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Original Research Article

Rumen bacterial cluster identification and its influence on rumen metabolites and growth performance of young goats

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ABSTRACT

Enterotypes, which are defined as bacterial clusters in the gut microbiome, have been found to have a close relationship to host metabolism and health. However, this concept has never been used in the rumen, and little is known about the complex biological relationships between ruminants and their rumen bacterial clusters. In this study, we used young goats ($n = 99$) as a model, fed them the same diet, and analyzed their rumen microbiome and corresponding bacterial clusters. The relationships between the bacterial clusters and rumen fermentation and growth performance in the goats were further investigated. Two bacterial clusters were identified in all goats: the P-cluster (dominated by genus *Prevotella*, $n = 38$) and R-cluster (dominated by *Ruminococcus*, $n = 61$). Compared with P-cluster goats, R-cluster goats had greater growth rates, concentrations of propionate, butyrate, and 18 free amino acids› and proportion of unsaturated fatty acids, but lower acetate molar percentage, acetate to propionate ratio, and several odd and branched chain and saturated fatty acids in rumen fluid ($P < 0.05$). Several members of Firmicutes, including *Ruminococcus*, *Oscillospiraceae NK4A214 group*, and *Christensenellaceae R-7 group* were significantly higher in the R-cluster, whereas Prevotellaceae members, such as *Prevotella* and *Prevotellaceae UCG-003,* were significantly higher in P-cluster ($P < 0.01$). Co-occurrence networks showed that R-cluster enriched bacteria had significant negative correlations with P-cluster enriched bacteria ($P < 0.05$). Moreover, we found the concentrations of propionate, butyrate and free amino acids, and the proportions of unsaturated fatty acids were positively correlated with R-cluster enriched bacteria ($P < 0.05$). The concentrations of acetate, acetate to propionate ratio, and the proportion of odd and branched chain and saturated fatty acids were positively correlated with P-cluster enriched bacteria ($P < 0.05$). Overall, our results indicated that rumen bacterial clusters can influence rumen fermentation and growth performance of young goats, which may shed light on modulating the rumen microbiome in early life to improve the growth performance of ruminant animals.

1. Introduction

Domestic ruminants are important protein-producing animals and make a huge contribution to meeting the increasing human demand for high-quality products (Clark and Mora García, 2017; Morand-Fehr et al., 2004). The microbes in the rumen provide energy, bacterial proteins, and vitamins by feed degradation and fermentation, which has a great impact on host metabolism (Jiang et al., 2022; Liu et al., 2021). Recent studies have shown that the rumen bacterial population is associated with production performance in dairy cows, including milk quality and feed efficiency (Wallace et al., 2019; Xue et al., 2020).

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Although the microbial community in the gut varies among individuals, there exists consistent clustering in the predominant microbes among similar individuals (Arumugam et al., 2011; Costea et al., 2018). Arumugam et al. (2011) first proposed the concept of "enterotypes" that suggested predicted clusters of gut microbiota can be used to describe the distribution of the gut microbial community. Further studies in humans have found that enterotypes were strongly associated with long-term dietary types and elicited broad influence on several diseases like obesity, hypertension, and diabetes (Christensen et al., 2018; Dinsmoor et al., 2021; Li et al., 2017; Molinaro et al., 2020; Wu et al., 2011). A recent study on the fecal microbiome in dairy cows reported that the milk yield and body weight were significantly higher in *unclassified Spirochaetaceae* enterotype cows than in *Bifidobacterium* enterotype cows (Tröscher-Mußotter et al., 2021). However, this research focuses primarily on enterotypes in the gut. To our knowledge, no research has been carried out to identify the bacterial clusters in the rumen and the relationship between bacterial clusters and host growth performance.

We hypothesized that there existed bacterial clusters in the rumen and the clusters would have an indispensable influence on individual variations in rumen fermentation and growth performance of young ruminants. The objective of this study was to identify the bacterial clusters in the rumen and to investigate the relationships of rumen bacterial clusters with rumen fermentation parameters and average daily gain (ADG) in young goats. This study will extend our understanding on how rumen microbiota contributes to host phenotypes and provide new insights into improving the growth performance of goats by manipulating the rumen bacterial clusters in their early life.

2. Materials and methods

2.1. Animal ethics statement

The use of the goats and the experimental protocol was approved by the Animal Use and Care Committee of Northwest A&F University (protocol number: NWAFAC1008) and the animal experiments compiled with the ARRIVE guidelines.

2.2. Experiment animals and sampling

The experiment was conducted at a local farm in Baoji (34°41′N, 109°09′E), China. A total of 99 healthy female Guanzhong goats were used in this experiment. They had no history of administration of any antimicrobial agents (antibiotics, antifungals, or antivirals) or infectious disease. Goats were all born in one week and their birth weights were immediately recorded after birth (3.02 ± 0.05 kg). Goat kids were raised with their dams in the first 3 d after birth, and then they were transferred to the lamb barn with bottle-feeding of mixed milk. Specifically, the goat kids were given alfalfa hay starting at 15 d old and a concentrate mixture starting at 30 d old. All goat kids were weaned at 3 months of age. After weaning, kids were fed ad libitum three times daily at 07:30, 13:00, and 19:00 with a ration consisting of forage and concentrate (60:40) and had free access to water. The ingredients and nutrient concentrations of the diet are presented in Table S1.

All goats were housed individually and allowed one week to acclimate to pens prior to dry matter intake (DMI) measurement at one week before 6 months old (173 ± 0.34 d). After the acclimatization period, amounts of feed offered and refused were weighed daily for seven consecutive days. Dairy goats were fed approximately 110% of their anticipated consumption, ensuring ad libitum feeding. Feed samples were dried for 24 h at 105 °C for dry matter (DM) analysis. DMI was recorded as the difference between the total feed offered and feed refused on a DM basis. When goats were 6 months plus one week old (187 ± 0.34 d), their body weights were recorded before the morning feeding. ADG was calculated as the difference between 6-month body weight and birth weight divided by the number of days. Rumen fluid samples of all young goats were also collected through an oral stomach tube before the morning feeding at 6 months of age. To avoid saliva contamination, the first 30 mL of rumen fluid was discarded before sampling. Samples were divided into two aliquots and stored in liquid nitrogen for 16S rDNA analysis and metabolite determination respectively.

2.3. Rumen volatile fatty acid (VFA) assay

The determination of VFA in rumen fluid was performed according to the protocol described by Li et al. (2014). In brief, thawed rumen fluid sample was centrifuged at 13,500 × *g* at 4 °C for 10 min. Two mL of supernatant was mixed with 200 μL of crotonic acid (1%, wt/vol) and then filtered through a 0.45-μm filter. The VFA concentrations in the filtrate were determined by gas chromatography (Agilent Technologies 7820A GC system, Santa Clara, CA) with a flame ionization detector and a fused silica column (AE-FFAP, 30 m × 0.25 mm × 0.33 μm, Agilent Technologies Inc., Santa Clara, CA).

