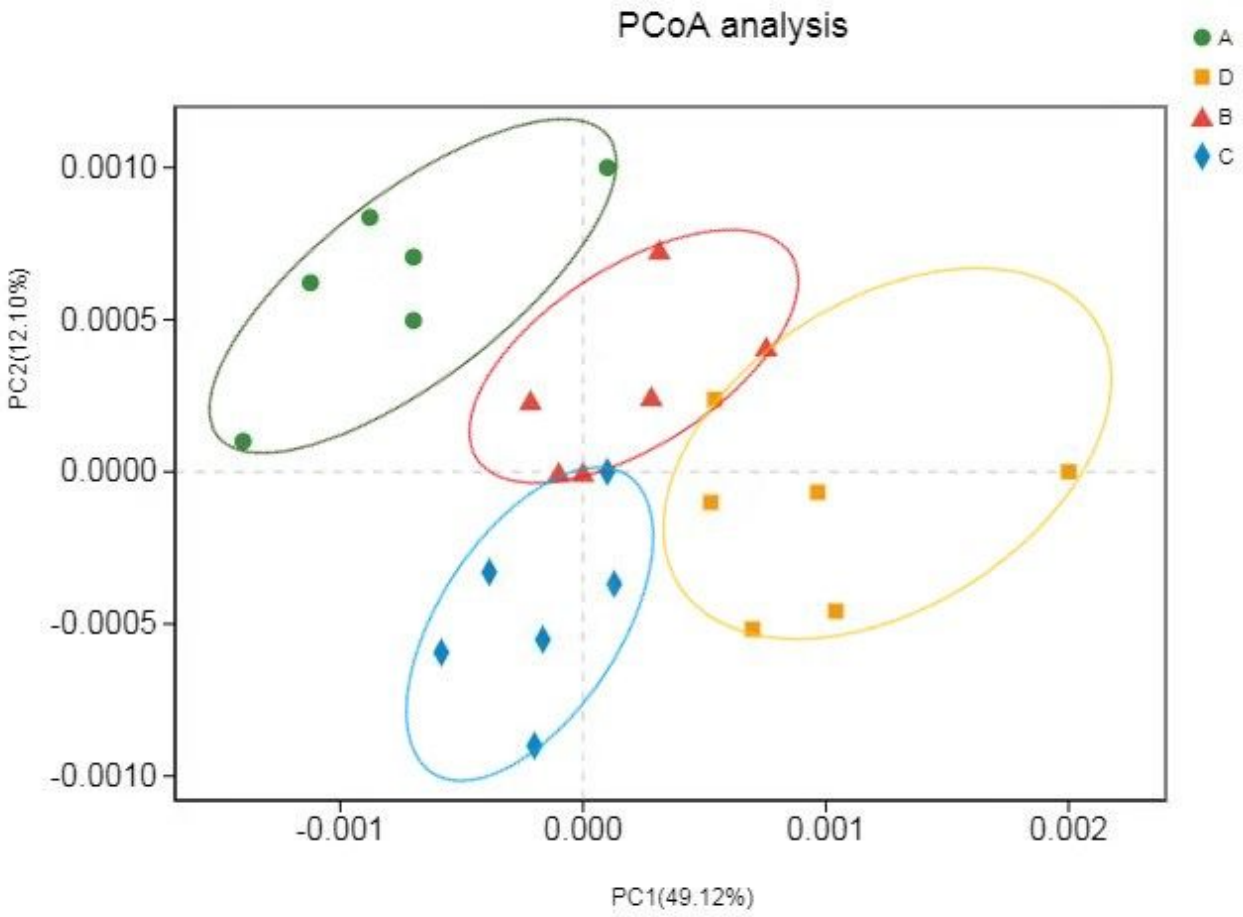

Figure 4

Ruminal fluid samples microbiota PCoA Analysis

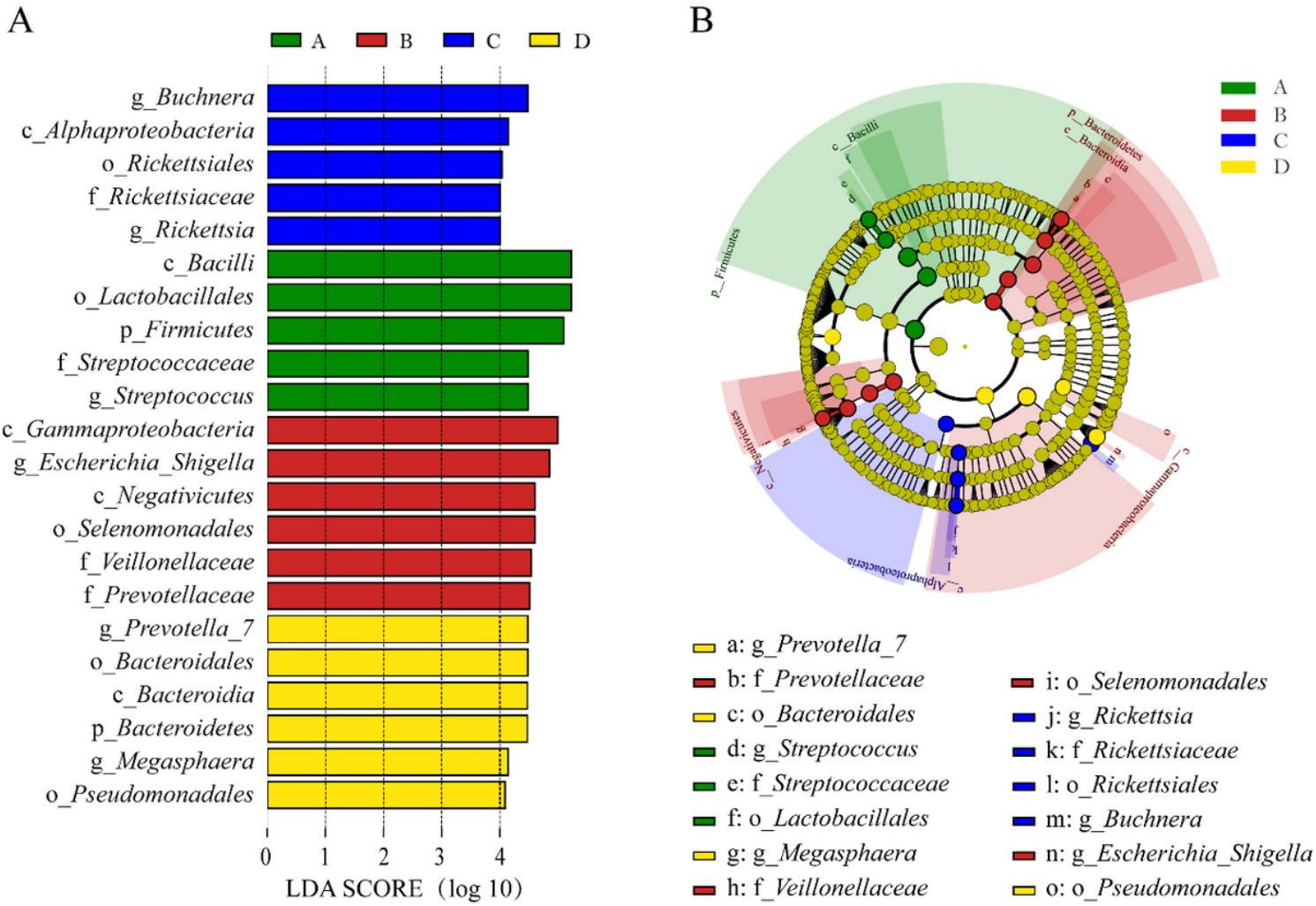

Figure 5

The linear discriminant analysis effect size (LEfSe) method identifies the significantly different abundant taxa of bacteria Note. B. The taxa with significantly different abundances among 4 groups were represented by colored dots, light green circles represent non-significance differences in abundance among 4 groups for that particular taxonomic group, and from the center to outward, they represent the kingdom, phylum, class, order, family, and genus levels. A: Only taxa meeting an LDA significance threshold of 4 were showed.

