Pakistan J. Zool., vol. 54(6), pp 2715-2726, 2022 DOI: https://dx.doi.org/10.17582/journal.pjz/20190318130344

Effect of Feeding Methods on Rumen Bacterial Flora in Chinese Tan Sheep Based on 16S rDNA High-throughput Analysis

Jie Zhang, Na Li, Tingting Guo, Dandan Hu, Xiaofeng XU and Lili Zhang*

Collage of Agriculture, Ningxia University, Yinchuan 750021, China

ABSTRACT

This study examined the effect of different feeding methods on the rumen bacterial flora of Tan sheep, a Chinese indigenous breed, using 16S rDNA high-throughput analysis. The rumen fluid was collected from 9-month-old Tan sheep that were either house-fed or grazed. The results showed that the diversity of rumen flora in the grazing group was significantly higher than that in the house-raised group ($P < 0.01$). In the grazing group, the abundance of 96 genera was significantly higher than that in the house-raised group ($P < 0.05$), whereas in the house-raised group, the abundance of only 24 genera was significantly higher than that in the grazing group ($P < 0.05$). At the phylum level, the abundance of Bacteroidetes, Firmicutes, Fibrobacteres, and Tenericutes in the rumen of grazing Tan sheep was significantly higher than that in the house-raised group ($P < 0.01$), whereas the abundance of Proteobacteria in the rumen of the house-raised group was significantly higher than that in the grazing group ($P < 0.01$). At the generic level, the abundance of *Fibrobacter, Ruminococcus*, Lachnospiraceae_XPB1014_group, and Lachnospiraceae_NA, which are associated with hemicellulose degradation, and *Desulfovibrio*, which is associated with lactic acid oxidation, was significantly higher in the grazing than in the house-raised group ($P < 0.01$). The abundance of the acid-producing Enterobacter, *Vibrio*, and *Selenomonas* in the house-raised group was significantly higher than that in the grazing group ($P < 0.05$). The abundance of *Prevotella*_7 and Succinivibrionaceae-UCG-001 in the house-raised group was 272.07 and 7605.75% higher than that in the grazing group, respectively, but the abundance of *Prevotella*_1 in the house-raised group was 3657.96% lower than that in the grazing group, with these differences highly significant ($P < 0.01$). The higher abundance of *Prevotella*_7 in the house-raised group and the higher abundance of *Prevotella*_1 in the grazing group are differences worthy of further study in Tan sheep.

Article Information
Received 18 March 2019
Revised 30 June 2019
Accepted 17 December 2019
Available online 30 June 2022
(early access)
Published 23 August 2022

Authors' Contribution
XX and LZ designed the experiment. JZ analyzed the data and wrote the article. TG, DH and NL performed experimental work and collected the data.

Key words
Tan sheep, Rumen, 16S rDNA, Microflora, Feeding model.

INTRODUCTION

All experimental sheep were slaughtered with non-penetrating pneumatic stun guns, strictly following the animal welfare requirements of western countries. The Tan sheep, a type of Mongolian sheep, is a unique local sheep breed and a national second-class protected breed in China. Tan sheep are famous not only for their fleece but also for their delicate meat, which, with light odor, even distribution of fat between the muscles, and rich nutritional value, has become the top grade of mutton. Since 2003, the feeding and management mode of Tan sheep in Ningxia, China, has changed from grazing the animals to house-raising them. However, Luo *et al*. (2016) found that the mutton from sheep that were grazed tasted better than the mutton from those that were house-raised, with the bitterness and astringency reduced with grazing. The contents of inosinic acid and inosine in the mutton from grazed sheep are higher than those in the mutton of house-raised sheep. The quality of mutton varies with different feeding methods, and the diet changes when sheep are house-raised. Similarly, Han (2015) found that diet is the main factor affecting rumen microbial flora. The principal co-ordinates analysis (PCoA) of rumen bacteria in goats fed grass were significantly different from those fed a 40:60 ratio of concentrate-to-roughage (Grilli *et al*., 2016). Since the composition of diet affects the rumen microflora, ruminants are greatly affected by a change in feeding method. In this study, 16S rDNA high-throughput sequencing was used to investigate the changes in the structure of the rumen bacterial flora of 9-month-old Tan sheep under the two different feeding modes, *i.e.* grazed and house-raised sheep, with the goal to provide a basis for studying the mechanism of changes in meat quality under different feeding conditions.

MATERIALS AND METHODS

Test animals and management

Twenty-four 4-month-old Tan sheep of similar weight

* Corresponding author: zhanglilinx@126.com
0030-9923/2022/0006-2715 $ 9.00/0

and in good health were selected and divided into two groups of grazed and house-raised sheep, with 12 animals in each group-half male and half female. The grazing Tan sheep fed on mixed pasture. The feed of the house-raised Tan sheep was a mixed diet of concentrate: roughage at the ratio of 3:7. The concentrate was composed of corn, soybean meal and bran while the roughage meal contained *Caragana korshinskii*, corn straw, licorice seedlings, and bitter bean seedlings.

Sample collection and processing

Five Tan sheep randomly selected from each group were slaughtered at nine months and the rumen fluid collected. Five samples were collected from the grazing group (G9-1, G9-2, G9-3, G9-4, G9-5), and five samples were collected from the house-raised group (R9-1, R9-2, R9-3, R9-4, R9-5). The ten samples were filtered using four layers of gauze, all rumen fluid from each sample were collected as far as possible. The collected rumen fluid was transferred to the laboratory, and 50 mL of filtered rumen fluid was separately collected from each sample after mixing it well in a centrifuge tube and later stored in a freezer at –80°C for microbial analysis and determination.

PCR amplification

After the genomic DNA (DNA extraction kit: HiPure Stool DNA Kits (Magen, Guangzhou, China)) had been extracted from the rumen samples, to ensure the accuracy and reliability of follow-up experiments, moved random and part samples from the ten samples were used for pre-PCR (PCR instrument (ETC811, EASTWIN, Beijing, China), PCR related reagent (TOYOBO, Japan), recovered and purified reagent: AMPure XP magnetic bead (Beckman Coulte, US) amplification experiments. These preliminary experiments were conducted to ensure that for most of the samples, the lowest cycle number could amplify the products to the appropriate concentration necessary to make full preparations for all the formal experiments. The purified PCR products were detected by 2% Agarose gel electrophoresis (Model DYY-6C (LIUYI, Beijing, China)). The amplified region was V3 + V4 of 16S rDNA. The primer sequences were 341F: CCTACGGGNGGCWGCAG and 806R: GGACTACHVGGGTATCTAAT.

Methods

Tan sheep are usually slaughtered between six and nine months for market consumption. To analyze the diversity of species in individual samples, the analysis of alpha diversity is based on ACE, chao1, Shannon, and Simpson diversity indices. The values of ACE and chao1 are used as measures of species richness and are estimated. The Shannon index combines the homogeneity and abundance of operational taxonomic units (OUT) to reflect species diversity, and the Simpson diversity index measures the probability that in two consecutive random samplings, the species selected will be different.

Beta diversity analysis is a follow-up analysis that uses weighted and unweighted Unifrac indices. The unweighted Unifrac index only considers the presence or absence of species, whereas the weighted Unifrac index considers not only the presence or absence of species but also intuitively, the abundance of species.

