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Abstract

Yaks (*Poephagus grunniens*), native to the Qinghai-Tibetan Plateau, are well adapted to the harsh alpine environment. It was reported that yaks digest low-quality forage more efficiently and require less protein and energy for maintenance than Qaidam cattle (*Bos taurus*). Given the central role of rumen bacteria in nutrient degradation, we hypothesized that interspecies differences in ruminal bacterial composition may underlie the yak's adaptation to low-nutrient diets. To test this hypothesis, we compared feed conversion efficiency, rumen fermentation characteristics and bacterial communities in the two species fattened on a low concentrate diet in a small-holder feedlot. Six yaks (211 ± 6.0 kg) and 6 cattle (210 ± 7.0 kg), all castrated males, were offered a diet consisting of 2.0 kg/day of concentrate and *ad libitum* oat hay pellets for 105 days, which included 10 days for dietary adaptation and 95 days for data recording. Dry matter intake ($P < 0.01$) and feed conversion ratio ($P < 0.05$) were greater in cattle than in yaks. Urinary purine derivative excretion and microbial nitrogen production were greater ($P < 0.05$) in cattle than yaks, but the purine nitrogen index and microbial protein synthesis efficiency were greater ($P < 0.01$) in yaks. Additionally, the ruminal concentrations of ammonia-N and free amino nitrogen were greater ($P < 0.05$) in yaks than cattle. The relative abundances of the fibrolytic bacteria *norank_f_UCG-011* and *Romboutsia*, were greater ($P < 0.05$) in yaks than cattle. These results suggest that interspecies differences in rumen microbial composition and N utilization confer a microbial protein synthesis advantage to yaks when fattened on a low concentrate diet in a small-holder feedlot. The advantages for the yaks over cattle in the present study, however, were less evident than reported for these species when grazing. Grazing yaks have a wide choice of dietary intake, which can differ substantially from co-grazing cattle, but under feedlot conditions they are offered the same feed, and their diets are similar. Dietary intake is a major determinant of rumen bacteria, which could explain why rumen bacteria and responses of yaks and cattle in feedlots would be closer than when grazing.

Keywords: Yaks; Qaidam cattle; Rumen fermentation; Rumen bacterial community; Microbial protein synthesis

INTRODUCTION

The Qinghai-Tibetan plateau (QTP) is known for its extreme environmental conditions, including prolonged cold, intense ultraviolet radiation, strong winds, and hypoxia. Due to a short forage-growing season, pasture quality is particularly poor during the long, harsh winters [1]. Yaks (*Poephagus grunniens*), native to the Asian highlands, are raised at elevations above 3,000 m above sea level (a. s. l.), and are well-adapted to these challenging conditions. They serve as a vital livelihood resource for local inhabitants by providing meat, milk, hides, dung, and transport [2].

Traditionally, yaks grazed year-round without supplements, and, in winter, this resulted in low forage intake and loss of body weight, which delayed slaughter [2]. Qaidam cattle (*Bos taurus*), introduced to the QTP before the third century AD, are raised at altitudes between 2,600 and 3,600 m a, s, l, [3]. They are less tolerant of the harsh conditions and low-quality forage and, thus, require dietary supplementation and shelter during winter. The two bovine species co-graze over much of their overlapping ranges [3].

In recent years, short-term intensive feedlot systems have been initiated to improve animal growth performance and economic returns on the QTP [4]. High-concentrate diets have enhanced growth rates and carcass yields in both yaks and cattle [5,6]; however, long-term use of such diets has been associated with risks of metabolic disorders, including ruminal acidosis and systemic inflammation [7]. Consequently, low- to medium-concentrate feeding regimes have attracted attention as a more sustainable and welfare-friendly alternative. For most small-holder feedlots on the QTP, a low-concentrate fattening strategy, characterized by restricted concentrate feeding combined with *ad libitum* roughage, appears to be the most economically viable option.

Although yaks and Qaidam cattle are both capable of subsisting on high-fiber diets, it has been reported that yaks are able to digest fibers to a greater extent, and produce greater amounts of volatile fatty acid (VFAs) and microbial protein than cattle when fed the same diet [8,9]. These advantages are likely due to their distinct rumen microbial community composition [10,11], suggesting that yaks may be better adapted to low-concentrate feeding systems. Based on previous studies, we hypothesized that a low-concentrate diet in small-holder feedlots could be an effective strategy for fattening yaks. This approach takes advantage of the yaks' ability to digest coarse feed, and, consequently, reduces rearing cost and improves production efficiency. To test our prediction, we compared dry matter intake, average daily gain, feed conversion rate and ruminal bacterial composition between yaks and Qaidam cattle receiving the same low-concentrate high roughage diet under small-holder feedlot conditions. The findings are expected to provide valuable insights for developing feeding strategies for stall-fed ruminants in the unique environmental context of the QTP.

MATERIALS AND METHODS

All animal-related procedures in this study were reviewed and approved by the Animal Ethics Committee of Lanzhou University, Gansu, China (Protocol number 202101050). Measurements were made from March to June

2021, at Wushaoling Yak Research Facility of Lanzhou University (37°14’20.54"N, 102°48‘34.32"E, altitude 3,154 m a. s. l), in Tianzhu Tibetan Autonomous County, Wuwei City, Gansu Province, China.

Experimental design, animals and diets

Six yaks (211 ± 6.0 kg) and six Qaidam cattle (210 ± 7.0 kg), all 4-year old castrated males, were maintained in individual metabolic cages (1.0 m × 2.2 m) with free access to water and received 2.0 kg/day of a fattening concentrate and *ad libitum* commercial oat hay pellets (Table 1). Due to the harsh environmental conditions on the Qinghai-Tibetan Plateau, yaks are typically fattened on pasture during June to September and are slaughtered over 4 years of age, when they weigh more than 250 kg. Therefore, we selected 4-year-old castrated animals at the fattening stage for this study. Feed was provided twice daily at 07:00 and 18:00 for 105 days, which included 10 days of dietary adaptation and 95 days of data recording.