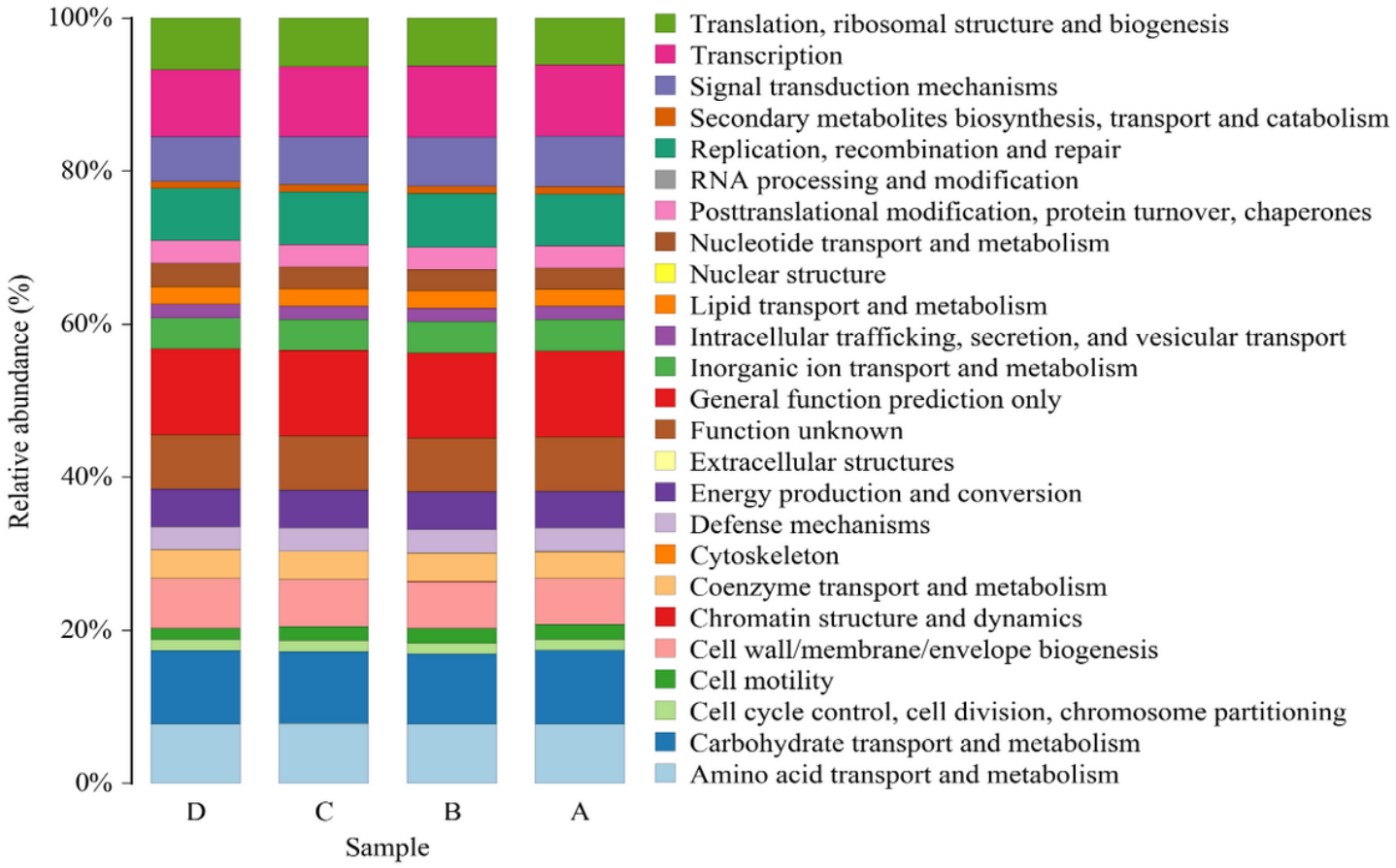

Figure 6

Ruminal fluid samples microbiota KEGG Analysis