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Effects of dietary epigallocatechin gallate (EGCG) supplementation on rumen fermentation parameters and microbiota in Hu sheep

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ABSTRACT. This study investigated the effects of dietary epigallocatechin gallate (EGCG) on rumen fermentation characteristics, and rumen microbiota in Hu sheep. Fifty-four healthy four-month-old male Hu sheep, with similar body weight, were randomly allocated to three groups (n = 18 per group): a control group (CON), and two experimental groups, L and H, which received a basal diet supplemented with 0, 300, or 1000 EGCG/head/day, respectively. The results showed that EGCG did not significantly affect ruminal pH, the concentrations of propionate, isobutyrate,

isovalerate, and valerate, or the acetate to propionate ratio ($P > 0.05$). However, the ammonia nitrogen concentration was significantly lower ($P < 0.05$) in both the L and H groups, whereas the concentrations of acetate, butyrate and total volatile fatty acids were significantly increased ($P < 0.05$). EGCG had no effect on the alpha or beta diversity of rumen bacteria ($P > 0.05$). Among the dominant phyla, the relative abundance of *Desulfobacterota* was significantly higher ($P < 0.05$) in the L and H groups. For *Verrucomicrobiota*, a significant increase was observed only in the H group relative to both the CON and L groups ($P < 0.05$). At the genus level, the abundance of *uncultured_rumen_bacterium* was significantly increased ($P < 0.05$) in the H group. Correlation analysis revealed significant associations between rumen fermentation parameters and specific microbial taxa. Acetate and butyrate levels were positively correlated with several bacterial groups involved in carbohydrate fermentation. Dietary EGCG supplementation influenced the rumen microbial community, resulting in lower ammonia nitrogen and higher volatile fatty acid concentrations, thereby improving rumen fermentation efficiency in Hu sheep.

Keywords: EGCG, Hu sheep, rumen fermentation, rumen microbiota

Introduction

Over the past few decades, animal nutrition research has increasingly focused on the physiological functions of the rumen and its microbiota, recognising that feed digestion and nutrient utilisation are strongly influenced by ruminal fermentation. Dietary manipulation is a key factor driving structural changes in the rumen microbial community. Therefore, investigating the effects of different diets or feed additives on rumen microbial composition contributes to the development of effective strategies to improve animal productivity (Zhou and Shen, 2025; Tian et al., 2025a).

Henderson et al. (2015) emphasised that, regardless of the geographical distribution or genetic background of the animals, dietary composition remains the dominant factor shaping the structure of the rumen microbial community. Numerous studies have demonstrated that the composition and abundance of the rumen microbiota are influenced not only by feed type but also by plant secondary compounds such as

phenolics, saponins, or essential oils (Vasta and Luciano, 2011). Plant-derived feed additives, an emerging category of functional supplements, can increase animal productivity by positively affecting the rumen ecosystem (Li et al., 2022; Gang et al., 2024). These additives are commonly administered as extracts, herbal mixtures, or essential oils and are widely used as growth promoters and rumen microbial modulators to improve rumen fermentation, animal health, and production performance (Qin et al.,2024). Among these compounds, polyphenols have been shown to affect rumen metabolism (Wang et al., 2024), promote growth, improve health status, and enhance product quality (Cimmino et al., 2018). These effects are closely associated with their significant influence on the rumen microbial environment.

Epigallocatechin gallate (EGCG), one of the most abundant polyphenolic catechins in green tea (Li et al., 2020), has been reported to exert anti-inflammatory, anticancer, and cardioprotective effects (Che et al., 2024). It is also involved in the regulation of lipid metabolism, immune function, and the maintenance of gut microbial balance (Zhang et al.,2021; Wu et al., 2023). However, most studies on EGCG have been conducted in monogastric animals or in the context of human medicine (Chen et al.,2024; Alam et al., 2024). Its potential application in ruminants, particularly in Hu sheep, remains largely unknown. Current evidence suggests that polyphenols can affect nutrient degradation and utilisation by altering rumen fermentation and the composition of the rumen microbiota (Ma et al., 2020). Therefore, this study systematically evaluated the effects of dietary EGCG supplementation on nutrient digestibility, rumen fermentation parameters (including volatile fatty acid profiles, ammonia nitrogen concentration, and rumen pH), and the structure of the rumen microbial community in Hu sheep. The findings provide information on the effects of EGCG on rumen function at the microbial and metabolic levels and support the development of plant polyphenol-based feed additives.