2.4. Rumen fatty acid assay

The compositions of fatty acids in rumen fluid were analyzed as described by Sun and Gibbs (2012). Briefly, a freeze-dried sample (0.5 g of dry matter) was directly methylated with 4 mL of 0.5 mol/L NaOH/methanol solution at 50 °C for 15 min, followed with 4 mL of HCl/methanol (5 mL/100 mL) solution at 50 °C for 1 h. The extract was dissolved in 2 mL of heptane and then introduced to a gas chromatograph (Agilent Technologies 7820A GC system, Santa Clara, CA) equipped with a fused silica capillary column (SP-2560, 100 m × 0.25 mm × 0.2 μm; Supelco Inc., Bellefonte, PA).

2.5. Rumen free amino acid assay

The compositions of free amino acids in rumen fluid were analyzed as described by Chen et al. (2022). Rumen fluid samples were deproteinized in sulfosalicylic acid (10%, wt/vol), centrifuged at 3,000 × *g* at 4 °C for 10 min, and filtered through a 0.45-μm filter. The supernatant was used for the determination of free amino acids in liquid chromatography and tandem mass spectrometry (Exion LC AC, QTRAP 5500, AB SCIEX, Framingham, MA) with a phase column (120 EC-C18, 4.6 mm × 100 mm × 2.7 μm; Agilent Technologies Inc., Santa Clara, CA).

2.6. Microbial DNA extraction and 16S rRNA gene sequencing

Total DNA in rumen fluid samples was extracted using the QIAamp DNA Stool Mini kit (QIAGEN, Germany) according to the manufacturer's protocol. The quality of DNA was checked in 1% agarose gel electrophoresis and the DNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The V3–V4 hypervariable region of bacterial 16S rDNA was amplified from extracted DNA using the forward primer 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and the reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′), with the reverse primer containing a 6-bp barcode. PCR products were separated in 2% agarose gel and then purified using AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA). Amplicons were then subjected to paired-end (PE300) sequencing on the Illumina MiSeq sequencing following the standard protocols.

2.7. Illumina sequencing data analysis

The raw sequences were merged with FLASH (v1.2.11) and quality filtered with fastp (0.19.6). Sequences were imported into QIIME2 v2021.8 for demultiplexing, and the amplicon sequence variants (ASV) table was constructed using DADA2. Bacterial 16S ASV were assigned a taxonomy using the SILVA database (version 138) as the reference, and the taxon abundance in each sample was determined for phylum, class, order, family, and genus. Alpha diversity measurements, including the richness estimates (Sobs, ACE, and Chao1) and diversity index (Shannon and Simpson), were calculated using QIIME 2. The microbial beta diversity was determined using the distance matrices generated from Bray–Curtis analysis, principal coordinate analysis (PCoA), and ANOMIS analysis.

2.8. Enterotype identification

The genus-level relative abundance profiles of samples were enrolled for enterotype analysis using Jensen–Shannon divergence and partitioning around medoid clustering (Arumugam et al., 2011; Wu et al., 2011). The optimal number of clusters was assessed by the Calinski-Harabasz (CH) index. PCoA was performed to visualize bacterial clusters in samples. Based on these analyses, the microbiomes in all kids in this study were identified into two separate clusters (enterotypes): cluster 1 (P-cluster) and cluster 2 (R-cluster), and these two enterotypes were used in subsequent analyses of their relationships with the other measurements.

2.9. Construction of microbial co-occurrence networks based on random matrix theory

A microbial co-occurrence network was constructed using a Random Matrix Theory (RMT)-based method as described by previous reports (Deng et al., 2012; Zhou et al., 2010). Briefly, the ASV that existed in less than 50% of all samples were excluded. The within-module degree (Zi) and among-module connectivity (Pi) were calculated to analyze the topological roles of different nodes in the network with the classification as follows: network hubs ($Zi > 2.5$; $Pi > 0.62$), module hubs ($Zi > 2.5$; $Pi < 0.62$), connectors ($Zi < 2.5$; $Pi > 0.62$); peripherals ($Zi < 2.5$; $Pi < 0.62$). The module separation was performed by fast-greedy modularity optimization procedure, and Module-EigenGene analyses were used to analyze module-environmental traits relationships based on the Pearson correlation matrix. The network structure was visualized using Cytoscape v3.7.1.

2.10. Statistical analysis

All goats were separated into P- and R-clusters according to their rumen enterotypes. The *t*-test for independent samples was used to analyze the variables, including ADG and DMI. Microbial taxonomy and rumen metabolites were compared using the Wilcoxon rank-sum test between two enterotypes. Spearman's rank correlation was used for most correlation analyses, including correlations between P-cluster enriched genera and R-cluster enriched genera and correlations between differential genera and metabolites in two bacterial clusters. Pearson correlation was used alone to analyze the memberships and module-environmental traits relationships in microbial co-occurrence networks using the RMT-based method. All data were expressed as the means ± SE. Differences were statistically significant at $P < 0.05$.

3. Results

3.1. Clusters of rumen microbiome in young goats

The clustering analysis of the microbiomes in rumen fluid is shown in Fig. 1. The microbiomes in all goats were separated into two clusters because the maximum Calinski-Harabasz index was reached when the cluster number was 2 (Fig. 1A). Cluster 1 ($n = 38$) was dominated by *Prevotella*, and cluster 2 ($n = 61$) was dominated by *Ruminococcus* (Fig. 1B). As shown in Fig. 1C, the relative abundance of *Ruminococcus* and *Prevotella* differed between the two clusters. In the following research, all goats were grouped according to cluster 1 (P-cluster) and cluster 2 (R-cluster).

3.2. Association of rumen bacterial clusters with growth rate

In this study, there was no difference in DMI between the two bacterial clusters ($P = 0.374$, Table 1), but ADG was greater in the R-cluster compared to P-cluster ($P = 0.023$, Table 1). Moreover, we analyzed the association of ADG with *Ruminococcus* and *Prevotella*, respectively (Fig. 2A and B). ADG of all goats ($n = 99$) was negatively correlated with the abundance of *Prevotella* in the rumen ($r = -0.26$, $P = 0.009$, Fig. 2B), indicating that the rumen enterotype may play an important role in the growth performance of young goats.

3.3. Rumen microbiome compositions between R-cluster and P-cluster

The rumen microbiome diversity and bacterial composition in the two bacterial clusters were further explored. Compared to the P-cluster, the R-cluster had higher community diversity with lower Simpson index and higher Shannon index ($P < 0.01$, Table 2). PCoA based on the Bray–Curtis distance showed that there was a significant difference in rumen microbial structure between the two clusters ($P = 0.001$, Fig. 2C).