The UPGMA (unweighted pair-group method with arithmetic means) means uses Mothur software, according to weighted and unweighted Unifrac matrix information. First, the two OTUs with the smallest distance are clustered, and then the two OTUs with the smallest distance are branched at 1/2 of the distance between the two OTUs. Then, the two OTUs with the smallest distance are clustered again, so as to complete the clustering of all OTUs and establish a complete phylogenetic tree. The more similar the samples are, the shorter the common branches.

The LEFse software was used to analyze the differences between groups. First, a Kruskal–Wallis rank sum test (a commonly used test method for the comparison of multiple samples) was performed among all groups of samples (Brown, 2015). Then, the screened differences were compared between the two groups by a Wilcoxon rank sum test (a commonly used test method for the group comparison of two samples) (Fang *et al*., 2012). Finally, the screened differences were sorted using the results of linear discriminant analysis (LDA).

HiSeq sequencing

The gel-cut products of the PCR amplification were recovered and quantified with a QuantiFluorTM fluorometer. The purified amplified products were mixed in equal amounts, connected with sequencing connectors, and used to construct sequencing libraries. A Hiseq2500 PE250 (illumine, San Diego, US) was used for sequencing at Guangzhou Kidio Biotechnology Co., Ltd.

Statistical analysis

Excel 2010 (Microsoft, US) was used for preliminary data processing, and the processed data were analyzed based on a random block design using the SAS (statistical analysis system) 8.2 statistical software package (SAS Institute Inc., US). The data are expressed as the mean ± standard error (SE). The criterion to judge whether a difference was significant was $P < 0.05$.

RESULTS

OTU dilution curve and OTU Shannon dilution curve

To verify the validity of the sequencing of the

diversity data of the rumen flora of Tan sheep in the grazing and house-raised groups, according to the relative ratio of OTUs of the measured sequence, the sequence less than the total number was randomly selected. To prepare the OTU dilution curve, the extracted sequence was sequenced, and the expected OTU number obtained. According to the same principle, a Shannon dilution curve was constructed to evaluate whether the sequencing depth covered all the groups in the sample. At the beginning of the sequencing, the OTU values increased sharply with the increase in sequencing depth (Figs. 1, 2). When the depth of sequencing reached 20000 reads, the curves changed more slowly but continued to increase, indicating that the sequencing was not complete. However, the Shannon dilution curve was saturated when the sequencing depth reached 20000 reads, indicating that the current sequencing depth was sufficient for the analysis of sample diversity; thus, a further increase in the sequencing quantity had no effect on species diversity.

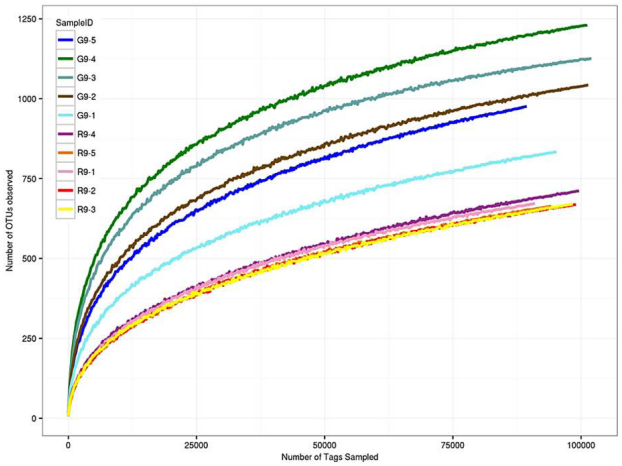

Fig. 1. Relationship between sequencing depth and number of OTUs (organizational taxonomic unit) for bacteria in rumen samples of grazed (G9) and house-raised (R9) Tan sheep. Different colored lines identify different samples by sample identification (ID) number.

OTU analysis of rumen bacterial flora

After removing the low-quality and nonbiological sequences from the original data and splicing the valid sequences, the OTU clustering analysis was conducted at a 97% similarity level using Mothur software. Venn diagrams were constructed according to the common and unique OTUs of each sample (Fig. 3). Based on the statistical analysis of the OTU number of each sample (Table I), significant differences were detected in the OTU number of rumen flora between grazing and house-raised sheep, with the number of OTUs in the grazing group significantly higher than that in the house-raised group ($P < 0.01$). The number of OTUs of rumen bacterial flora in the grazing group was 1771, whereas the number of rumen bacterial flora OTUs in the house-raised group was 1188. The two groups shared 842 OTUs.

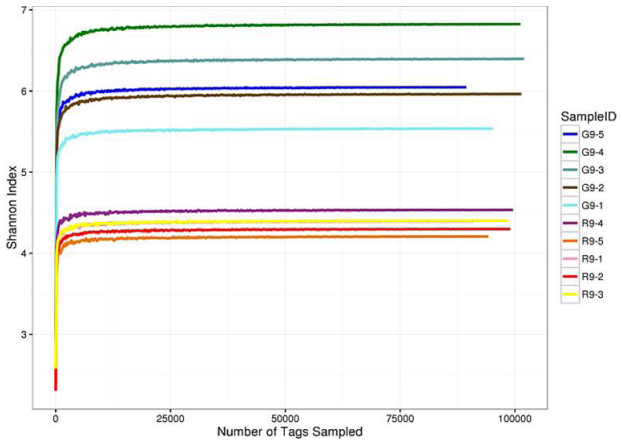

Fig. 2. Relationship between Shannon and number of OTUs (organizational taxonomic unit) for bacteria in rumen samples of grazed (G9) and house-raised (R9) Tan sheep. Different colored lines identify different samples by sample identification (ID) number.

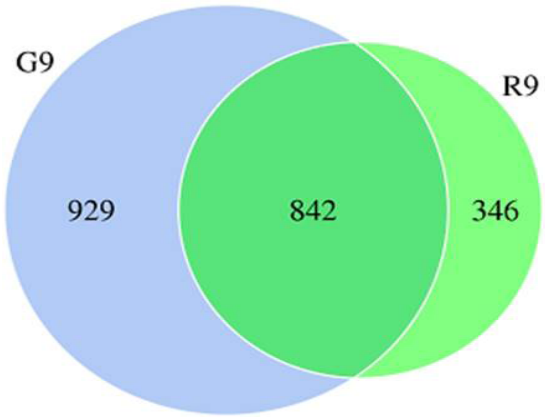

Fig. 3. Venn diagram of common and unique rumen microorganism 0TUs in grazed (G9) and house-raised (R9) Tan sheep. Blue represents the OTUs of grazing group and green represents the OTUs of house-raising group, the intersecting part is a common OTUs.

Species classification analysis

The sequences were annotated to link the OTUs with species information, and each OTU represented a set of classification levels. The classification of microbial species includes seven grades: boundary, phylum, class, order, family, genus, and species. The two groups of samples were divided into species classification trees according

to the distribution of each level (Fig. 4). At each level, the abundance in the two groups was different. Nineteen phyla were found in the rumen fluid of sheep in the two feeding conditions, with 18 phyla in the grazing group and 16 phyla in the house-raised group. Three phyla were specific to the grazing group, Planctomycetes, Chloroflexi, and SR1, and one phylum was specific to the house-raised group, Fusobacteria. The bacteria in the two groups were concentrated in several phyla (Fig. 4), with 99.98% of the bacteria distributed in the Bacteroidetes, Firmicutes, Proteobacteria, and Fibrobacteres. The most abundant phylum in the grazing group was *Fibrobacteres* (1.38%), whereas the most abundant phylum in the house-raised group was Proteobacteria (20.36%).