Materials and methods

Animal ethics statement

The animal experiment and the experimental protocol were approved by the

International Animal Care and Use Committee of Fujian Academy of Agricultural Sciences (FAAS), Fujian Province, China (Approval No.202402FJ017).

Experimental design and feeding management

The present study was conducted from September to December 2024 at a commercial farm in Fujian, China. A total of 54 healthy four-month-old male Hu sheep with similar body weight (23.98 ± 1.01 kg) were randomly assigned to three groups using a single-factor completely randomised design: a control group (CON), and two experimental groups, L and H. Each group consisted of 18 sheep, with each sheep considered one replicate. The experimental diet was formulated according to the nutrient requirements for growing-fattening meat sheep with a target daily weight gain of 0.1 kg, as specified in the Nutrient requirements of meat-type sheep and goat (NY/T 816-2021). The ingredient composition and nutrition levels are presented in Table 1. The CON group received the basal diet, while the L and H groups were fed the basal diet supplemented with 300 and 1000 mg/head/day of EGCG, respectively. The experiment lasted 100 days, and consisted of a 10-day adaptation phase followed by a 90-day experimental period.

The feeding regimen followed the farm's standard management practices. The sheep were fed twice daily, at 07:00 and 15:00, with feed offered in amounts sufficient to maintain feed refusals at 5–10%. Each morning before feeding, EGCG was thoroughly mixed with a small quantity of concentrate and offered to the L and H groups prior to the basal diet. The CON group received an equal amount of the concentrate without EGCG, following the same procedure.

Sample collection

Rumen fluid was collected on the first day after the end of the trial period, prior to morning feeding. From each group, five experimental sheep were randomly selected, and approximately 100 ml of rumen fluid was obtained per animal using an oral rumen fluid collector. Each sample was divided into three portions: one portion was used immediately for ruminal pH measurement. The remaining two portions were filtered through four layers of sterile gauze into sterile 50 ml centrifuge tubes, rapidly frozen in liquid nitrogen, and stored at −80 °C for further analysis.

Determination of Basal Diet Nutritional Composition

The contents of dry matter (DM), crude ash (Ash), and gross energy (GE) in the basal diet were determined according to the methods described by Zhu et al. (2021). Metabolisable energy (ME) was calculated using the following equation: ME (MJ/kg DM) = 0.046 + 0.820 × [17.211 − 0.135 × NDF (%DM)], as specified in Nutrient requirements of meat-type sheep and goat (NY/T 816-2021). Crude protein (CP) content was determined according to the national standard GB/T 6432-2018. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined according to the method of Van Soest et al. (1991).

Analysis of Rumen Fermentation Parameters

Following collection and filtration through four layers of sterile gauze, rumen fluid pH was measured directly using a portable pH meter (PHB-1, Hangzhou Oulong Instruments Co., Ltd., Hangzhou, China). The ammonia nitrogen (NH_3-N) concentration was determined by a colorimetric method, with absorbance measured at 625 nm using a spectrophotometer. The concentrations of volatile fatty acids (VFAs) were determined by gas chromatography (GC-4000A, Beijing East & West Analytical Instruments Co., Ltd., Beijing, China).

Assessment of Rumen Microbial Diversity

High-throughput 16S ribosomal RNA gene sequencing

Total genomic DNA was extracted from 15 rumen fluid samples using the Guide S96 Magnetic Soil/Stool DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions. The V3-V4 hypervariable region of the bacterial 16S rRNA gene were amplified using the following primer pairs; 338F: 5'-ACTCCTACGGGAGGCAGCA-3' and 806R: 5'-GGACTACHVGGGTWTCTAAT-3'. The PCR products were verified by agarose gel electrophoresis and purified using the Omega DNA Purification Kit (Omega Inc., Norcross, GA, USA). The purified PCR products were subjected to paired-end (2 × 250 bp) sequencing on an Illumina Novaseq 6000 platform.

Bioinformatic Analysis

Qualified sequences with ≥ 97% similarity were clustered into operational taxonomic units (OTUs) using USEARCH (version 10.0). Taxonomic annotation of the OTUs was performed using the Naive Bayes classifier implemented in QIIME2 (Bolyen et al., 2019) with the SILVA database (Callahan et al., 2016) (release 138.1) and a confidence threshold of 70%. Alpha diversity indices were calculated using QIIME2. Beta diversity was assessed by principal coordinate analysis (PCoA). One-way analysis of variance was used to compare bacterial abundance and diversity among the groups. Linear discriminant analysis effect size (LEfSe) was used to identify differentially abundant taxa. Sequencing data were analysed using the BMKCloud platform (https://www.biocloud.net).