The taxonomic differences between the two clusters were analyzed at phylum, family, and genus levels, respectively (Tables 3–5). At the phylum level, the abundance of Bacteroidota was lower, but Firmicutes was greater in the R-cluster compared with P-cluster ($P < 0.01$, Table 3). At the family level, the abundance of Prevotellaceae was lower in the R-cluster, while the abundances of Ruminococcaceae and other Firmicutes members, such as Lachnospiraceae, Oscillospiraceae, and Christensenellaceae, were greater in the R-cluster compared with the P-cluster ($P < 0.05$, Table 4). At the genus level, the abundances of *Prevotella*, *Prevotellaceae UCG-003*, and *Succiniclasticum* were lower in the R-cluster, whereas the abundances of *Ruminococcus*, *Oscillospiraceae NK4A214 group*, *Christensenellacea R-7 group*, *Candidatus Saccharimonas*, *unclassified Clostridia*, and *unclassified Lachnospiraceae*, were greater in the R-cluster than P-cluster ($P < 0.01$, Table 5).

3.4. Microbial interactions differ between P-cluster and R-cluster

The top 60 genera (relative abundance >0.1%, detected in >50% of all samples) of the rumen microbiome were used to explore the bacterium–bacterium interactions in the two clusters (Table S2). There were positive associations among P-cluster enriched genera and among R-cluster enriched genera, while multiple negative associations were found between P-cluster enriched genera and R-cluster enriched genera (Spearman's correlation, $P < 0.05$, Fig. 3). Notably, several genera that were enriched in the R-cluster, such as *Oscillospiraceae NK4A214 group*, *Christensenellacea R-7 group*, unclassified *Clostridia*, and unclassified *Lachnospiraceae*, were negatively correlated with the members of Prevotellaceae that were

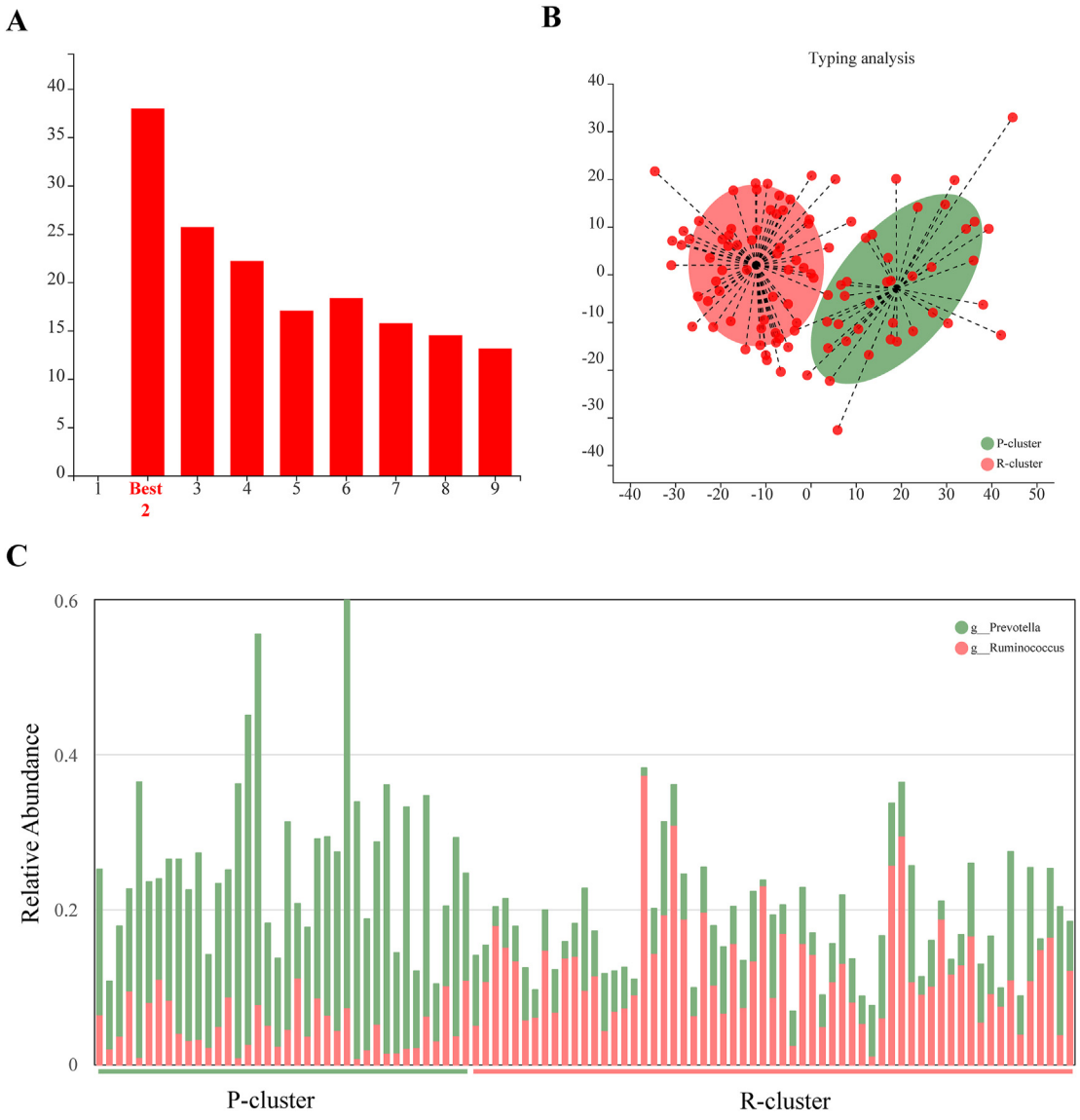


Fig. 1. Rumen bacterial clustering in young goats ($n = 99$). (A) Optimal number of rumen bacterial cluster separation. The x axis shows cluster number; the y axis shows Calinski-Harabasz index. (B) Principal coordinate analysis (PCoA) plot of bacterial clusters based on Jensen-Shannon distance. (C) Histogram showing the relative abundances of *Ruminococcus* and *Prevotella* in the two clusters.

enriched in the P-cluster (Spearman's correlation, $P < 0.05$, Fig. 3 and Fig. S1). In addition, *Ruminococcus* showed increased participation in the network of the R-cluster compared to P-cluster (Fig. 3, Table S3 and Table S4).

We also performed an RMT-based network analysis to further evaluate the potential microbial modules and identify the keystone bacteria in the P-cluster and R-cluster (Fig. 4A and B). In the P-cluster, two nodes (ASV) belonging to *Ruminococcus* and one node (ASV) belonging to *Prevotellaceae UCG-003* were regarded as the connectors linking the different modules together. One node (ASV) belonging to *Ruminococcus* was considered as a module hub to cohere their own modules. In R-cluster only 2 module hubs were identified, which belonged to *norank UCG-010* and *Selenomonas* (Fig. 4C and D).

Table 1
The feed intake and growth performance of goats in P-cluster and R-cluster (g/d).