Table I.- Number of OTUs for bacteria in the rumen fluid of grazing and house-raised Tan sheep.

Item	Grazing group	House-raised group
OTUs	1043.20±67.61^{A}	677.40±8.75^{B}

Note: In the same row, values without superscript letters are not significantly different ($P > 0.05$); whereas values with different small-letter superscripts are significantly different ($P < 0.05$). For values with different capital-letter superscripts, the differences are highly significant ($P < 0.01$). The same applies to below.

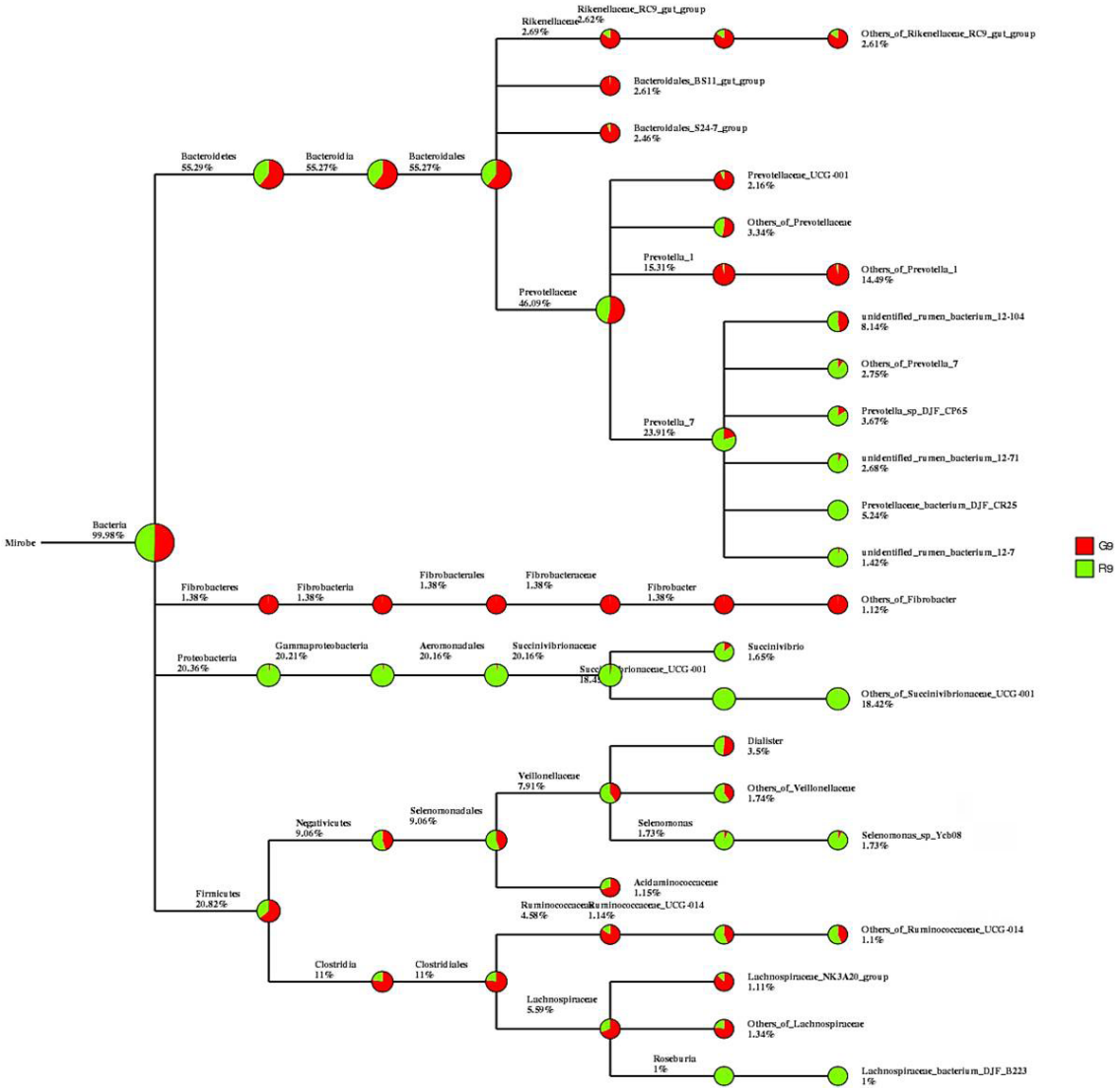

Fig. 4. Species classification of rumen microorganisms in both grazed (G9) and house-raised (R9) Tan sheep. Red represents the grazing group and green represents the house-raising group, and different branches represent different classification levels, successively including boundary, phylum, class, order, family, genus and species.

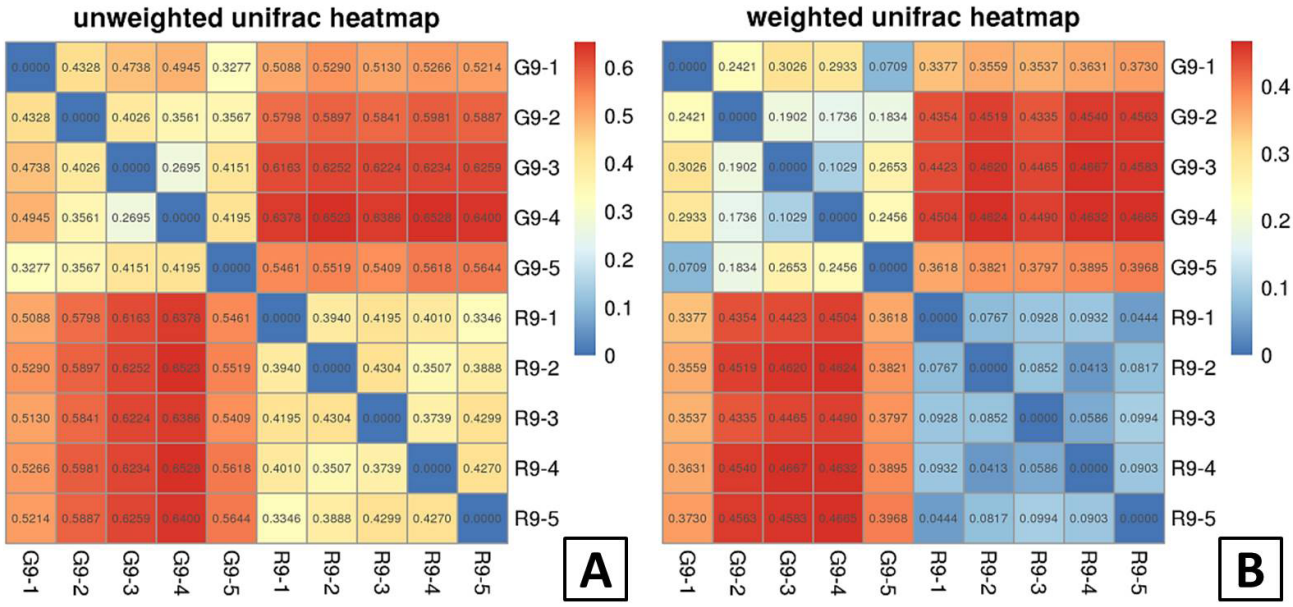

Fig. 5. Heat map of microbial Beta diversity index in rumen of grazed (G9) and house-raised (R9) Tan sheep. The color ranges from blue to red representing the range of 0 ~ 0.5. The larger the value, the greater the species abundance. A, unweighted; B, weighted.