Statistical analysis

Data were collated and processed using Microsoft Excel. Statistical analyses were performed using SPSS 26.0 software (IBM Corp., Armonk, NY, USA). Differences between the groups were analysed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons. Differences were considered statistically significant at $p < 0.05$.

Results

Rumen fermentation parameters

As shown in Table 2, supplementation with different levels of EGCG had no significant effects ($P > 0.05$) on ruminal pH, the concentrations of propionate, isobutyrate, isovalerate, or valerate, nor on the acetate-to-propionate ratio. However, the NH_3-N concentration was significantly lower ($P < 0.05$) in both the L and H groups compared to the CON group. In contrast, acetate, butyrate and total VFA levels were significantly higher ($P < 0.05$) in the EGCG-supplemented groups than in the CON group.

Alpha and beta diversity of rumen bacteria

As shown in Table 3, dietary supplementation with EGCG at different levels did not exert any significant effect ($P > 0.05$) on the Ace, Shannon, Simpson, or Chao1 indices of rumen bacteria in Hu sheep. Principal coordinate analysis (PCoA) of beta diversity (Figure 1) revealed no significant differences ($P > 0.05$) in bacterial

community clustering between the control and EGCG-supplemented groups ($p > 0.05$).

Effects of EGCG on the relative abundance of rumen bacteria in Hu sheep

As presented in Table 4, the predominant bacterial phyla were *Actinobacteriota*, *Bacteroidota*, *Desulfobacterota*, *Fibrobacterota*, *Firmicutes*, *Patescibacteria*, *Proteobacteria*, *Spirochaetota*, *Synergistota*, and *Verrucomicrobiota*. Dietary EGCG supplementation significantly increased ($P < 0.05$) the relative abundance of *Desulfobacterota* in both the L and H groups compared with the CON group. The relative abundance of *Verrucomicrobiota* was significantly higher ($P < 0.05$) in the H group than in both the CON and L groups.

At the genus level, the most abundant genera included *Bacteroidales_bacterium_Bact_22, Christensenellaceae_R_7_group, Lachnospiraceae_NK3A20_group, NK4A214_group, Prevotella, Rikenellaceae_RC9_gut_group, Ruminococcus, unclassified_Bacteroidales_RF16_group, unclassified_F082,* and *uncultured_rumen_bacterium* (these genus-level names follow the SILVA database taxonomy; "uncultured" indicates taxa not yet obtained in pure culture, 'unclassified' indicates taxa that could not be reliably assigned to a known family or genus, and group names prefixed with a family name represent unformalized phylogenetic lineages within that family). Among them, the relative abundance of *uncultured_rumen_bacterium* was significantly increased ($P < 0.05$) in the H group compared to the CON and L groups.

Identification of core responsive rumen bacteria by LEfSe analysis

Linear Discriminant Analysis Effect Size (LEfSe) analysis identified 11 bacterial taxa that differed significantly among the experimental groups (Fig. 2) (with prefixes denoting taxonomic levels: p_ = phylum, c_ = class, o_ = order, f_ = family, g_ = genus, and s_ = species). These biomarkers included *g_Prevotellaceae_UCG_003*, *s_unclassified_Prevotellaceae_UCG_003*, *c_Gammaproteobacteria*, *o_Enterobacterales*, *f_Succinivibrionaceae*, *g_Selenomonas*, *p_Verrucomicrobiota*, *g_Butyrivibrio, f_UCG_010, g_unclassified_UCG_010, and s_unclassified_UCG_010.*

Correlation analysis between rumen fermentation parameters and microbiota

Association between rumen fermentation parameters and the rumen microbiota are

presented in Figure 3. At the phylum level, NH_3-N and isobutyrate were negatively correlated with *Actinobacteriota*. Acetate and propionate were negatively correlated with *Firmicutes* but positively correlated with *Fibrobacterota*. Acetate, propionate, butyrate, and valerate demonstrated positive correlations with *Proteobacteria*, while acetate, propionate, and valerate were positively correlated with *Spirochaetota*.

At the genus level, propionate was positively correlated with *Prevotella*, and isovalerate was positively correlated with *unclassified_Bacteroidales_RF16_group*. In contrast, acetate, propionate, and butyrate showed negative correlations with both *Ruminococcus* and *Bacteroidales_bacterium_Bact_22*. Additionally, acetate and propionate were negatively correlated with *Lachnospiraceae_NK3A20_group*.