Item	P-cluster	R-cluster	*P*-value
DMI	938 ± 37	941 ± 34	0.374
ADG	105.97 ± 2.34	112.34 ± 1.87	0.023

DMI = dry matter intake; ADG = average daily gain.
Data are expressed as the means ± SE.

3.5. Rumen metabolites differ between P-cluster and R-cluster

The differences in the metabolites in rumen fluid between the P-cluster and R-cluster are shown in Tables 6–8. Compared with the P-cluster, the R-cluster had greater concentrations of propionate, butyrate, and molar percentages of propionate and butyrate, but lower acetate percentage and acetate to propionate ratio ($P < 0.05$, Table 6).

As shown in Table 7, free amino acid concentration in rumen fluid differed between the P-cluster and R-cluster. Interestingly, the concentrations of all 18 amino acids were significantly higher in the R-cluster compared with the P-cluster ($P < 0.05$). Furthermore, the concentrations of branched chain amino acids (BCAA) and total

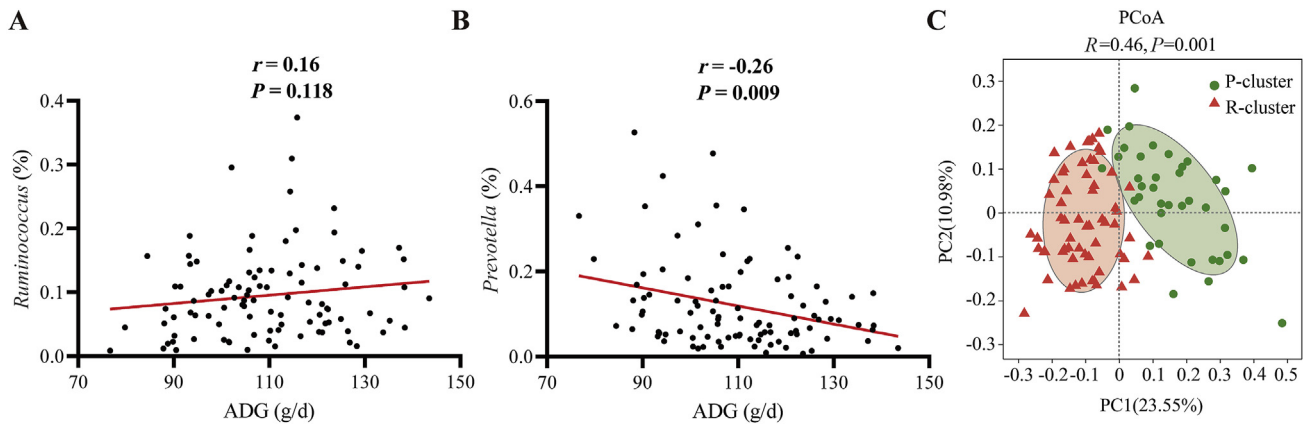


Fig. 2. The relationship between rumen bacterial clusters and the growth rates in young goats. (A) Spearman's correlation between ADG and the relative abundance of *Ruminococcus*. (B) Spearman's correlation between ADG and the relative abundance of *Prevotella*. (C) Principal-coordinate analysis (PCoA) plot of the rumen microbiome, based on Bray–Curtis distance matrix.

Table 2
Alpha diversity of rumen microbiota in P-cluster and R-cluster.

Estimators	P-cluster	R-cluster	*P*-value
Ace	110.07 ± 1.79	107.80 ± 1.28	0.404
Chao1	109.67 ± 1.78	107.62 ± 1.30	0.468
Sobs	109.24 ± 1.75	106.85 ± 1.26	0.371
Shannon	2.99 ± 0.04	3.14 ± 0.03	0.002
Simpson	0.11 ± 0.01	0.08 ± 0.00	<0.001

The data are expressed as the means ± SE.

Table 3
The relative abundance of rumen microbiota in P-cluster and R-cluster at the phylum level (%).

Taxa name	P-cluster	R-cluster	*P*-value
Firmicutes	38.72 ± 1.22	57.43 ± 1.11	<0.001
Bacteroidota	55.78 ± 1.30	35.04 ± 1.11	<0.001
Patescibacteria	2.53 ± 0.35	3.84 ± 0.36	0.003
Actinobacteriota	0.75 ± 0.15	1.71 ± 0.37	0.020
Spirochaetota	1.07 ± 0.17	0.96 ± 0.21	0.023
Proteobacteria	0.38 ± 0.16	0.19 ± 0.03	0.082
unclassified_k_norank_d_Bacteria	0.11 ± 0.02	0.27 ± 0.03	<0.001
Synergistota	0.14 ± 0.03	0.19 ± 0.03	0.071
Desulfobacterota	0.17 ± 0.02	0.12 ± 0.01	0.043
Verrucomicrobiota	0.17 ± 0.03	0.13 ± 0.02	0.567

The data are expressed as the means ± SE.

Table 4
The relative abundance of rumen microbiota in P-cluster and R-cluster at the family level (%).

Taxa name	P-cluster	R-cluster	*P*-value
Prevotellaceae	27.97 ± 1.96	9.24 ± 0.64	<0.001
F082	11.83 ± 1.21	10.88 ± 0.79	0.707
Ruminococcaceae	6.03 ± 0.57	13.52 ± 0.91	<0.001
Rikenellaceae	8.32 ± 0.70	8.19 ± 0.50	0.853
Lachnospiraceae	6.00 ± 0.35	9.50 ± 0.50	<0.001
Oscillospiraceae	5.80 ± 0.38	8.20 ± 0.35	<0.001
Selenomonadaceae	5.90 ± 0.62	6.97 ± 0.70	0.535
Christensenellaceae	3.24 ± 0.34	7.15 ± 0.38	<0.001
Muribaculaceae	4.92 ± 0.71	4.37 ± 0.47	0.762
Saccharimonadaceae	2.50 ± 0.35	3.83 ± 0.36	0.003
norank_o_Clostridia_UCG-014	2.72 ± 0.40	2.31 ± 0.38	0.191
unclassified_c_Clostridia	1.41 ± 0.15	2.88 ± 0.29	<0.001
Acidaminococcaceae	2.98 ± 0.50	0.55 ± 0.09	<0.001
Eubacterium_coprostanoligenes_group	1.39 ± 0.15	1.94 ± 0.20	0.039
Bacteroidales_RF16_group	1.52 ± 0.19	1.07 ± 0.09	0.054

The data are expressed as the means ± SE.

essential amino acids (TEAA) were significantly higher in the R-cluster ($P < 0.01$).