Table II.- Richness and diversity indices of the bacteria in rumen fluid samples for grazing and house-raised groups of Tan sheep.

Item	Index	Grazing group	House-raised group
Richness indices	ACE	1312.29±51.66^{A}	1000.00±21.85^{B}
	chao1	1292.90±54.46^{A}	961.35±24.42^{B}
Diversity indices	Shannon	6.15±0.22^{A}	4.37±0.055^{B}
	Simpson	0.95±0.0063^{A}	0.89±0.0033^{B}
Coverage		0.997±0.00019	0.997±0.000047

Alpha diversity analysis

In a comparison of samples, the species richness and diversity are higher with greater values of ACE, chao1, and the Shannon index and with the Simpson diversity index close to 1. The coverage rate of bacteria in both groups of sheep exceeded 99% (Table II), which indicated that the data of both groups were valid and fully reflected the actual bacterial flora in the rumen samples. For the richness of rumen bacterial flora, the ACE and chao1 indices for the grazing group were significantly higher than those of the house-raised group ($P < 0.01$). For the diversity of rumen bacterial flora, the Shannon and Simpson indices were significantly higher in the grazing group than those in the house-raised group ($P < 0.01$). Thus, the richness and diversity of rumen bacterial flora of the grazing Tan sheep were significantly higher than those of the house-raised Tan sheep.

Beta diversity analysis

Beta diversity index

The Pheatmap package in R language was used, and the intergroup weighted and unweighted Unifrac indices are displayed in thermal graphics (Fig. 5). The distance values in the house-raised group were between 0.3 and 0.4 (Fig. 5A), indicating little difference in the abundance of species of bacteria in the house-raised group. The distance values in the grazing group were between 0.4 and 0.5, which indicated that the uniformity of bacterial species abundance in the grazing group was lower than that in the house-raised group. Thus, because the distances between the bacterial groups of the grazing group were higher than those between the bacterial groups of the house-raised group, the diversity of rumen bacterial flora in the grazing group was greater than that in the house-raised group. After abundance was weighted, the distance within the house-raised group was between 0.0 and 0.1, which further indicated that the species abundance of bacteria in the house-raised group was more homogeneous. The weighted distance in the grazing group was between 0.2 and 0.3, which indicated that the uniformity of species abundance of the flora in the grazing group was relatively low, although the species diversity of the grazing group was significantly higher than that in the house-raised group.

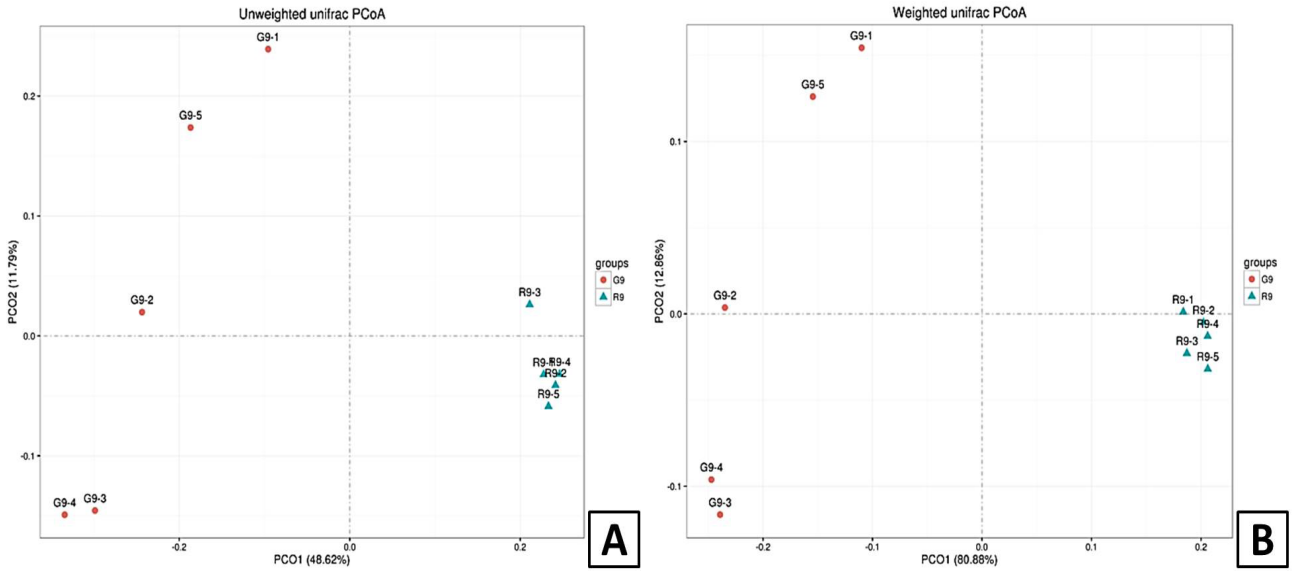

Fig. 6. PCoA (principal co-ordinates analysis) of rumen microorganisms in grazed (G9) and house-raised (R9) Tan sheep. The red circles represent grazing samples and the blue triangles represent house-raising samples. A, unweighted; B, weighted.

Principal co-ordinates analysis (PCoA)

Based on the weighted and unweighted Unifrac data between samples, PCoA graphics were prepared (Fig. 6). The more similar the samples were, the closer the distance in the PCoA diagram. The rumen samples from the sheep in different environments showed differences in aggregation distribution for both unweighted (Fig. 6A) and weighted analyses (Fig. 6B). The bacterial flora in the grazing group showed a dispersed distribution, whereas the flora in the house-raised group showed an aggregated distribution, which indicated that the homogeneity of bacterial flora in the house-raised group was higher than that in the grazing group. For PCO1, the two groups of samples were completely separated, and the difference between the two groups was significant ($P < 0.05$); thus, the bacterial flora structure in the rumen of the two groups was significantly different.

UPGMA cluster analysis

No common branch was observed at the phylum and genus levels between the grazing group and the house-raised group (Fig. 7), although a common branch was observed within each group. This result showed that a difference existed between the house-raised and grazing groups, but the difference was small within each group. At the phylum level, the *Fibrobacteres* and *Spirochaetae* were both distributed in the grazing group, whereas the *Fibrobacteres*, *Tenericutes*, *Spirochaetae*, and *Verrucomicrobia* were at almost undetectable levels under house-raised conditions. Most *Proteobacteria* were found in the house-raised group, whereas *Bacteroidetes* and *Firmicutes* were more abundant in the grazing group than in the house-raised group.