Procedures and collection of samples

Body weights were recorded proior to the morning feeding on the first and final days to calculate average daily gain (ADG). The daily intake of concentrate and oat pellets were recorded to calculate total dry matter intake (DMI), and feed conversion efficiency (FCR) was determined as the ratio of DMI to ADG. Every 15 days, 300 g samples of both oat hay pellets and concentrate feed were collected, sealed in airtight plastic bags, and stored at -20℃ for subsequent analysis. On the final day of the trial and 3 hours after morning feeding, approximately 30 mL of rumen fluid were collected from each animal using an oral stomach tube (Anscitech Co. Ltd., Wuhan, China) connected to a vacuum pump. To minimize saliva contamination, the tube was rinsed thoroughly between animals, and the first 10 mL of rumen fluid were discarded. Rumen pH was measured immediately using a portable pH meter (PB-10, Sartorius Co., Göttingen, Germany) and then the fluid was filtered through 4 layers of cheesecloth, and aliquots were prepared for further analysis. Specifically, 10 mL were mixed with 5 mL of 25 % (w/v) metaphosphoric acid and stored at -20°C for VFAs analysis, while 5 mL were mixed with 5 ml of 0.5 mmol/L hydrochloric acid for analysis of ruminal nitrogen components. The remaining portion was stored at -80°C for bacteria identification.

During the last week of the experimental period, the metabolic cages were cleaned thoroughly before morning feeding to prepare for total collection of urine and feces. Fecal samples were collected in a clean tray positioned at the rear of each cage, while urine was collected using a latex funnel attached to the animal, which allowed the urine to flow into a plastic tray located underneath the metabolism crate. Daily urine from individual animal was collected into a bucket containing 50 mL of 9.0 mol/L H_2SO_4 to maintain the pH below 2.5. Total feces and urine were collected

from d 100 to d 105, and 10% of each daily output were stored at -20°C. The 5-day fecal collections were pooled for each animal, but the daily urinary samples were maintained separately for each day.

Laboratory analyses

Feed and fecal samples were oven-dried at 65℃ for 72 hours ground to pass through a 1 mm screen (JFSO-100, Topu Yunnong Instrument, Hangzhou, China), and stored at room temperature in airtight plastic bags. The contents of dry matter (DM; AOAC method 925.45) and organic matter (OM; AOAC method 942.05) were analyzed following standard procedures outlined by the Association of Official Analytical Chemists [12]. Ether extract (EE) was measured using a reflux apparatus (Ankom XT 15, Fairport, NY, USA), with petroleum ether extraction at 90 ℃ for 1 hour (AOAC method 920.29) [12]. Urinary and feed nitrogen (N) contents were measured with a nitrogen analyzer (K1100, Hannon Instruments, Jinan, China), and crude protein (CP) was estimated by multiplying the total N content by 6.25. Neutral detergent fiber (aNDF), analyzed with a heat stable amylase, and acid detergent fiber (ADF) were quantified using an automated fiber analysis system (Ankom Technology, Fairport, NY, United States), as described by Robertson and Soest [13] and Van Soest and Robertson [14], respectively. Non-fibrous carbohydrates (NFC) were calculated as DM minus the sum of individual nutrient content.

Ruminal VFA concentrations were measured by gas chromatography (Shimadzu 2010 Plus, Shimadzu Corporation, Kyoto, Japan) equipped with an AT-FFAP capillary column (30 m × 0.32 mm × 0.5 mm), following Liu [15]. The injector and detector temperatures were set at 200°C and 250°C, respectively. The oven temperature increased from 90°C to 120°C at 10 °C/min, held for 3 minutes, then raised to 180°C at 10 °C/min and held for 5 minutes. Rumen soluble protein nitrogen (SPN) concentration was determined using the Lowry method [16], and ammonia-N and free amino nitrogen (FAN) concentrations were measured using a spectrometer (SpectraMax M5, Molecular Devices, San Jose, CA, USA) at absorbance wavelengths of 630 nm and 570 nm, respectively [17].

Urinary purine derivatives (PDs) were quantified using high-performance liquid chromatograph (HPLC; Agilent 1260, Lexington, MA, USA) equipped with a reverse-phase column (250 × 4.6 mm, Synergi 4 μm Hydro-RP 80 A, Phenomenex, Torrance, CA, USA), as described by Shingfield and Offer [18]. Rumen microbial N synthesis in rumen fluid was estimated based on the urinary PD, following Chen and Gomes [19]:

$$Y = 0.84\ X + 0.15\ BW^{0.75} \times \exp(-0.25X).$$

$$\text{microbial N (g N/d)} = \frac{X\ (\text{mmol/d}) \times 70}{0.116 \times 0.83 \times 1000}$$

where: Y (mmol/d) = PD excreted in urine; X (mmol/d) = duodenal absorption of microbial purines; and e (mmol/BW 0.75 daily) = endogenous PD excretion. The purine derivatives nitrogen index (PNI) was calculated as the ratio of

purine derivative nitrogen to total urinary nitrogen [20], and rumen microbial protein synthesis (MPS) efficiency was calculated as the ratio of microbial N to digestible organic matter intake (DOMI).

Microbial DNA extraction and sequencing

Total genomic DNA was extracted from the rumen fluid samples using the E.Z.N.A® DNA kit (Omega Bio-tek, Norcross, GA, United States), following the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, Wilmington, DE, USA), with an acceptable A260/A280 ratio ranging from 1.8 to 2.2. DNA integrity was further verified via 1% agarose gel electrophoresis.

Polymerase Chain Reaction (PCR) amplification and subsequent bioinformatic analyses were done by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The V3-V4 hypervariable regions of the 16S rRNA gene were amplified using the universal primer pair 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR products were visualized on a 2% agarose gel, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified with a Quantus™ Fluorometer (Promega, Madison, WI, USA). Equimolar concentrations of purified amplicons were pooled and sequenced (2 × 300 bp) using the Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA).

Microbiome bioinformatic analyses were used QIIME2. Raw paired-end reads were first demultiplexed with the *demux* plugin and primers were removed using *cutadapt* plugin. Quality filtering, denoising, read merging, and chimera removal used DADA2. To ensure comparability with previous ruminant microbiome studies, the sequences were clustered de novo into operational taxonomic units (OTUs) using VSearch (q2-vsearch). Representative OTU sequences were classified using a naïve Bayes classifier trained on 16S rRNA data from the SILVA-138 database. The OTU counts were normalized to the number of reads in the sample with the fewest reads. Subsequent analyses of alpha and beta diversities used normalized data.

Statistical analyses

Data processing used Microsoft Excel (version 2021). Statistical comparisons between the yaks and cattle were carried out using an independent samples two-tailed t-test in SPSS software (version 26.0; SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at $P < 0.05$, and results are reported as means ± standard error of the mean (SEM).

Alpha diversity indices, including ACE index and Shannon diversity index, were calculated using the QIIME2 diversity plugin, and analyzed by the non-parametric Kruskal-Wallis test and Wilcoxon rank test using R packages (v3.4.1). Beta diversity was assessed via constrained principal component analysis (PCA) of Bray–Curtis distance

matrices, implemented by the *capscale* and *anova.cca* functions in the vegan package in R. Statistical significance was determined by permutation testing.