The different free fatty acids between the two clusters with a proportion greater than 1.0% in rumen fluid are included in Table 8. C16:0, C18:1t11, C18:1c9, unsaturated fatty acids (UFA), long chain fatty acids (LCFA), and monounsaturated fatty acids (MUFA) were significantly greater in the R-cluster ($P < 0.01$), while several odd and branched chain fatty acids (OBCFA, including C14:0iso, C15:0iso, C15:0anteiso, C15:0, and C16:0iso), saturated fatty acids (SFA), medium chain fatty acids (MCFA), and ratio of SFA to UFA were in lower in the R-cluster compared with the P-cluster ($P < 0.05$).

3.6. Association between rumen metabolites and bacterial clusters

Because the significant differences in the rumen metabolites, we further performed correlation analysis on the bacterial genus (relative abundance > 0.1%) and the metabolites that differed between the R-cluster and P-cluster. In the P-cluster, we found that the *Oscillospiraceae NK4A214 group* and *Lachnospiraceae ND3007 group* had positive correlations with UFA and MUFA, while OBCFA and SFA correlated with them negatively ($P < 0.05$, Fig. 5A). Moreover, the *Oscillospiraceae NK4A214 group* had positive correlations with several amino acids, including total BCAA and TEAA ($P < 0.05$, Fig. 5A). Three genera including *Candidatus*

Table 5
The relative abundance of rumen microbiota in P-cluster and R-cluster at the genus level (%).

Taxa name	P-cluster	R-cluster	*P*-value
Prevotella	21.46 ± 1.79	6.24 ± 0.48	<0.001
norank_f_F082	11.83 ± 1.21	10.88 ± 0.79	0.707
Ruminococcus	5.06 ± 0.51	12.23 ± 0.91	<0.001
Rikenellaceae_RC9_gut_group	7.90 ± 0.68	7.79 ± 0.48	0.927
Oscillospiraceae_NK4A214_group	4.74 ± 0.39	7.48 ± 0.34	<0.001
Christensenellaceae_R-7_group	3.23 ± 0.34	7.14 ± 0.38	<0.001
norank_f_Muribaculaceae	4.92 ± 0.71	4.37 ± 0.47	0.762
Candidatus_Saccharimonas	2.50 ± 0.35	3.83 ± 0.36	0.003
norank_f_norank_o_Clostridia_UCG-014	2.72 ± 0.40	2.31 ± 0.38	0.191
unclassified_f_Selenomonadaceae	1.90 ± 0.26	2.98 ± 0.38	0.058
Selenomonas	2.61 ± 0.45	2.19 ± 0.31	0.521
unclassified_c_Clostridia	1.41 ± 0.15	2.88 ± 0.29	<0.001
unclassified_f_Lachnospiraceae	1.38 ± 0.11	2.61 ± 0.23	<0.001
Prevotellaceae_UCG-003	2.35 ± 0.20	1.42 ± 0.12	<0.001
Succiniclasticum	2.98 ± 0.50	0.55 ± 0.09	<0.001

The data are expressed as the means ± SE.

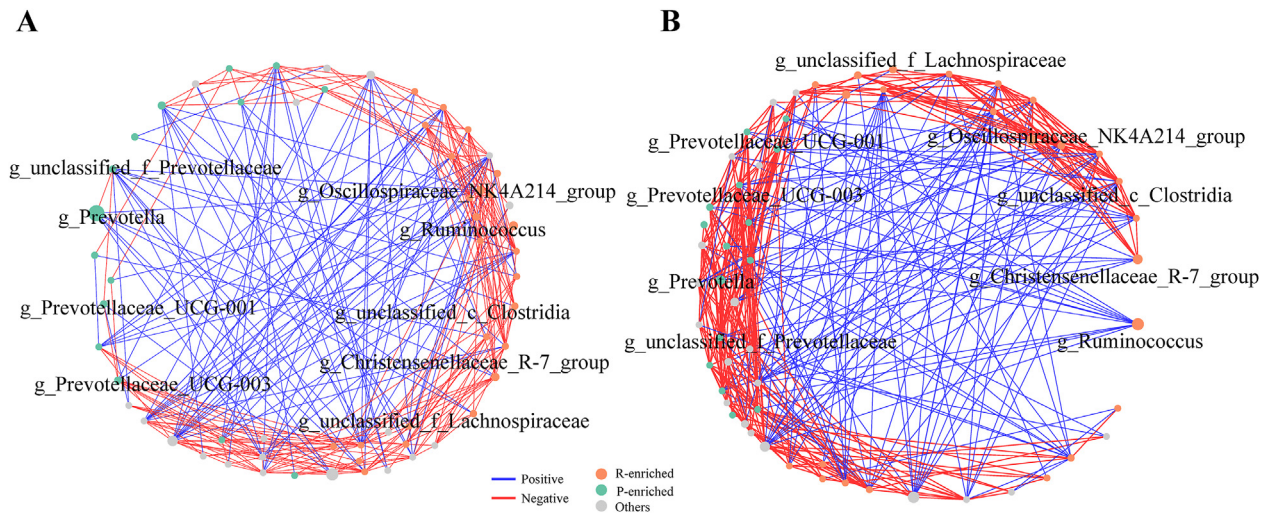
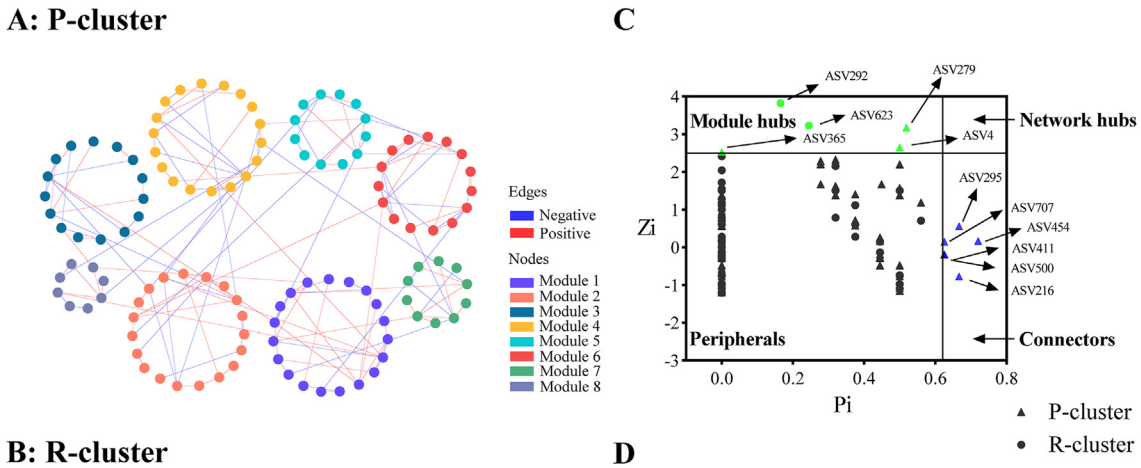


Fig. 3. Co-occurrence network of the top 60 rumen microbial genera (relative abundance >0.1%, detected in >50% of all samples) in young dairy goats with different bacterial clusters. (A) Co-occurrence network of the top 60 rumen microbial genera in the P-cluster. (B) Co-occurrence network of the top 60 rumen microbial genera in the R-cluster. Red lines represent positive correlations and blue lines represent negative correlations. The colors of nodes indicate the clusters in which the genera were enriched.