In the two groups, 187 genera were identified, and between the groups, 33 of the genera showed highly significant differences ($P < 0.01$) and 42 of the genera showed a significant difference ($P < 0.05$). *Prevotella*_1, Prevotellaceae_UCG-001, Fibrobacter, and Rikenellaceae_RC9_gut_group were more abundant in the grazing group than in the house-raised group. Compared with the grazing group, the abundance of *Prevotella*_7, Succinivibrionaceae_UCG-001, *Selenomonas*, and Succinivibrio was higher in the house-raised group. The proportion of unclassified genera in the grazing group was significantly higher than that in the house-raised group. The abundance of Fibrobacter and *Ruminococcus*, associated with cellulose degradation, Lachnospiraceae_XPB1014_group and Lachnospiraceae_NA, associated with hemicellulose degradation, and Desulfovibrio was significantly higher in the grazing group than that in the house-raised group ($P < 0.01$). The abundances of the acid-producing *Enterobacter, Selenomonas*, and Veillonellaceae_NA, which can utilize lactic acid, were significantly higher in the house-raised group than those in the grazing group ($P < 0.05$). For the distribution of *Prevotella*_1 and *Prevotella*_7, the abundance of *Prevotella*_7 was higher in the house-raised group than that in the grazing group ($P < 0.01$), whereas the abundance of *Prevotella*_1 was higher in the grazing group than that in the house-raised group ($P < 0.01$), which is a difference worthy of further study.

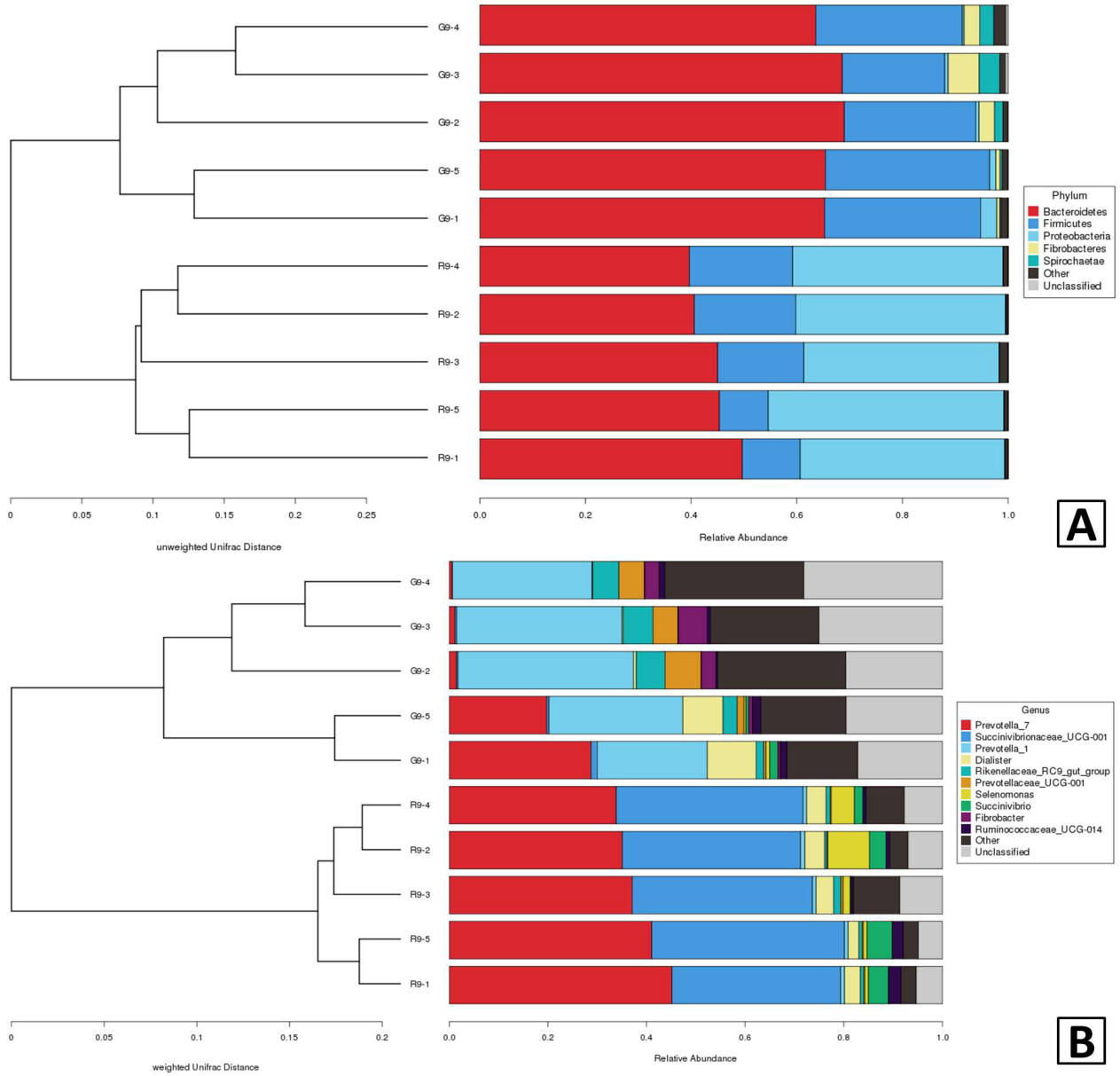

Fig. 7. UPGMA (unweighted pair-group method with arithmetic means) cluster analysis of rumen microorganisms in grazed (G9) and house-raised (R9) Tan sheep. A, the phylum level, different colors represent different phylum; B, shows the genus level, different colors represent different genera. The closer the branch distance is, the higher the similarity between samples will be.

Variation in the structure of bacterial flora between the two groups

Figure 8A shows the species with significant differences in abundance in the different groups, and the length of the bar chart represents the size of the impact of the different species (LDA Score). Then, the evolutionary branch (Fig. 8B) was obtained by mapping the differences to a taxonomic tree with a known hierarchical structure. Based on the LEFse analysis of the differences of bacterial flora between the groups, the specific, main bacterial flora was identified between the groups. In the grazing group, 96 categories were significantly higher than those in the house-raised group ($P < 0.05$) (Fig. 8A), whereas 24 categories in the house-raised group were significantly higher than those in the grazing group ($P < 0.05$). Six flora groups played an important role in the house-raised group, and 36 flora groups played an important role in the grazing group (Fig. 8B).