Discussion

Rumen fermentation parameters

Rumen fermentation parameters are key indicators of dietary nutrient utilisation efficiency. Ruminal pH, a comprehensive measure of ruminal homeostasis, remained within the optimal range (6.00–7.00) in all groups (Viennasay et al., 2020), with no significant differences observed. This indicates that EGCG supplementation did not disrupt normal ruminal fermentation, consistent with previous reports on other phenolic compounds (Tian et al., 2025b). Ammonia nitrogen (NH_3-N), the end product of nitrogen metabolism, serves as a precursor for microbial protein synthesis. The significantly lower NH_3-N concentration in EGCG-supplemented groups aligns with previous studies on tea polyphenols (Zhong et al., 2019), suggesting improved microbial nitrogen utilisation. Volatile fatty acids (VFAs), which supply 70–80% of the energy requirements for ruminants (Wu et al., 2024) were also influenced by EGCG addition. The concurrent increase in acetate and butyrate concentrations indicates enhanced degradation of structural carbohydrates. The acetate-to-propionate ratio, which reflects the rumen fermentation profile and energy utilisation efficiency (Hu et al., 2024), remained stable despite the increase in total VFA concentration. This demonstrated improved fermentation efficiency without changes in the overall fermentation profile. These beneficial effects were accompanied by stable ruminal pH, suggesting that EGCG promoted efficient fermentation without compromising ruminal

homeostasis. The specific increase in acetate levels further reflected improved fibre digestion and a favourable alterations in microbial metabolism, which could contribute to enhanced growth performance and feed efficiency in Hu sheep.

Rumen microbiota

The rumen hosts a complex microbial ecosystem comprising bacteria, protozoa, and fungi, which collectively degrade fibrous plant materials and convert nutrients into available forms. These microbial communities ferment structural carbohydrates such as cellulose and hemicellulose into soluble sugars and short-chain fatty acids (Palma-Hidalgo et al., 2021). Moreover, the rumen microbiota contributes to maintaining ruminal homeostasis, particularly through pH regulation, which supports efficient digestion and helps prevent metabolic disorders such as acidosis (Kazemi, 2021). The composition and diversity of the rumen microbiota are influenced by multiple factors, including diet, host genetics, and environmental conditions, reflecting the close relationship between the microorganisms and host nutrition and health (Ahmad et al., 2022). In the present study, EGCG supplementation did not significantly affect the alpha diversity indices of the rumen microbiota in Hu sheep. This suggested that EGCG did not substantially alter the overall microbial community but rather affected the abundance of specific microbial groups while preserving overall species richness and evenness. Beta diversity analysis (PCoA) further indicated clustering patterns without significant separation between the groups, supporting this observation. These findings confirm previous reports on dietary tea polyphenols in Hu sheep (Wang et al., 2024).

In this study, supplementation with EGCG significantly increased the relative abundance of the phyla *Desulfobacterota* and *Verrucomicrobiota*, as well as the genus-level taxon *uncultured_rumen_bacterium*.*Desulfobacterota* includes sulphate-reducing bacteria such as *Desulfovibrio*, a predominant genus of this type of bacteria in the rumen (Zhao et al., 2020). Therefore, the increased abundance of this phylum may indicate an increased potential for sulphur metabolism. This may, in turn, influence ruminal energy metabolism and contribute to the stability of the rumen microbial community (van Leeuwen et al., 2024). *Verrucomicrobiota* is recognised for their role in the degradation of complex polysaccharides (Gharechahi et al., 2023). Its increased

abundance suggests that EGCG may improve the degradation of complex dietary carbohydrates, which may optimise nutrient utilisation by the host. Similarly, the enrichment of *uncultured_rumen_bacterium* holds biological significance. Taxonomically, these OTUs branch into lineages affiliated with the phyla *Bacteroidota* and *Firmicutes*, both of which are primary fibrolytic bacteria. Their proliferation strongly indicates enhanced fiber digestion and improved energy harvest for the host, reflecting a beneficial adaptive response to EGCG supplementation.

LEfSe analysis identified several taxa that were enriched in the EGCG-supplemented groups, including members of the class *Gammaproteobacteria*, the family *Succinivibrionaceae*, and the genera *Butyrivibrio* and *Selenomonas*. These taxa have been associated with fibre degradation and carbohydrate fermentation, suggesting that EGCG may improve the fibre-degrading capacity of Hu sheep when fed roughage-based diets. *Prevotella*, which is primarily involved in the degradation of hemicellulose, starch, and protein, contributes to propionate and acetate production (Zhang et al., 2023). Members of the family *Succinivibrionaceae* can utilise H_2 to produce succinate, a precursor of propionate (McCabe et al., 2015), and their increased abundance may partly explain the upward trend (though statistically non-significant) in ruminal propionate levels. *Butyrivibrio*, a key butyrate-producing genus, also showed higher abundance following EGCG supplementation, likely contributing to the elevated ruminal butyrate concentration observed in this study (Zhou and Shen, 2025). Collectively, these findings indicate that EGCG may influence the rumen microbial community by selectively enriching specific functional taxa, which may in turn alter rumen metabolism. Further studies are needed to clarify the mechanisms underlying the effects of EGCG on rumen function and productivity in Hu sheep.