D

P-cluster		
Module hubs	ASV4	Ruminococcus
Module hubs	ASV279	Rikenellaceae_RC9_gut_group
Module hubs	ASV365	norank_f_F082
Connectors	ASV295	Lachnospiraceae_NK3A20_group
Connectors	ASV500	Ruminococcus
Connectors	ASV707	Ruminococcus
Connectors	ASV216	Selenomonas
Connectors	ASV411	Rikenellaceae_RC9_gut_group
Connectors	ASV454	Prevotellaceae_UCG-003
R-cluster		
Module hubs	ASV292	norank_f_UCG-010
Module hubs	ASV623	Selenomonas

Fig. 4. Module analysis of co-occurrence network based on amplicon sequence variants (ASV) (detected in >50% of all samples) in young goats with different bacterial clusters. (A and B) Visualization of microbial co-occurrence networks and their module distribution using the fast greedy modularity optimization method in the P-cluster and R-cluster. Red lines mean positive correlations, and blue lines mean negative correlations. The colors of nodes indicate the module to which the ASV belongs. (C) Distribution of bacterial ASV based on their network roles. Nodes in the network were classified as peripherals, modular hubs, or connectors based on Zi and Pi indices. Zi: within-module connectivity; Pi: among-module connectivity. (D) Keystone ASV in P-cluster and R-cluster networks.

Table 6
Volatile fatty acid (VFA) concentrations in rumen fluid of P-cluster and R-cluster.

Item	P-cluster	R-cluster	*P*-value
Absolute concentration, mmol/L			
Acetate	62.31 ± 1.85	62.91 ± 1.34	0.557
Propionate	18.05 ± 1.33	20.69 ± 0.98	0.033
Isobutyrate	0.85 ± 0.04	0.86 ± 0.02	0.871
Butyrate	9.27 ± 0.44	11.14 ± 0.37	0.002
Isovalerate	1.48 ± 0.10	1.65 ± 0.05	0.032
Valerate	0.96 ± 0.05	1.00 ± 0.03	0.326
Total VFA	92.92 ± 2.54	98.25 ± 2.21	0.059
Proportion, %			
Acetate	67.26 ± 1.18	64.3 ± 0.57	0.002
Propionate	19.07 ± 1.22	20.71 ± 0.68	0.011
Isobutyrate	0.93 ± 0.05	0.89 ± 0.02	0.569
Butyrate	10.09 ± 0.45	11.39 ± 0.3	0.014
Isovalerate	1.62 ± 0.11	1.70 ± 0.05	0.308
Valerate	1.03 ± 0.04	1.02 ± 0.02	0.796
Acetate to propionate ratio	4.08 ± 0.26	3.33 ± 0.12	0.004

The data are expressed as the means ± SE.

Table 7
Free amino acid concentrations in rumen fluid of P-cluster and R-cluster.

Item	P-cluster	R-cluster	*P*-value
Aspartate	419.94 ± 31.33	692.56 ± 31.50	<0.001
Glutamate	308.5 ± 30.08	589.04 ± 42.78	<0.001
Serine	237.22 ± 21.33	487.53 ± 30.14	<0.001
Histidine	88.38 ± 8.09	170.17 ± 14.15	<0.001
Glycine	290.75 ± 30.99	618.14 ± 41.90	<0.001
Threonine	157.42 ± 21.56	319.74 ± 21.35	<0.001
Arginine	54.22 ± 9.95	62.97 ± 4.89	0.013
Alanine	616.97 ± 61.10	1180.86 ± 77.02	<0.001
Tyrosine	159.44 ± 12.58	315.17 ± 17.46	<0.001
Cystine	46.07 ± 2.77	96.36 ± 6.46	<0.001
Valine	273.21 ± 25.81	510.21 ± 27.94	<0.001
Methionine	137.62 ± 12.86	288.92 ± 21.08	<0.001
Tryptophan	35.5 ± 3.84	56.83 ± 4.47	<0.001
Phenylalanine	130.84 ± 9.82	236.07 ± 12.83	<0.001
Isoleucine	243.3 ± 23.74	492.48 ± 30.94	<0.001
Leucine	295.01 ± 24.72	538.74 ± 28.99	<0.001
Lysine	608.65 ± 50.47	1221.3 ± 71.82	<0.001
Proline	105.23 ± 12.32	194.89 ± 21.66	<0.001
BCAA	811.51 ± 73.47	1541.44 ± 86.38	<0.001
TEAA	2024.15 ± 169.20	3897.42 ± 210.40	<0.001

BCAA = branched chain amino acids; TEAA = total essential amino acids.
The data are expressed as the means ± SE.

Table 8
Free fatty acid concentrations in rumen fluid of P-cluster and R-cluster.

Item	P-cluster	R-cluster	*P*-value
C14:0iso	1.58 ± 0.90	0.96 ± 0.49	<0.001
C15:0iso	2.82 ± 1.01	2.29 ± 0.74	0.009
C15:0anteiso	6.01 ± 1.52	5.03 ± 1.41	0.005
C15:0	2.81 ± 0.76	2.26 ± 0.67	0.001
C16:0iso	2.94 ± 1.23	2.35 ± 1.05	0.022
C16:0	40.34 ± 2.53	42.18 ± 3.10	0.004
C18:1t11	2.80 ± 1.10	3.38 ± 1.18	0.022
C18:1c9	10.69 ± 2.41	13.13 ± 2.72	<0.001
OBCFA	20.38 ± 4.15	16.89 ± 3.84	0.007
UFA	24.80 ± 3.77	27.38 ± 3.68	0.010
SFA	75.20 ± 3.77	72.62 ± 3.68	0.010
MUFA	17.86 ± 2.63	20.93 ± 2.81	<0.001
MCFA	64.19 ± 4.27	61.97 ± 4.92	0.039
LCFA	35.16 ± 4.3	37.44 ± 4.58	0.038
SFA:UFA ratio	3.13 ± 0.67	2.72 ± 0.51	0.010

OBCFA = odd and branched chain fatty acids; UFA = unsaturated fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; MCFA = medium chain fatty acids; LCFA = long chain fatty acids.
The data are expressed as the means ± SE.

Saccharimonas, *Ruminococcus gauvreauii group* and *Streptococcus* showed positive correlations with the concentration and molar percentage of propionate, but they correlated with the molar percentage of acetate and acetate to propionate ratio negatively ($P < 0.05$, Fig. 5A). Unlike these R-cluster enriched genera, we found that some P-cluster enriched genera like *Prevotella* correlated with OBCFA positively and several amino acids negatively ($P < 0.05$, Fig. 5A).