Differential bacterial taxa between species were identified by linear discriminant analysis effect size (LEfSe), which combined the Kruskal-Wallis test with subsequent tests assessing biological consistency and effect size relevance. Taxa with a linear discriminant analysis (LDA) score > 2 were considered significantly enriched. Spearman's rank correlation coefficient tested relationships between the relative abundances of the top 30 most prevalent ruminal bacterial genera and VFA concentrations, using the *corrplot* package in R. Functional predictions of microbiota metabolic pathways were generated using PICRUSt2 (v2.2.0), with pathway abundance annotated against the KEGG database.

RESULTS

Dry matter intake, average daily gain and feed conversion ratio

Total DMI was greater ($P < 0.01$) in cattle than in yaks (7.28 vs 5.56 kg/d), while ADG did not differ ($P > 0.05$) between species (526 vs 521 g/d). FCR was lesser ($P < 0.05$) in yaks than in cattle (12.1 vs 15.1 g/g; Figure 1).

Rumen pH and volatile fatty acids

Ruminal pH, total VFA concentration, and the molar proportions of acetate, propionate, butyrate, and iso-VFAs did not differ ($P > 0.05$) between yaks and Qaidam cattle (Table 2).

Urinary purine derivatives (PD) excretion, purine nitrogen index (PNI) and rumen microbial protein synthesis (MPS) and nitrogen components

The ruminal concentrations of ammonia-N and FAN were greater ($P < 0.05$) in yaks than cattle, while the concentration of SPN did not differ ($P > 0.05$) between species. Urinary total PD excretion and rumen microbial N production were greater ($P < 0.05$) in cattle than yaks but PNI and MPS efficiency were greater ($P < 0.01$) in yaks than Qaidam cattle (Table 3).

Collection sequencing data

A total of 748,225 raw 16S rRNA reads were obtained from the 12 rumen fluid samples. After quality filtering, chimera removal, and reads merging, 687,839 high-quality sequences were retained, with an average read length of 417 bp. Clustering at 97% sequence identity yielded 1,790 OTUs, of which 1,526 were shared between species, while yaks had 139 and cattle had 125 unique OTUs (Figure 2). Principal component analysis (PCA) of rumen bacterial communities revealed only modest separation between the two species, with PC1 and PC2 explaining 15.4% and 14.0% of the total variance, respectively. Alpha diversity indices (ace and Shannon) did not differ ($P > 0.05$) between species.

Ruminal bacterial composition

A total of 21 bacterial phyla were identified across all samples, with six phyla having relative abundances > 0.1 % (Figure 3). Bacteroidetes was the dominant phylum in both species, accounting for 52.3% in yaks and 57.4% in cattle, while Firmicutes followed, with relative abundances of 41.8% in yaks and 36.9% in cattle. The Firmicutes: Bacteroidetes ratio did not differ ($P > 0.05$) between species. Cattle had a greater relative abundance of Fibrobacterota ($P < 0.05$) and a lesser abundance of Elusimicrobiota ($P < 0.05$) than yaks.

A total of 286 genera were identified, with the 30 most abundant presented in Figure 4. Dominant genera included *Prevotella* (23.5% in yaks vs. 32.5% in cattle), *Rikenellaceae_RC9_gut_group* (10.0% vs. 10.3%), and *Christensenellaceae_R-7_group* (7.33% vs. 7.21%). Relative abundances of *norank_f__UCG-011, Atopobium, Family_XIII_AD3011_group, Oscillospira, norank_f__Peptococcaceae, Quinella, Catenibacterium, Romboutsia,* and *Corynebacterium* were greater ($P < 0.05$) in yaks than cattle, whereas abundances of *Succiniclasticum, Howardella, Pseudobutyrivibrio, Lachnospiraceae_FCS020_group,* and *GAG_352* were greater ($P < 0.05$) in cattle than yaks.

LEfSe analysis identified 47 discriminative taxa between the two groups (Figure 5) and, based on an LDA score threshold of ± 2.0, 35 genera were enriched in yaks and 12 in cattle. Genera enriched in yaks included *norank_f__UCG-011, Family_XIII_AD3011_group, Atopobium, Oscillospira,* and *Quinella* and in cattle included *Succiniclasticum, Alloprevotella, Pseudobutyrivibrio,* and *Lachnospiraceae_FCS020_group.*

Correlations between ruminal bacteria and fermentation parameters

In total, 24 positive and 25 negative correlations ($P < 0.05$) emerged between bacterial genera and rumen fermentation variables (Figure 6). *Prevotella* was correlated positively with the molar proportions of propionate (r = 0.699; $P = 0.011$), and negatively with the acetate: propionate (A:P) ratio (r = -0.622; $P = 0.031$). *Rikenellaceae_RC9_gut_group* was correlated positively with the A:P ratio (r = 0.825; $P < 0.010$), while *Lachnospiraceae_NK3A20_group* was correlated positively with ruminal pH (r = 0.609; $P = 0.035$) and ammonia-N concentration (r = 0.594; $P = 0.042$), and negatively with the molar proportions of acetate (r = - 0.650; $P = 0.022$) and propionate (r = - 0.636; $P = 0.026$) and total VFA concentration (r = - 0.587; $P = 0.045$). *Succiniclasticum* was correlated negatively with the molar proportions of iso-VFAs (r = - 0.664; $P = 0.018$), and concentrations of ammonia-N (r = - 0.902; $P < 0.001$) and FAN (r = - 0.747; $P < 0.010$), while *Veillonellaceae_UCG-001* was correlated negatively with the concentrations of ammonia-N (r = - 0.615; $P = 0.033$) and FAN (r = - 0.786; $P < 0.01$). *Butyrivibrio* was correlated negatively with the molar proportions of acetate (r = - 0.664; $P = 0.018$) and propionate (r = - 0.748; $P < 0.01$), and total concentrations of VFAs (r = - 0.678; $P = 0.015$), and positively with ruminal pH (r = 0.697; $P = 0.012$).

Prevotellaceae_UCG-001 was correlated negatively with ammonia-N (r = - 0.650; P = 0.022) and concentration of FAN (r = - 0.740; P < 0.010), while *DNF00809* was correlated positively with ruminal pH (r = 0.704, P = 0.011) and negatively with total VFAs (r = - 0.608, P = 0.036). *UCG-001* was correlated negatively with the molar proportion of butyrate (r = - 0.629, P = 0.028), *Family_XIII_AD3011_group* was correlated positively with iso-VFAs (r = 0.636, P = 0.026), ammonia-N (r = 0.699, P = 0.011), and FAN (r = 0.632, P = 0.027), and *Saccharofermentans* was correlated positively with ruminal pH (r = 0.578, P = 0.049) and negatively with propionate (r = –0.657, P = 0.020).