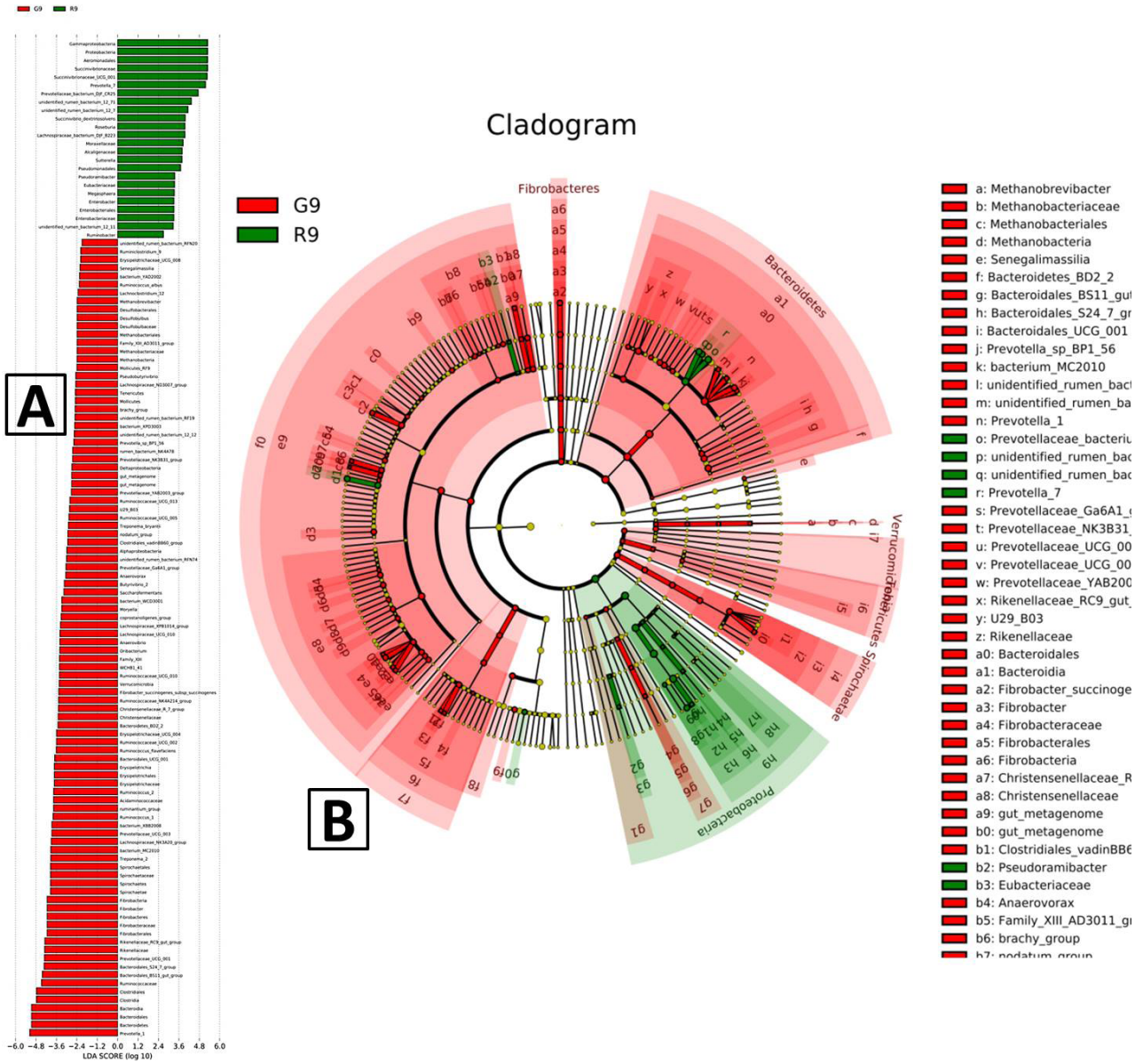

Fig. 8. Rumen microorganism LEFse difference analysis of grazed (G9) and house-raised (R9) Tan sheep. Red represents the grazing group and green represents the house-raising group. A, linear discriminant analysis (LDA); B, evolutionary branching. In the evolutionary branching diagram, the circles that radiate from inside to outside represent the taxonomic level from phylum to genus (or species). Each small circle at the different classification levels represents a classification at that level. The diameter of the small circle is proportional to the relative abundance. For the coloring scheme, the species that are not significantly different are uniformly colored yellow.

DISCUSSION

The rumen, as an important organ of nutrient digestion and absorption in ruminants, provides energy for ruminants through special processes of digestion and absorption. Bacteria, fungi, ciliates, protozoa, and other microorganisms inhabit in the rumen, but the most abundant species are bacteria. Bacteria play an important role in the digestion and absorption of three major nutrients (carbohydrate, fat and protein) and in the synthesis of vitamins in ruminants. The composition balance of rumen flora depends on the diversity and abundance of rumen bacteria.

According to the Venn diagrams of the OTU cluster

analysis, species specific to the grazing group and the house-raised group were identified, in addition to those in common. At the generic level, the difference between the two groups was significant, and the diversity of rumen bacteria in Tan sheep decreased under the house-raised mode of feeding. This result is consistent with that of Han (2015) who found that diet is the main factor affecting the microbial flora. In the rumen ecosystem, changes in the levels of crude dietary fiber cause changes in the bacteria of the rumen. With an increase in the intake of fiber in the diet, more time is spent chewing and the salivary secretions of ruminants increase, which can effectively neutralize rumen pH and provide a more suitable environment for the growth of bacteria (Sun *et al*., 2013; Wang, 2017; Yang *et al*., 2015).

In this study, the difference in the diversity of rumen microflora between the house-raised and grazing groups of Tan sheep can be explained by the difference in the feeding methods. In the house-raised group, the feed was coarse feed and concentrate, whereas in the grazing group, Tan sheep only ate coarse feed. The diversity of rumen microorganisms is greatly impacted by differences in diet, and the supply of concentrate reduced the diversity of rumen microorganisms in the house-raised group. Grilli *et al*. (2016) found that in goats fed grass, based on PCoA, rumen bacteria were significantly different from those fed with the ratio 40:60 concentrate-to-roughage. The abundance and homogeneity of rumen bacteria in goats fed grass were significantly higher than those fed with the 40:60 concentrate-to-roughage ratio. In this study, the alpha diversity and the beta diversity of the rumen flora were significantly higher in the grazing group than those in the house-raised group, but the homogeneity in the house-raised group was significantly higher than that in the grazing group. To explain this result, the grazing group had free-choice to select what to eat, which led to a variety of foods consumed and therefore lower homogeneity of the rumen flora. By contrast, the house-raised group was continuously fed the same diet, which increased the homogeneity of the rumen flora.

Under the experimental conditions, 19 total phyla were identified in the two groups of rumen microflora, with 18 phyla in the grazing group and 16 phyla in the house-raised group. Planctomycetes, Chloroflexi, and SR1 were specific to the grazing group, whereas *Clostridium* spp. was exclusive to the house-raised group. Of the bacteria, 99.98% were distributed in the Bacteroidetes, Firmicutes, Proteobacteria, and Fibrobacteres. The highest relative abundance was for Bacteroidetes. However, for the distribution of the four groups of flora with the highest abundance, the differences were not significant between the grazing and house-raised groups. This result is consistent with that of Li *et al*. (2018), and similarly, Wang (2017) reported that the dominant bacteria at different levels of NDF are *Bacteroidetes*, *Firmicutes*, and Proteobacteria. Bacteroidetes are the primary bacteria to degrade nonfibrous carbohydrates among rumen microorganisms, with genes associated with the degradation of nonfibrous polysaccharides (Xu and Li, 2013). Yang *et al*. (2017) reported that the number of Proteobacteria bacteria in mammals has a large impact on growth and metabolism. Firmicutes primarily affect carbohydrate enzymes.