Correlation analysis between rumen fermentation parameters and microbial community

Several significant correlations were identified between rumen fermentation parameters and bacterial phyla. Acetate and propionate concentrations were negatively correlated with *Firmicutes* but positively correlated with *Fibrobacterota*. Although *Firmicutes* includes numerous fibre-degrading bacteria (Lin et al., 2023), its negative

correlation with these major VFAs under EGCG intervention suggests changes in the abundance or activity of specific members of this phylum. In contrast, *Fibrobacterota*, which comprises highly specialised cellulolytic bacteria (Wang et al., 2023), was positively correlated with acetate and propionate, indicating that EGCG may promote a more efficient fibre-degradation by specialised fibrolytic bacteria. Several VFAs were positively correlated with *Proteobacteria* and *Spirochaetota*. The former comprises metabolically versatile bacteria capable of rapidly fermenting non-fibrous carbohydrates such as starch and sugars to produce VFAs (Pitta et al., 2016), while the latter has also been associated with starch and soluble sugar degradation (Hernández et al., 2022). Their positive correlations with acetate, propionate, and valerate suggest that EGCG may improve the fermentation of non-fibrous carbohydrates. NH_3-N, an indicator of protein degradation, and isobutyrate, a branched-chain VFA derived mainly from branched-chain amino acid degradation, were negatively correlated with *Actinobacteriota*. This implies that EGCG may reduce protein degradation and nitrogen loss by influencing bacteria associated with these processes. At the genus level, *Prevotella*, a major genus involved in starch and sugar degradation that produces propionate as one of its main fermentation products (Strobel, 1992), was positively correlated with propionate concentrations, supporting its role in propionate synthesis. In contrast, *Ruminococcus* and *Bacteroidales_bacterium_Bact_22* showed negative correlations with acetate, propionate, and butyrate. This may reflect changes in the abundance or activity of specific members of these genera under EGCG supplementation or competition with other fibrolytic bacteria, such as *Butyrivibrio* in the modified rumen environment (Fondevila and Dehority, 1996). Similarly, *Lachnospiraceae_NK3A20_group* was negatively correlated with acetate and propionate, indicating that EGCG may affect members of this functionally diverse group involved in rumen fermentation. Collectively, these findings suggest that EGCG promotes favourable changes in the rumen microbial community, which may contribute to improved rumen fermentation and nutrient utilisation.

Conclusions

Dietary supplementation with epigallocatechin gallate (EGCG) influenced the

rumen microbiota community by selectively increasing the abundance of bacterial groups associated with carbohydrate fermentation, thereby reducing ammonia nitrogen concentration and increasing volatile fatty acid production. These changes improved rumen fermentation efficiency without affecting microbial diversity. The findings suggests that EGCG has the potential to serve as a natural feed additive for sheep. Both supplementation levels (300 and 1000 mg/head/day) produced similar effects. However, considering cost-effectiveness, supplementation with 300 mg per head per day may be more preferable.

Conflict of interest

The Authors declare that there is no conflict of interest.

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Table 1. Composition and nutrient levels of basal diets, DM basis

Items	Content, %
Ingredients	
Soybean meal	14.00
Corn	21.00
Peanut seedling	17.00
Hybrid penisetum	35.00
Garlic skin	10.50
NaCl	0.50
Premix[1)]	2.00
Total	100.00
Nutrient levels[2)]	
GE, MJ/kg	15.63
ME, MJ/kg	9.06
Ash	9.91
CP	13.23
NDF	46.07
ADF	25.69
Ca	0.98
TP	0.54

DM – dry matter, GE – gross energy, ME – metabolizable energy, CP – crude protein, NDF – neutral detergent fibre, ADF – acid detergent fibre, TP – total phosphorus, [1)] provided per kg of diets: mg: Cu 20.0, Fe 80.0, Mn 30.0, Zn 80.0, I 1, Se 0.30, Co 0.5, VE 50.0; IU: VA 20 000, VD 5 000; [2)] ME was a calculated value, while the others were measured values