PICRUSt2 prediction of functions

The top 40 predicted functional pathways of the rumen bacterial communities in yaks and cattle were identified using PICRUSt2 (Table S1). Among these, only one predicted metabolic pathway differed (P < 0.05) between the two species. Overall, the most abundant predicted function was metabolic pathways (18.8%), followed by biosynthesis of secondary metabolites (9.42%) and biosynthesis of amino acids (4.27%).

DISCUSSION

Dry matter intake, growth performance and feed conversion efficiency

In the present study, DMI was 30.6% greater in cattle than yaks, yet ADG did not differ between the species. This would indicate that: 1) the maintenance energy requirements of cattle were greater than for yaks and, therefore, yaks had more energy available for growth than cattle; and/or 2) yaks were able to utilize the energy and the nutrients more efficiently than cattle for maintenance and growth. There is support for the former option as it was reported that yaks had lesser energy maintenance requirements and that N requirements for maintenance of yaks were substantially lesser than for cattle [21,22]. In addition, it was reported that enteric methane losses were lesser in yaks than cattle [23]. The relative abundances of *Quinella*, an important hydrogen-utilizing bacteria occurring in ruminants with low methane emissions [24], and Elusimicrobiota, which converts H_2 into ferric compounds, were greater in yaks than cattle, indicating that energy losses via methane emission [25] were lesser in yaks than cattle.

Furthermore, the concentration of appetite peptide A, a neuropeptide in the gastrointestinal tract that stimulates feeding behavior [26], was lesser in yaks than in cattle [27]. These differences between species indicate that yaks consume less DM, lose less energy via methane production and allocate more energy and N toward maintenance and growth, ultimately contributing to their lesser FCR. These findings imply that under low-concentrate fattening

conditions, such as those commonly used in small-holder feedlot systems on the QTP, yaks require lesser DMI than Qaidam cattle and, thus, yak production could be more economical than cattle production under these conditions.

Rumen fermentation parameters

Rumen pH is a critical determinant of microbial growth, fiber degradation, biohydrogenation, and methanogenesis [28]. In the present study, ruminal pH values were 6.86 and 6.91 in yaks and cattle, respectively, both within the range of 6.2-7.2, which is optimal for microbial activity and efficient fermentation [29]. These values indicated that both species maintained a healthy rumen environment under the experimental dietary conditions.

VFAs are the primary end-products of microbial fermentation in the rumen and supply approximately 70–80% of the host's energy requirements [30]. VFA profiles are largely shaped by dietary nutrient composition and fermentation efficiency. An ultra-deep metagenomic sequencing study demonstrated greater VFA-yielding pathways of rumen microbial genes in yaks than in cattle [31], and previous in vivo [32] and in vitro [31] studies reported that total VFA concentration was greater in yaks than in cattle. In the present study, the relative abundances of *Catenibacterium*, which hydrolyze glucose and produce acetate and butyrate [33], and *norank_f__Pseudonocardiaceae*, which ensure the stable VFA generation [34], were greater in yaks than cattle. However, in the present study, there was no difference in VFA concentration between yaks and cattle. This discrepancy may be due to the difference in rumen volume between species as the fractional absorption rates of all VFAs were reduced by an increase in rumen volume [35]. As DMI was substantially greater in cattle than yaks, we reasoned that the rumen volume was greater in cattle and, therefore, the VFAs remained longer in the rumen in cattle than yaks, resulting in a greater concentration and, thus, increasing the concentration in cattle.

Urinary PD excretion and rumen microbial N synthesis efficiency

In ruminants, urinary PDs are derived primarily from the intestinal absorption of nucleic acid purines, the majority of which originate from microbial protein. Thus, urinary PD excretion is a widely accepted proxy for microbial protein synthesis [19]. In the present study PD excretion and microbial N yield were lesser in yaks than in cattle, which could be explained by the greater DMI by cattle than yaks. MPS is dependent on ruminal fermentable substrate [36], which was considerably greater in the cattle than yaks. However, PNI, a useful indicator for evaluating the efficiency of dietary digestible N converted into rumen MCP [37] and the efficiency of MPS were greater in yaks than cattle. In addition, the concentration of ruminal ammonia-N, a major intermediate generated from microbial degradation of dietary protein, peptides, amino acids, and host-derived urea [38] and the concentration of FAN, a byproduct of proteolytic and peptidolytic activity by bacteria such as *Butyrivibrio fibrisolvens* and *Prevotella albensis* [39,40], were

greater in yaks than cattle. FAN contributes approximately 20–50% [41,42] of the N used for microbial protein synthesis. Therefore, the elevated FAN and ammonia-N levels in yaks enhance the availability of nitrogenous substrates for microbial growth, The greater PNI and MPS efficiency, combined with greater concentrations of ruminal ammonia-N and FAN, supported the premise that enhanced N utilization is an important adaptation of yaks to the chronically limited crude protein availability on the QTP.

Ruminal bacterial community composition

The composition of the ruminal bacteria, which are essential for host productivity, immunity, and survival, is closely modulated by diet [43]. In the present study, alpha diversity indices, including ACE and Shannon diversity indices, and bacterial richness did not differ between the yaks and Qaidam cattle, which is consistent with previous reports where Bacteroidetes and Firmicutes were the predominant phyla, and *Prevotella* the most abundant genus [44,45]. Firmicutes and Bacteroidetes are known to respond to dietary energy levels and the forage: concentrate ratio. Bacteroidetes degrade primarily carbohydrates and proteins, whereas Firmicutes degrade mainly fibrous and non-fibrous polysaccharides [46,47]. The Firmicutes: Bacteroidetes (F:B) ratio, often considered an indicator of feed conversion efficiency, typically increases with high-grain diets [48-50]. In the present study, the F: B ratio was greater in yaks (0.65) than in cattle (0.59), suggesting a potentially greater conversion efficiency in yaks than in cattle.