In this study, the abundance of Proteobacteria increased significantly in the house-raised group, but the abundance of Bacteroidetes, Firmicutes, Fibrobacteres, and Tenericutes was significantly reduced. In addition, the abundance of Spirochaetae and Verrucomicrobia was reduced significantly in the house-raised group. Moreover, Fibrobacters, Tencuteries, Spirochaetae, and Verrucomicrobia were almost nonexistent in this feeding condition. According to reports such as those by Han (2015) and Lin *et al*. (2016), the supplementation of concentrate increases the number of *Proteus* and decreases the number of Bacteroides in the rumen (Wang *et al*., 2016, 2018). Hook *et al*. (2011) show that the level of roughage intake not only affects the number of individual bacteria but also affects the total amount of microorganisms in the entire rumen ecosystem and results in changes in microbial flora diversity. When the rumen receives a certain level of feed for a long time, the rumen environment reaches a relatively stable state. A dynamic, balanced microbial ecosystem operates in the rumen, with close relationships among the microorganisms, mutually affecting one another. This dynamic balancing process changes the colonization, growth, and reproduction of microorganisms (Lu, 2017; Tang, 2018; Wu *et al*., 2016).

Under the experimental conditions, the rumen for the two feeding groups contained 187 genera of bacterial flora, with 179 genera in the grazing group and 137 genera in the house-raised group. Fifty genera were specific to the grazing group, and eight genera were specific to the house-raised group. The results showed that the abundance of functional microflora in the rumen changed between the house-raised and grazing groups. Compared with the grazing group, the abundance of Succinivibrionaceae_UCG-001 increased significantly and that of *Selenomonas* tended to increase in the house-raised group. These results are consistent with those of Zhang *et al*. (2018) for the rumen microorganisms of adult and lamb cashmere goats in grazing and house-raised groups during fattening, with the supply of concentrate improving the biosynthesis of lactic acid in the rumen. Fernando *et al*. (2010) show that many types and different quantities of cellulase occur in the rumens of cattle fed high-fiber diets, and when fed

a diet high in concentrate, the number of *Bacteroidetes* in the rumen increases. Similarly, with an increase in the proportion of concentrate, Megasphaera and others increase significantly, which shows that the rumen can re-establish the corresponding microecological system according to the change in dietary composition (Plaizier *et al*., 2017). In the current experiment, the abundance of Fibrobacter and *Ruminococcus*, associated with cellulose degradation, Lachnospiraceae_XPB1014_group and Lachnospiraceae_NA, associated with hemicellulose degradation, and Desulfovibrio was significantly higher in the grazing group than that in the house-raised group. The abundances of the acid-producing Enterobacter, *Selenomonas*, and Veillonellaceae_NA, which can utilize lactic acid, were significantly higher in the house-raised group than those in the grazing group. Thus, the structure of the bacterial flora changed in the house-raised group, and the abundances of the primary functional bacteria were different from those in the grazing group.

Prevotella reportedly composes up to 60–70% (Meyer *et al*., 2008) of rumen microorganisms. These bacteria are highly active hemifibrillar decomposers (Kamra, 2005) that play an important role in the degradation of nonstructural carbohydrates and proteins (Evans *et al*., 2011; Kiyoshi *et al*., 1999). In addition, *Prevotella* can also digest and utilize starch, xylan, and pectin (Evans *et al*., 2011; Kopecny *et al*., 2003; Matsui *et al*., 2000).

Pitta *et al*. (2014) found that the abundance of rumen Fibrobacter and *Coccus* of buffalo fed 100% fiber feed increased significantly, whereas *Prevotella*_7 was the most abundant when the concentrate: roughage ratio = 1:1. Among the bacteria identified in this experiment, the abundance of *Prevotella*_7 was significantly higher in the house-raised group than that in the grazing group, whereas the abundance of *Prevotella*_1 was significantly higher in the grazing group than that in the house-raised group. In addition, other bacteria related to *Prevotella* show differences. Zhang *et al*. (2018) found that the relative abundance of Prevotellaceae_UCG-001 and Prevotellaceae_UCG-003 increased significantly in the rumen of goats fed high-fiber diets. Grilli *et al*. (2016) showed that the abundance of *Prevotella* increased significantly in the rumen of goats fed high fiber ratio diets. These bacteria belong to the Bacteroidetes and Prevotellaceae and share the same homology with *Prevotella*. Thus, their functions in the rumen should be similar. However, *Prevotella*_1 was less abundant in the house-raised group than in the grazing group. The distribution of *Prevotella*_1 and *Prevotella*_7 between the two feeding groups was significantly different. This difference could be explained by the interaction between bacteria; for example, *Prevotella*_1 may has a synergistic effect on the degradation of fibers. However, to determine the specific mechanism, further study is needed.

CONCLUSIONS

Under the experimental conditions, the two groups of rumen bacterial flora included 187 genera, with 179 genera in the grazing group and 137 genera in the house-raised group. Fifty genera were specific to the grazing group, and eight genera were specific to the house-raised group. Under the different feeding methods, the diversity of the rumen bacterial flora of Tan sheep changed significantly. Compared with the grazing group, the diversity of the rumen bacterial flora of Tan sheep in the house-raised group decreased significantly. The structure of the microflora also changed significantly, and the abundance of microflora that played an important role changed significantly in the house-raised group. For the distribution of *Prevotella_1* and *Prevotella_7*, the abundance of *Prevotella_7* was higher in the house-raised group than that in the grazing group, whereas the abundance of *Prevotella_1* was higher in the grazing group than that in the house-raised group, which is a difference worthy of further study.

ACKNOWLEDGMENTS

This research work was funded by the National Nature Science Foundation of China (Grant No. 31660668).

Statement of conflict of interest

The authors declares that there is no conflict of interests regarding the publication of this article.

REFERENCES

Brown, B.M., 2015. Kruskal–wallis, multiple comparisons and efron dice. *Austral. N.Z. J. Stat*., **44**: 427-438. https://doi.org/10.1111/1467-842X.00244

Evans, N.J., Brown, J.M., Murray, R.D., Getty, B., Birtles, R.J., Hart, C.A. and Carter, S.D., 2011. Characterization of novel bovine gastrointestinal tract treponema isolates and comparison with bovine digital dermatitis treponemes. *Appl. environ. Microbiol*. ,**163**: 138–147. https://doi.org/10.1128/AEM.00993-10

Fernando, S.C., Purvis, H.T., Najar, F.Z., Sukharnikov, L.O., Krehbiel, C.R., Nagaraja, T.G., Roe, B.A. and Desilva, U., 2010. Rumen microbial population dynamics during adaptation to a high-grain diet. *Appl. environ. Microbiol*., **76**: 7482-7490. https://doi.org/10.1128/AEM.00388-10

Fang, Z., Du, R. and Cui, X., 2012. Uniform approximation is more appropriate for Wilcoxon Rank-Sum test in gene set analysis. *PLoS One*, **7**: 0031505. https://doi.org/10.1371/journal.pone.0031505

Grilli, D.J., Fliegerová, K., Kopečný, J., Lama, S.P., Egea, V., Sohaefer, N., Pereyra, C., Ruiz, M.S., Sosa, M.A., Arenas, G.N. and Mrázek, J., 2016. Analysis of the rumen bacterial diversity of goats during shift from forage to concentrate diet. *Anaerobe*, **42**: 17-26. https://doi.org/10.1016/j.anaerobe.2016.07.002

Han, X.F., 2015. *Effects of age and dietary forage-to concentrate rations on rumen microbial flora of the Shaanbei white Cashmere goat*. Dissertation, Northwest A&F University.