Table2. Effects of dietary epigallocatechin gallate (EGCG) addition levels on rumen fermentation parameters of Hu sheep

Items	CON Group	L Group	H Group	SEM	*P*-value
pH value	6.63	6.61	6.68	0.034	0.900
Ammonia nitrogen (NH_3-N), mg/dl	32.12[a]	9.33[b]	10.30[b]	4.276	0.033
Acetate (AA), mmol/l	23.40[b]	31.74[a]	32.71[a]	1.712	0.036
Propionate (PA), mmol/l	6.20	8.93	8.39	0.660	0.210
Isobutyrate (IBA), mmol/l	1.34	0.93	1.17	0.087	0.152
Butyrate (BA), mmol/l	4.85[b]	7.23[a]	7.23[a]	0.472	0.045
Isovalerate (IVA), mmol/l	1.73	1.45	1.76	0.156	0.696
Valerate (VA), mmol/l	0.76	0.88	0.97	0.100	0.723
Acetate to Propionate ratio	3.81	3.76	4.04	0.161	0.780
Total VFA (TVFA), mmol/l	38.27[b]	51.16[a]	52.23[a]	2.660	0.044

CON Group – control group fed the basal diet, L Group – fed the basal diet supplemented with 300 mg/h/day EGCG; H Group – fed the basal diet supplemented with 1000 mg/h/day EGCG; SEM – standard error of the mean, $P > 0.05$ (no statistically significant); [ab] – different letters indicate significant differences between mean values for a given behaviour ($P < 0.05$)

Table 3. Effects of dietary epigallocatechin gallate (EGCG) addition levels on Alpha diversity index of rumen bacterial of Hu sheep

Items	CON Group	L Group	H Group	SEM	*P*-value
ACE	2267.1013	1901.5643	2194.8672	78.34776	0.127
Shannon	9.2075	9.1325	9.4596	0.08717	0.295
Simpson	0.9918	0.9947	0.9967	0.00114	0.215
Chao	2260.0252	1894.4819	2188.848	78.25072	0.126

CON Group – control group fed the basal diet, L Group – fed the basal diet supplemented with 300 mg/h/day EGCG, H Group – fed the basal diet supplemented with 1000 mg/h/day EGCG, SEM – standard error of the mean, $P > 0.05$ (no statistically significant)

Table 4. Effects of dietary epigallocatechin gallate (EGCG) on the abundance of bacteria at the phylum and genus levels in the rumen of Hu Sheep

Items	CON Group	L Group	H Group	SEM	*P*-value
Phylum levels					
Actinobacteriota	0.0166	0.0360	0.0174	0.0048	0.187
Bacteroidota	0.4463	0.4332	0.4452	0.0080	0.783
Desulfobacterota	0.0047^{b}	0.0064^{a}	0.0098^{a}	0.0009	0.036
Fibrobacterota	0.0076	0.0177	0.0123	0.0022	0.164
Firmicutes	0.4504	0.4226	0.4231	0.0112	0.546
Patescibacteria	0.0097	0.0078	0.0078	0.0011	0.753
Proteobacteria	0.0081	0.0126	0.0108	0.0012	0.312
Spirochaetota	0.0110	0.0232	0.0178	0.0028	0.217
Synergistota	0.0088	0.0087	0.0056	0.0020	0.789
Verrucomicrobiota	0.0246^{b}	0.0160^{b}	0.0338^{a}	0.0028	0.021
Genus levels					
Bacteroidales_bacterium_Bact_22	0.0598	0.0061	0.0050	0.0135	0.168
Christensenellaceae_R_7_group	0.0448	0.0535	0.0448	0.0038	0.603
Lachnospiraceae_NK3A20_group	0.0406	0.0317	0.0274	0.0041	0.434
NK4A214_group	0.0315	0.0408	0.0363	0.0025	0.330
Prevotella	0.1387	0.1889	0.1506	0.0115	0.179
Rikenellaceae_RC9_gut_group	0.0747	0.0603	0.0811	0.0060	0.375
Ruminococcus	0.0493	0.0282	0.0336	0.0048	0.178
unclassified_Bacteroidales_RF16_group	0.0303	0.0231	0.0251	0.0032	0.679
unclassified_F082	0.0336	0.0233	0.0415	0.0034	0.088
uncultured_rumen_bacterium	0.0833^{b}	0.0848^{b}	0.1187^{a}	0.0063	0.019

CON Group – control group fed the basal diet, L Group – fed the basal diet supplemented with 300 mg/h/day EGCG; H Group – fed the basal diet supplemented with 1000 mg/h/day EGCG; SEM – standard error of the mean, $P > 0.05$ (no statistically significant); ^{ab} – different letters indicate significant differences between mean values for a given behaviour ($P < 0.05$)