The relative abundances of *norank_f_UCG-011* [51] and *Romboutsia* [34], genera associated with fiber degradation, were greater in yaks than in cattle, which supported the premise that yaks are more efficient in degrading fibers than cattle. In a previous study in grazing animals, the relative abundances of *Ruminococcaceae_NK4A214_group*, *Prevotella*, *Ruminococcus*, *Butyrivibrio*, and *Rikenellaceae_RC9_gut_group*, *Pseudobutyrivibrio* and *Fibrobacter succinogenses* were greater in yaks than cattle [52,53]. These genera degrade fibers; however, the relative abundances of these genera did not differ between the yaks and cattle in the present study and the relative abundance of *Fibrobacterota*, which degrades lignocellulosic materials [54,55], was even greater in cattle than in yaks. These differences between the grazing ruminants and feedlot ruminants in the present study could be attributed to the dietary intake, as dietary intake is a major factor affecting the composition of the ruminal bacteria community [43]. Under grazing conditions, the diets of the yaks and cattle could have differed considerably, as they were able to select from a wide array of plant species, but under stall-fed conditions, the composition of the dietary intakes were similar, as both species were offered the same diet and there was little choice.

The relative abundance of *Succiniclasticum*, a bacteria that ferments succinate and converts it to propionate, an important precursor of glucose in the rumen [56], was greater in cattle than yaks. It was reported that the relative

abundance of *Succiniclasticum* increases linearly with increasing dietary energy levels, and that an increase in NFC content favors the growth of this genus [9], and this could explain the difference in relative abundances between yaks and cattle.

PICRUSt2 prediction of functions

Using PICRUSt2 to predict the potential functions of the rumen bacterial communities, a wide range of metabolism-related pathways were identified. Among these, metabolic pathways at KEGG level 3 were the most prominent, reflecting their crucial role in livestock survival, growth, and reproduction. The functional predictions indicated an enhanced enrichment of pathways related to pantothenate and coenzyme A (CoA) biosynthesis in cattle than yaks. Pantothenate, vitamin B5, is the key precursor for the biosynthesis of CoA, a universal and essential cofactor involved in a myriad of metabolic reactions, including the synthesis of phospholipids, the synthesis and degradation of fatty acids, and the operation of the tricarboxylic acid cycle [57]. The pathways related to carbohydrate degradation, energy metabolism, amino acid metabolism, and lipid metabolism did not differ between the two species. To further elucidate the functional impacts of low-concentrate diets on rumen microbial activity and metabolite production in yaks and cattle, integrative studies employing metagenomics and metabolomics are warranted. Such approaches could provide a more comprehensive understanding of the underlying microbial mechanisms and host-microbe interactions in these species.

Conclusions

Under identical dietary conditions, DMI was lesser in yaks than Qaidam cattle, but ADG did not differ between species and FCR was lesser in yaks than cattle. The greater ruminal concentrations of ammonia-N and FAN, along with the greater PNI and MPS efficiency in yaks, indicated more effective N utilization and microbial protein production. Additionally, differences in rumen microbial composition indicated that yaks harbor distinct microbial communities. Under small-holder feedlot conditions with low-concentrate diets, yaks exhibited better fattening performance tha Qaidam cattle. These characteristics highlight the adaptive capacity of yaks to seasonal fluctuations and low-quality forage resources typical of the QTP.

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464

Table 1. Ingredients and chemical composition of the experimental diets.

Items	Oat hay	Concentrate
Ingredients, g/kg		
Corn	-	400
Soybean meal	-	90
Cottonseed meal	-	120
Corn DDGS	-	85
Wheat bran	-	72
Distiller's grains	-	40
Corn germ meal	-	50
Sprayed corn bran	-	70
Soybean oil	-	10
$CaCO_3$	-	22
NaCl	-	10
$NaHCO_3$	-	15
Urea	-	6
Premix[1]	-	10
Total	-	1000
Chemical composition, g/kg		
DM	975	971
CP	115	235
aNDF	533	231
ADF	338	114
EE	31	41
NFC	202	402
Ash	119	91
ME (MJ/kg)[2]	8.29	12.15

DM, dry matter; OM, organic matter; CP, crude protein; aNDF, neutral detergent fiber; ADF, acid detergent fiber; NFC, non-fibrous carbohydrates; ME, metabolizable energy.

[1] The constitutes were provided as per kg of the premix: VA 800 000 IU, VD 500 000 IU, VE 10 000 IU, Fe 4000 mg, Zn 5000 mg, Cu 600 mg, Mn 2500 mg, Se 50 mg, Co 40 mg, I 50 mg.

[2] The ME was calculated based on the Tables of Feed Composition and Nutritive Values in China [58]

Table 2. Ruminal pH and volatile fatty acids in fattening yaks and cattle.

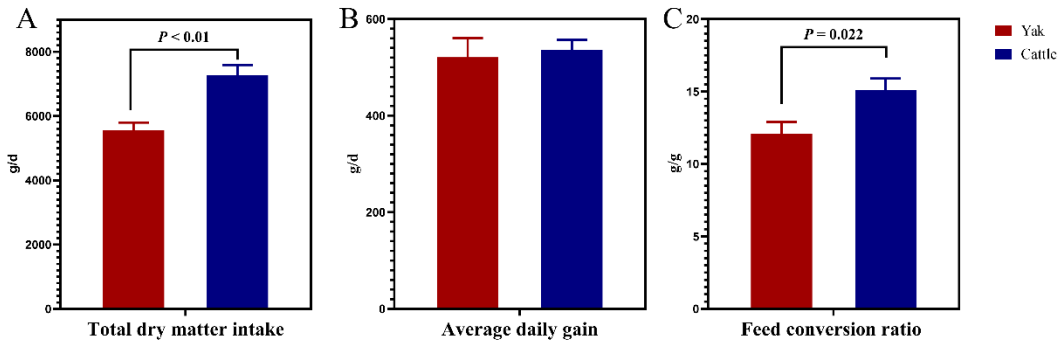
Items	Animal species		SEM	*P*-value
	Yak	Cattle		
pH	6.86	6.91	0.096	0.808
Total VFA, mmol/L	76.4	71.4	3.84	0.542
Individual VFAs, mol/100 mol				
Acetate	70.2	70.4	0.29	0.782
Propionate	15.6	15.7	0.31	0.830
Butyrate	11.5	11.8	0.24	0.614
Iso-VFA	2.73	2.15	0.182	0.120
Acetate/Propionate	4.52	4.52	0.102	0.995

VFA, volatile fatty acids.

Table 3. Urinary purine derivatives excretion, purine nitrogen index, microbial protein synthesis efficiency and ruminal nitrogen components in fattening yaks and cattle.

Items	Animal species		SEM	*P*-value
	Yak	Cattle		
Ruminal N components concentration, mg/100 mL				
Ammonia-N	9.76	7.28	0.494	0.011
SPN	36.7	34.6	2.11	0.630
FAN	7.38	4.67	0.645	0.027
Total urinary PD excretion, mmol/d	150	171	2.0	< 0.01
PNI	0.154	0.137	0.002	0.035
Rumen microbial N production, g/d	121	138	2.2	0.015
Rumen MPS efficiency, g MN/kg DOMI	35.2	30.7	0.21	< 0.01

N, nitrogen; SPN, soluble protein nitrogen; FAN, free amino nitrogen; PD, purine derivatives; PNI, purine nitrogen index; MPS, microbial protein synthesis; DOMI, digestible organic matter intake.