Haas, B.J., Gevers, D., Earl, A.M., Feldgarden, M., Ward, D.V., Giannoukos, G., Ciulla, D., Tabbaa, D., Highlander, S.K., Sodergren, E., Methé, B. and DeSantis, T.Z., 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res*., **21**: 494-504. https://doi.org/10.1101/gr.112730.110

Hook, S.E., Steele, M.A., Northwood, K.S., Dijkstra, J., France, J., Wright, A.D.G. and McBride, B.W., 2011. Impact of subacute ruminal acidosis (SARA) adaptation and recovery on the density and diversity of bacteria in the rumen of dairy cows. *FEMS Microbiol. Ecol*., **78**: 275-284. https://doi.org/10.1111/j.1574-6941.2011.01154.x

Kamra, D., 2005. Rumen microbial ecosystem. *Curr. Sci*., **89**: 124-135.

Kiyoshi, T., Roustam, I.A., Takafumi, N., Ogata, K., Nakamura, M., Matsui, H. and Benno, Y., 1999. Rumen bacterial diversity as determined by sequence analysis of 16S rDNA libraries. *FEMS Microbiol. Ecol*., **29**: 159-169. https://doi.org/10.1111/j.1574-6941.1999.tb00607.x

Kopecny, J., Zorec, M., Mrazek, J.Y., Kobayashi, Y. and Marinsek-Logar, R., 2003. *Butyrivibrio hungatei* sp. nov. and *Pseudobutyrivibrio xylanivorans* sp. nov. butyrate-producing bacteria from the rumen. *Int. J. Syst. Evol. Microbiol*., **53**: 201-209. https://doi.org/10.1099/ijs.0.02345-0

Li, Y.Q., Xi, H.M., Zeng, H.F., Zhang, L., Chen, M.J. and Han, Z.Y., 2018. Diversity changes of bacterial community in rumen of dairy cows *in vitro* fermentation analyzed by 16S rRNA sequencing technology. *Chinese J. Anim. Nutr*., **30**: 4059-4070.

Lin, B., Liang, X., Li, L.I., Wei, S.J., Li, P. and Zou, C.X., 2016. Dietary forage to concentrate ratio affects ruminal bacterial and methanogen community composition of water buffaloes. *Chinese J. Anim. Nutr*., **28**: 3101–3109.

Lu, J.Y., 2017. *Effects of roughage sources on production performance and ruminal microflora of finishing lamb*. Dissertation, Lanzhou University.

Luo, Y.L., Wang, B.H., Jin, Z.M., Liu, X.W. and Jin, Y., 2016. Effects of two feeding conditions on nutritional quality of Sunit sheep meat. *Fd. Sci*., **37**: 227-231. https://doi.org/10.7506/spkx1002-6630-201619038

Matsui, H., Ogata, K., Tajima, K., Nakamura, M., Nagamine, T., Aminov, R.I. and Benno, Y., 2000. Phenotypic characterization of polysaccharidases produced by four *Prevotella* type strains. *Curr. Microbiol*., **41**: 45-49. https://doi.org/10.1007/s002840010089

Meyer, M., Stenzel, U. and Hofreiter, M., 2008. Parallel tagged sequencing on the 454 platform. *Nat. Protoc*., **3**: 267. https://doi.org/10.1038/nprot.2007.520

Pitta, D.W., Kumar, S., Veiccharelli, B., Parmar, N., Reddy, B. and Joshi, C.G., 2014. Bacterial diversity associated with feeding dry forage at different dietary concentrations in the rumen contents of Mehshana buffalo (*Bubalus bubalis*) using 16S pyrotags. *Anaerobe*, **25**: 31-41. https://doi.org/10.1016/j.anaerobe.2013.11.008

Plaizier, J.C., Shucong, L., Tun, H.M. and Khafipour, E., 2017. Nutritional models of experimentally-induced subacute ruminal acidosis (SARA) differ in their impact on rumen and hindgut bacterial communities in dairy cows. *Front. Microbiol*., **7**: 2128. https://doi.org/10.3389/fmicb.2016.02128

Sun, G.P., Yan, S.M., He, H., Wu, T.M., Wang, Y.D., Liu, S.Q., Han, S. and Suri, G.G., 2013. Effects of different feeding methods on rumen fermentation function of Arbas white Cashmere goat. *Feed Indust*., **34**: 51-54.

Tang, P., 2018. *Effect of dietary energy and protein levels on growth performance, rumen microbial flora and metabolomics of Shanbei white cashmere goats*. Dissertation, Northwest A&F University.

Wang, L.L., 2017. *The study of different NDF level diet on the apparent nutrients digestibility and the structure and composition of rumen microorganisms in goats*. Dissertation, Sichuan Agricultural *University.*

Wei, Y., Chen, C.X., Li, X.W., Cong, L.X., Zhang, H. and Liu, G.W., 2018. Effect of different crude fiber source diets on rumen microflora of sika deer. *Chinese J. Vet. Sci*., **38**: 217–221.

Wang, Y.Y., Zhao, Z.Y., Wang, X.T., Wang, X.T., Chen, Y.L. and Yang, Y.X., 2016. Effect of dietary

nutrient levels on the number of related microbes, pH and VFA levels in rumen of Tan sheep aged from 150 to 180 days. *Chinese J. Anim. Vet. Sci*., **47**: 2060–2070.

Wang, Y.Y., 2017. *Effects of dietary nutrient levels on the bacterial flora, pH and VFA levels in rumen of Tan sheep*. Dissertation, Northwest A&F University.

Wu, D.Q., Jiang, G.T., Zhang, X., Wang, X.R. and Dai, Q.Z., 2016. Advances in developmental changes and evolution of rumen cellulolytic bacteria flora. *Anim. Husb. Vet. Med*., **48**: 108–111.

Xu, X.N. and Li, W.L., 2013. Effect of dietary concentrate ratio on rumen microorganism, environment and feed digestibility of sheep. *Cereal Feed Indust*., **1**: 57-59.

Yang, H.B., Liu, H., Zhan, J.S., Lin, M. and Zhao, G.Q., 2015. Effects of diet pellets with different concentrate-roughage ratios on rumen fermentation parameters and microorganism abundance in weaned bull calves. *Acta Pratacult. Sin*., **24**: 131-138.

Yang, L., Wang, B.H. and Jin, Y., 2017. Advances in studies on rumen flora and nutrient deposition in ruminants. *Heilongjiang Anim. Sci. Vet. Med*., **15**: 85-87.

Zhang, J., Sun, G.P., Han, S. and Yan, S.M., 2018. Effects of grazing fattening and indoor fattening on the number of microorganisms in the rumen of adult goats and lambs of cashmere goats. *Feed Indust*., **39**: 32–39.

Zhang, X.J. and Wang, L.Z., 2018. Effects of dietary neutral detergent fibre level on structure and composition of rumen bacteria in goats. *Chinese J. Anim. Nutr*., **30**: 1377–1386.