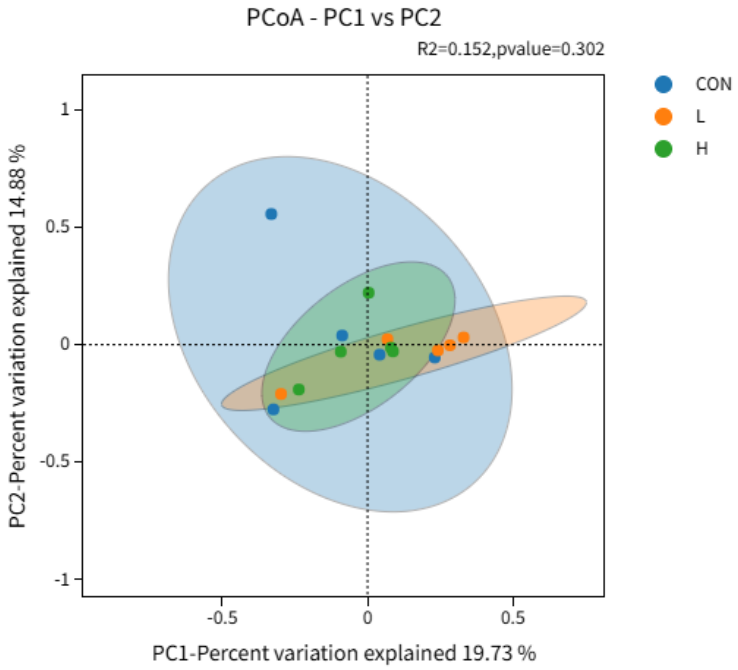
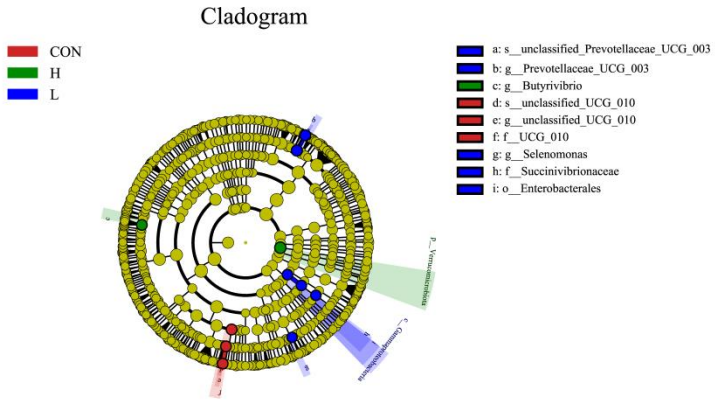


Figure 1. PCoA of rumen microbial community of Hu sheep

PCoA – principal coordinates analysis; PC1 – first principal component; PC2 – second principal component; CON – control group fed the basal diet; L – fed the basal diet supplemented with 300 mg/h/d EGCG; H – fed the basal diet supplemented with 1000 mg/h/d EGCG

A



B

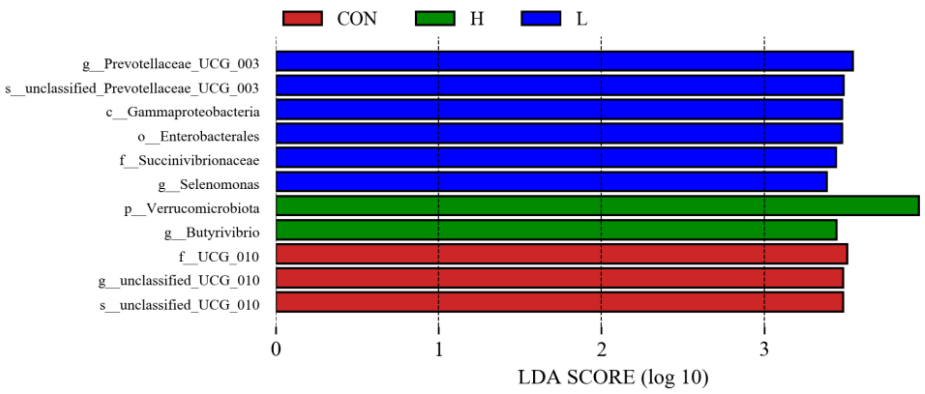


Figure 2. Identification of key microbiota in Hu sheep rumen fluid

(A) Cladogram of annotated differential species; (B) Score plot of differential species based on LDA analysis. LDA – linear discriminant analysis; CON – control group fed the basal diet; L – fed the basal diet supplemented with 300 mg/h/day EGCG; H – fed the basal diet supplemented with 1000 mg/h/day EGCG; EGCG – dietary epigallocatechin gallate