482

483 **Figure 1.** Dry matter intake, average daily gain, and feed conversion ratio of yaks and cattle.

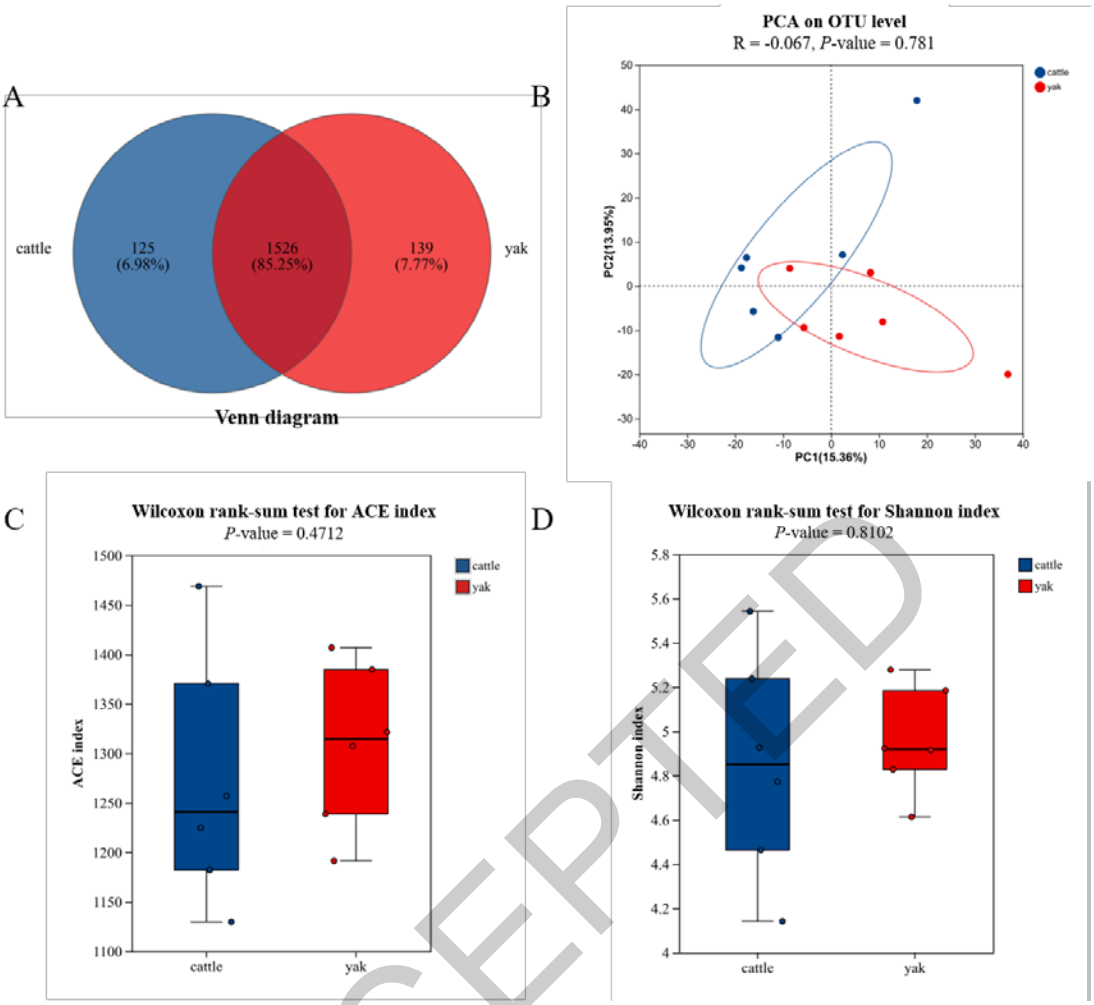
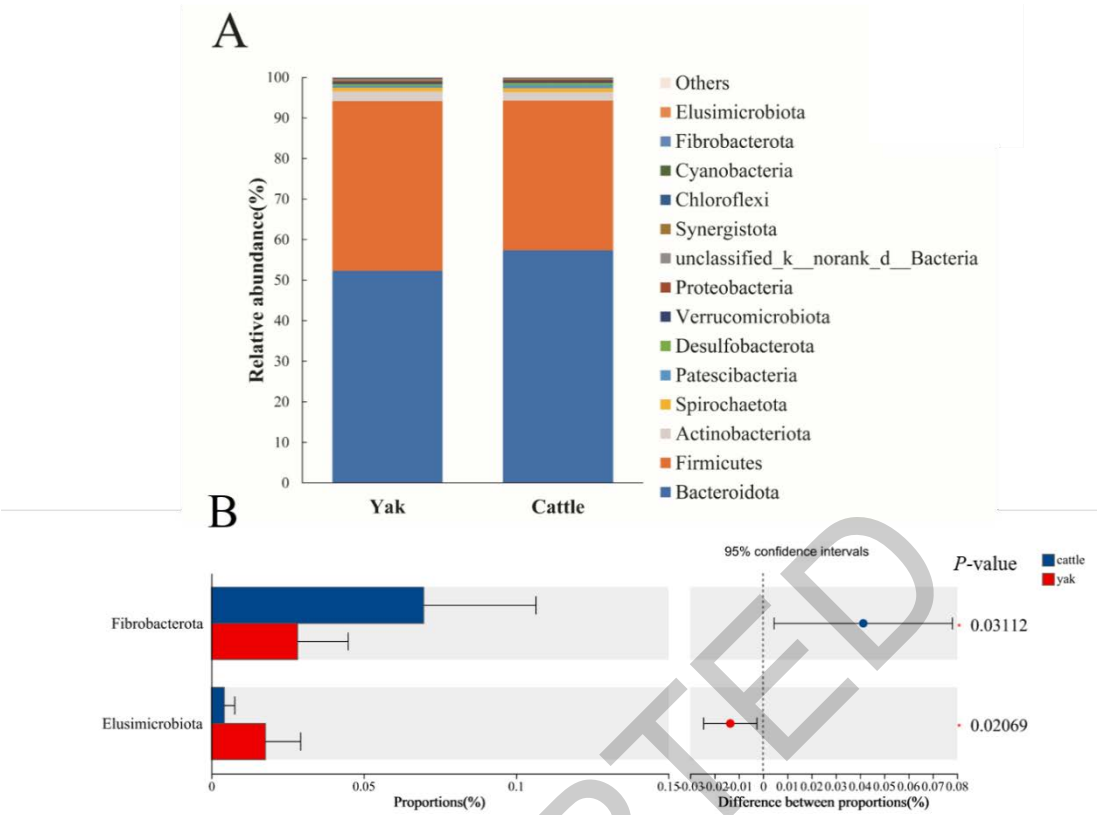