In the R-cluster, the *Lachnospiraceae ND3007 group* had negative correlations with several OBCFA and SFA, but had positive correlations with UFA and almost all amino acids ($P < 0.05$, Fig. 5B). On the contrary, some P-cluster enriched genera like *Succiniclasticum* and *Norank Lachnospiraceae* had positive correlations with several OBCFA and SFA, but had negative correlations with UFA and almost all amino acids ($P < 0.05$, Fig. 5B).

3.7. Relationship between microbial modules and rumen metabolites

The ADG and rumen metabolite data were submitted to Module-EigenGene analyses to calculate their correlations with the microbial modules. In the P-cluster, modules 1 and 2 were positively correlated with ADG and the molar percentage of propionate, but negatively correlated with the molar percentage of acetate and acetate to propionate ratio ($P < 0.05$, Fig. 6A). Many ASV of modules 1 and 2 were assigned to R-cluster enriched genera, such as *Ruminococcus*, *Christensenellaceae R-7 group*, and *Oscillospiraceae NK4A214 group* (Table S5).

In the R-cluster, modules 3 and 4 were positively correlated with the concentration and molar percentage of propionate, but negatively correlated with the molar percentage of acetate and acetate to propionate ratio. Conversely, module 2 showed the opposite result compared with modules 3 and 4. In addition, module 2 negatively correlated with almost all amino acids, but positively correlated with OBCFA. Meanwhile module 5 positively correlated with amino acids, but negatively correlated with OBCFA ($P < 0.05$, Fig. 6B). It was notable that modules 3, 4, and 5 contained many ASV that belonged to R-cluster enriched genera, like *Ruminococcus*, *Oscillospiraceae NK4A214 group*, *Christensenellaceae R-7 group*, whereas module 2 was dominated by ASV belonging to *Prevotella* and *Rikenellaceae RC9 gut group* (Table S6).

4. Discussion

Enterotypes are the strongest differentiator for gut microbial community structures and provide an attractive framework for understanding microbial variation in host metabolism and health (Arumugam et al., 2011; Costea et al., 2018; Hills et al., 2019). However, to our knowledge, rumen bacterial clustering (enterotype) has never been reported in the rumen of ruminant animals. In the present study, the rumen microbiome of 99 young goats was able to be identified as two different clusters. The respective bacterial clusters were characterized by different community structures, microbial diversity, and microbial interactions. In addition, rumen bacterial clusters were associated with the rumen metabolites and growth performance of young goats.

Previous research has found that the patterns of enterotypes vary across species and within the same species (Arumugam et al., 2011; Guo et al., 2021; Tröscher-Mußotter et al., 2021; Yuan et al., 2020). For example, a total of three enterotypes were identified in humans: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2), and *Ruminococcus* (enterotype 3) (Arumugam et al., 2011). Some studies suggested that the gut microbial composition of commercial hybrid

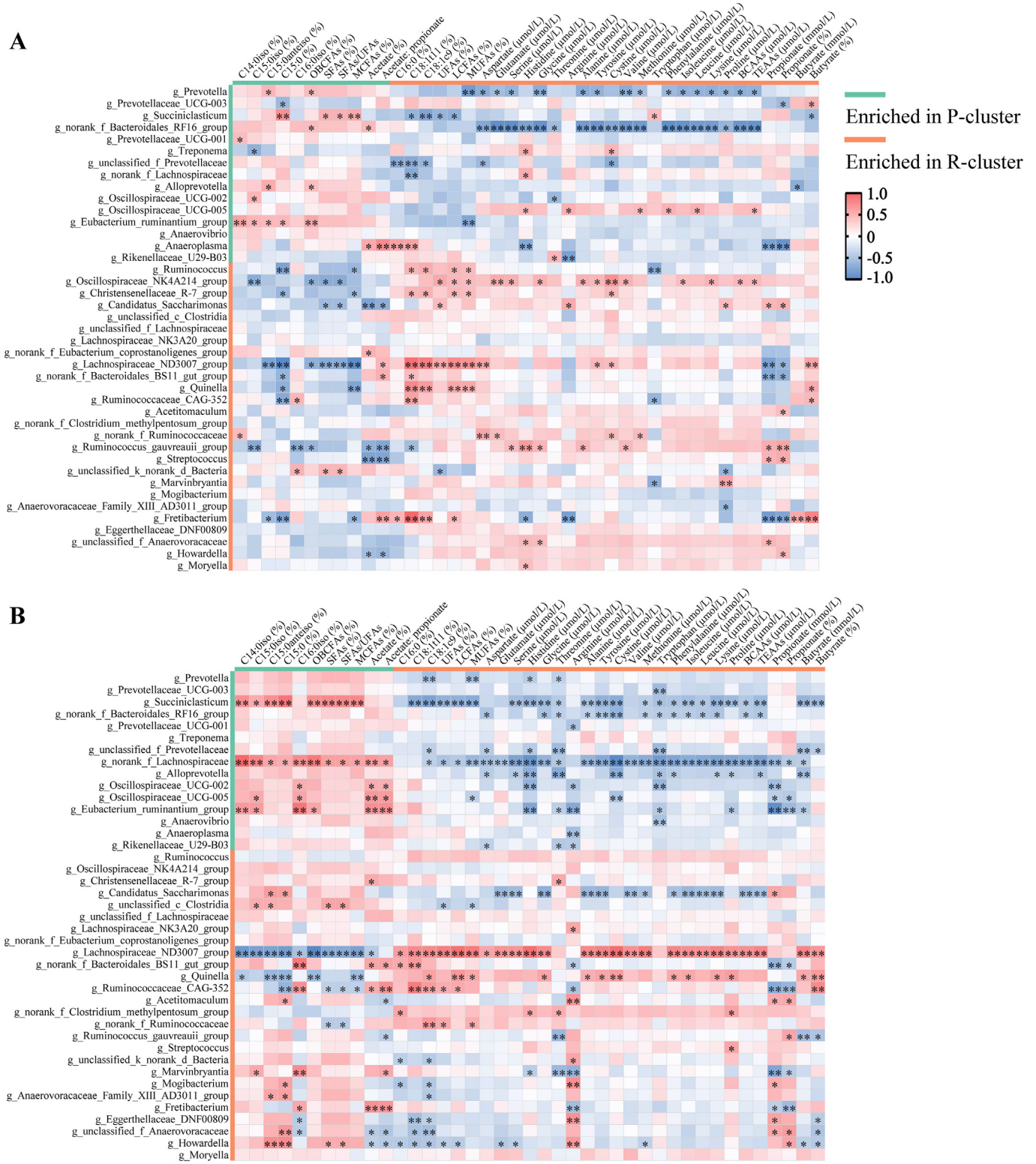


Fig. 5. Association analysis between differential genera (relative abundance >0.1%) and differential rumen metabolites in P-cluster (A) and R-cluster (B). Spearman's correlation, $^{*}P < 0.05$, $^{**}P < 0.01$.

pigs is structured in two enterotypes, while one native Chinese breed, called Jinhua pigs, can be clustered into three enterotypes (Ramayo-Caldas et al., 2016; Xu et al., 2021). All of these suggest that the enterotypes could be influenced by host genetic factors, and other nongenetic factors, such as diet, life style, and environmental stress.