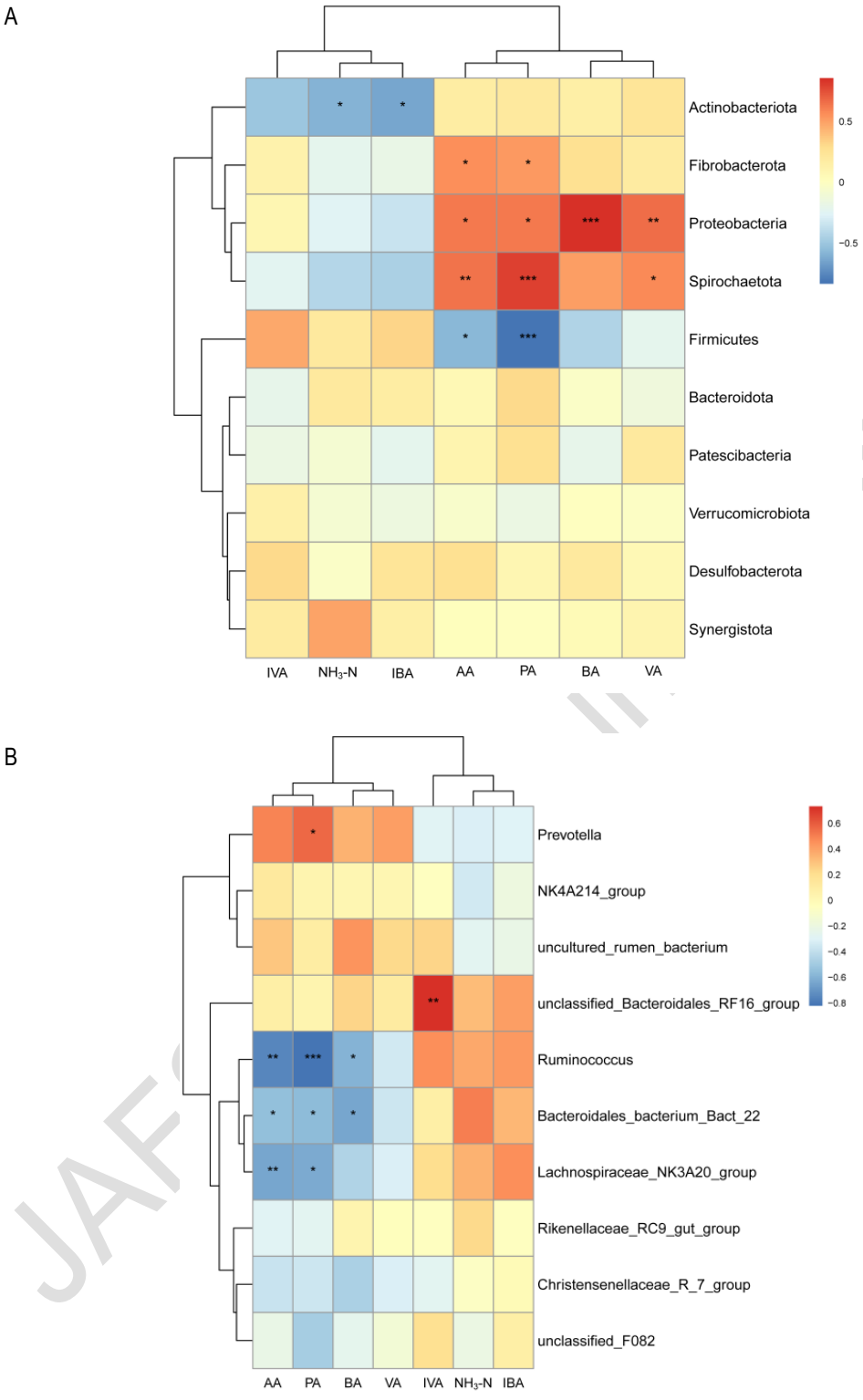


Figure 3. Heat map of correlation analysis between rumen fermentation parameters and microbiota. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

(A) Correlation analysis between rumen fermentation parameters and the top ten abundant phyla; (B) Correlation analysis between rumen fermentation parameters and the top ten most abundant genera. AA – acetate, PA – propionate, BA – butyrate, VA – valerate, IVA – isovalerate, IBA – isobutyrate