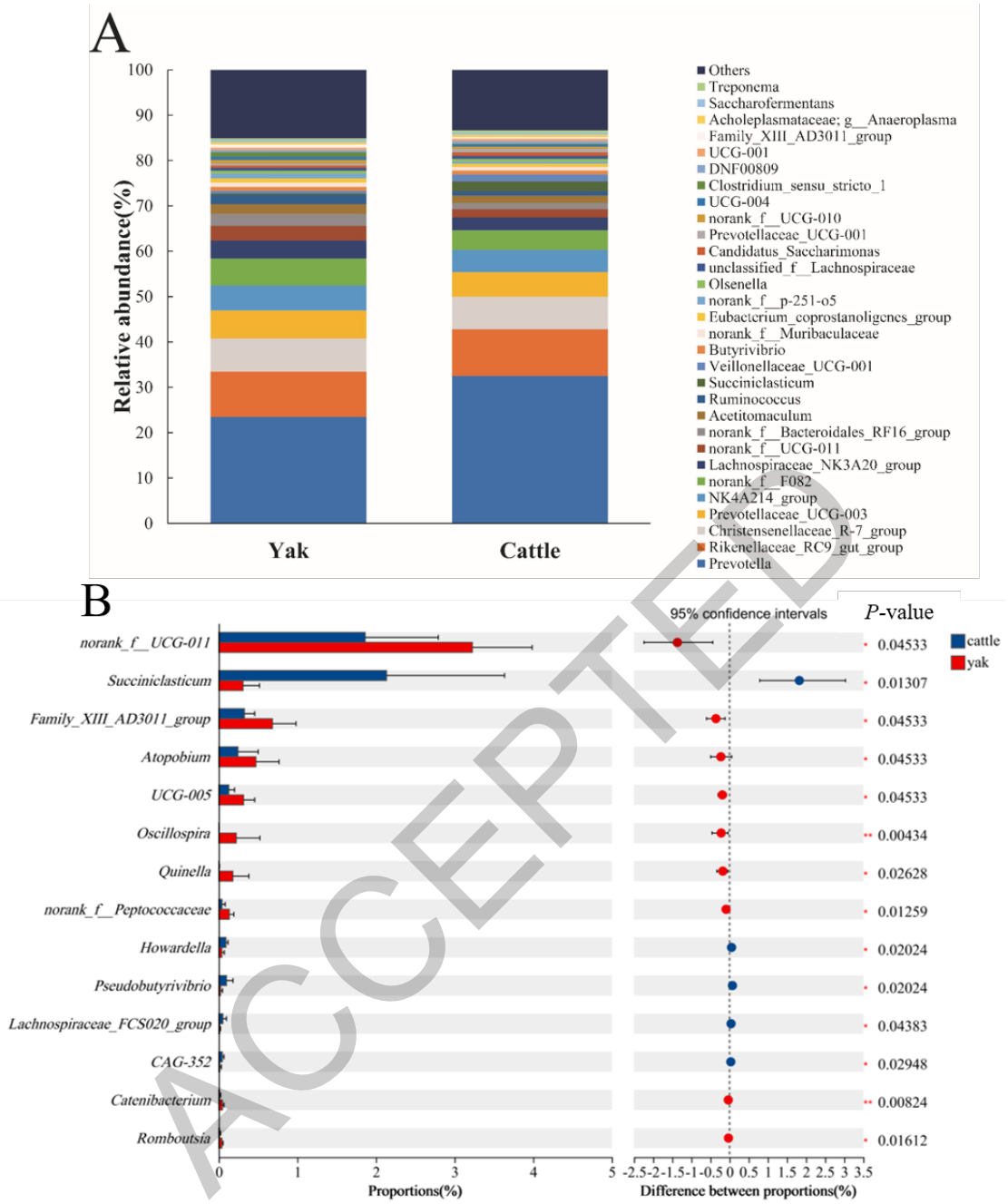
Figure 2. Alpha and beta diversity of the ruminal bacterial community in fattening yaks and cattle. A. Venn diagram; B. Principal Component Analysis (PCA) of the rumen bacteria; C. ACE index; D. Shannon index.



488

489 **Figure 3.** Bacterial relative abundances of yaks and cattle at phylum level. A. Relative

490 abundance; B. Differential rumen bacterial species.



491

492 **Figure 4.** Bacterial relative abundances of yaks and cattle at genus level. A. Relative abundance;

493 B. Differential rumen bacterial species.

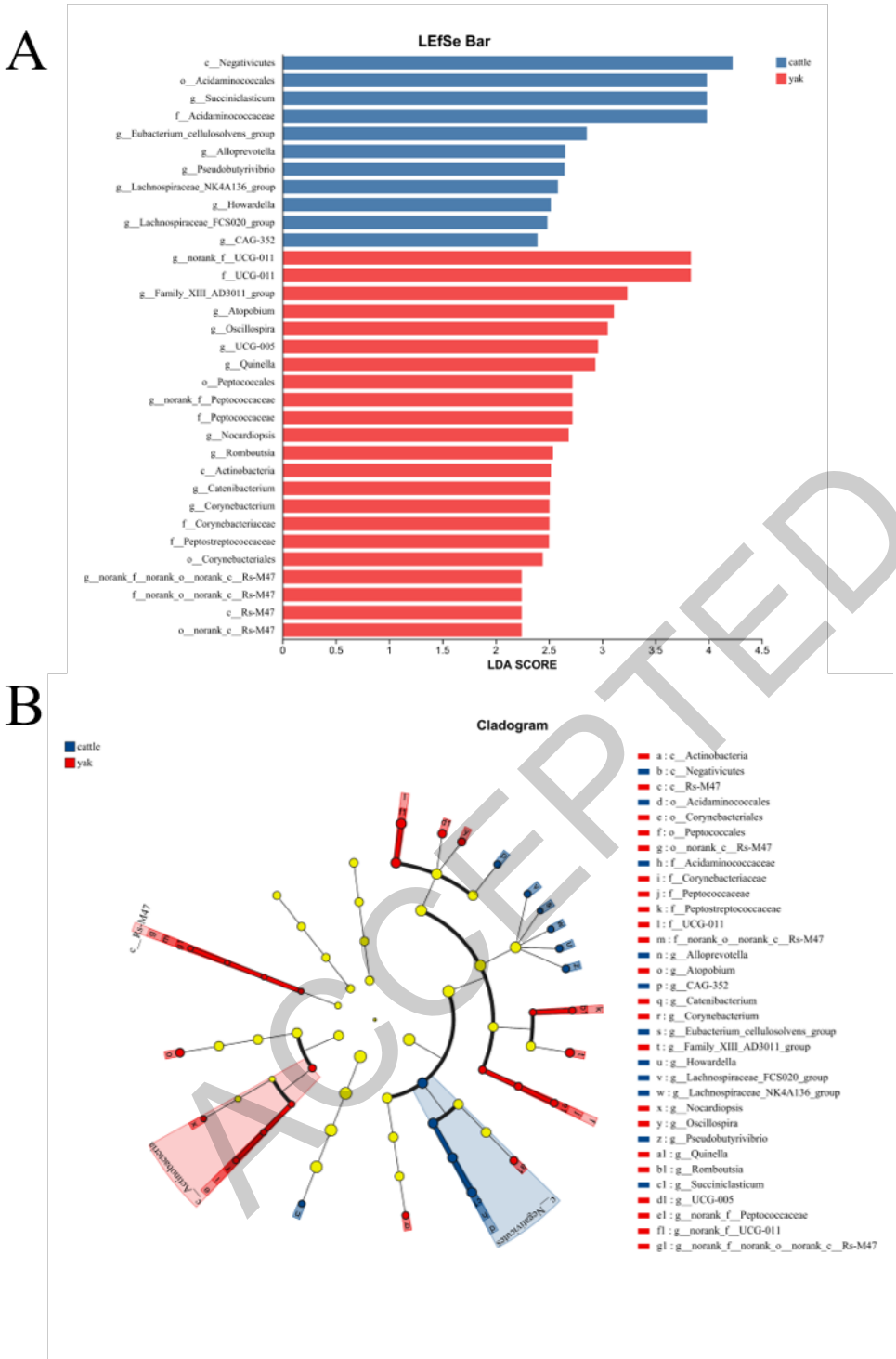


Figure 5. Linear discriminant analysis effect size (LEfSe) analysis of rumen microflora between yaks and cattle. (A) Linear discriminant analysis; (B) Cladogram reported. Prefixes represent abbreviations for the taxonomic rank of each taxon, phylum (p_) class (c_) order (o_), family (f_) and genus (g_).

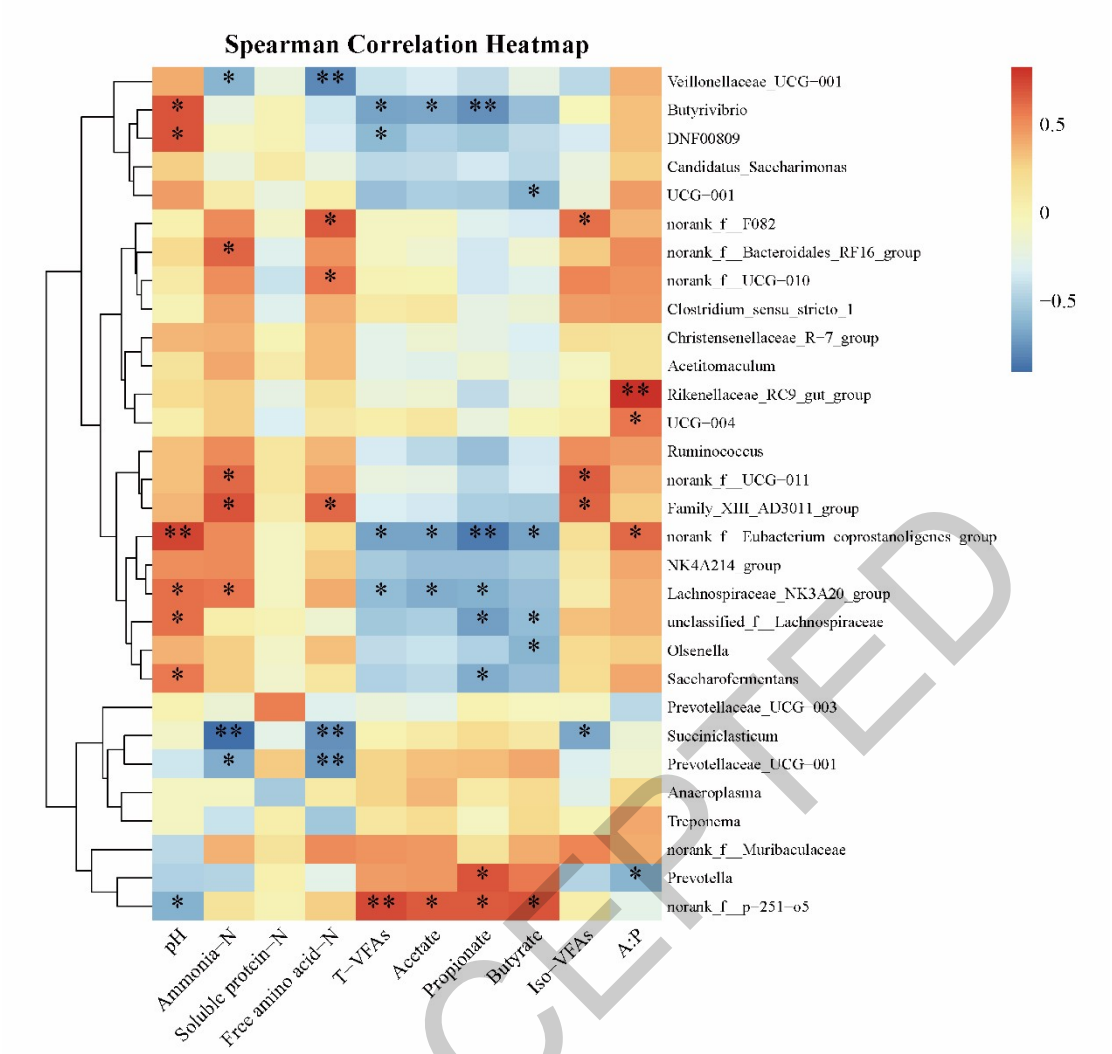


Figure 6. Spearman's rank correlation analysis between the bacteria at the genus level (TOP 30) and rumen fermentation parameters. According to Spearman's rank correlation coefficient, the $P < 0.05$ is marked with "*" and $P < 0.01$ is marked with "**".