In our study, the goats in the P-cluster had higher relative abundances of several Prevotellaceae members, but their ADG was

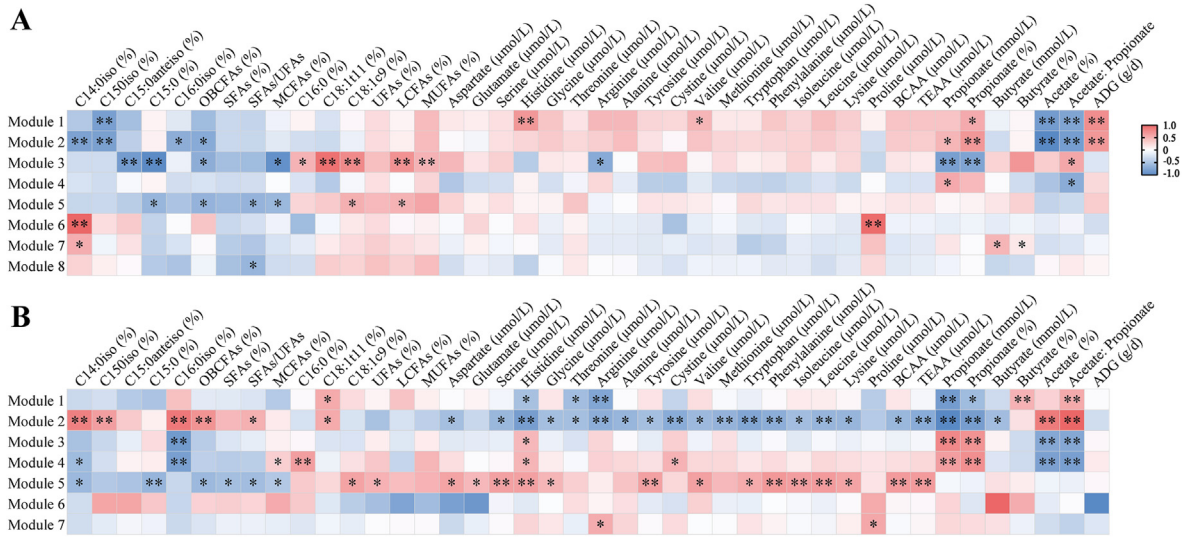


Fig. 6. Association analysis between microbial modules and rumen metabolites in young goats with different bacterial clusters. (A) Heatmap showing association between microbial modules and differential rumen metabolites in the P-cluster. (B) Heatmap showing association between microbial modules and differential rumen metabolites in R-cluster. Pearson's correlation, $*P < 0.05$, $**P < 0.01$.

lower. This was similar to an obese mice model fed with a high-fat high-sucrose diet, in which Firmicutes/*Ruminococcus* enterotype was changed into a *Prevotella*/Akkermansiaceae enterotype when the mice became thinner (Rodríguez-Daza et al., 2020). Moreover, some other researchers stratified enterotypes and found that *Prevotella* contributes to weight loss in humans (Christensen et al., 2019; Hjorth et al., 2020; Song et al., 2020). Ruminal *Prevotella* generally possesses extensive repertoires of polysaccharide utilization loci and carbohydrate active enzymes targeting various plant polysaccharides (Accetto and Avguštin, 2019; Emerson and Weimer, 2017). Some researchers suggested that the ruminal *Prevotella* was beneficial to raise acetate production and reduce animal feed efficiency (Anderson and Fernando, 2021; Bandarupalli and St-Pierre, 2020; Carberry et al., 2012; Dai et al., 2021). In our study, we also found the molar percentage of acetate and the ratio of acetate to propionate were higher in the P-cluster. In addition, we found that these Prevotellaceae members may have great impacts on rumen lipid metabolism. The relative proportions of OBCFA and SFA in rumen fluid were lower in the P-cluster compared to the R-cluster. Some previous studies have proven that variations in those lipids can reflect the changes in the biohydrogenation process and rumen fermentation patterns, and OBCFA were related to the relative proportions of cellulolytic and amylolytic bacteria in the rumen (Vlaeminck et al., 2006; Xin et al., 2021; Zhang et al., 2019).

We found that *Ruminococcus* and other Firmicutes, such as *Oscillospiraceae NK4A214 group*, *Christensenellaceae R-7 group*, *unclassified Clostridia*, and *unclassified Lachnospiraceae*, were enriched in the R-cluster. Those R-cluster enriched genera belong taxonomically to Clostridia and have been reported to produce propionate and butyrate (Low et al., 2022; Reichardt et al., 2014; Sun et al., 2019; Waters and Ley, 2019), which is consistent with our results that R-cluster goats have higher ruminal propionate and butyrate in both concentration and proportion. In ruminants, up to 70% of energy is sourced from VFA (Baldwin and Connor, 2017). In contrast to hydrogen production in the process of acetate fermentation, the production of propionate and butyrate during rumen fermentation is accompanied by hydrogen sequestration, which is considered to be energy efficient (Auffret et al., 2020; Shabat et al., 2016; van Lingen et al., 2016). What's more, we observed that some R-cluster enriched genera had positive correlations with ruminal AA, including several EAA and BCAA, which have an important influence on animal health, growth, and production performance (Cao et al., 2021; Nichols et al., 2022; Webb et al., 2020). For example, the *Lachnospiraceae ND3007 group* was found to have a positive correlation with methionine and lysine, which were most frequently identified as the two most limiting AA in ruminants (Richardson and Hatfield, 1978; Schwab and Broderick, 2017).

Our results also highlighted the co-occurrence and co-exclusion of the differential bacteria in the two bacterial clusters, which is consistent with previous studies carried out in humans and pigs, especially the co-exclusion between *Prevotella* and *Ruminococcus* (Arumugam et al., 2011; Ramayo-Caldas et al., 2016). Using RMT-based network analysis, we also found that the ASV belonging to *Prevotella* and *Ruminococcus* were the keystone bacteria in some important microbial modules. Although the most microbial interactions among other differential bacteria were less of a focus in previous research, they may play indelible roles in shaping the formation of the two rumen bacterial clusters. For example, the *Oscillospiraceae NK4A214 group*, *Christensenellaceae R-7 group*, *unclassified Clostridia*, and *unclassified Lachnospiraceae*, which were the predominant R-cluster enriched bacteria, were found to have multiple negative correlations with several P-cluster enriched bacteria. Furthermore, different microbe-metabolite interaction patterns were found between these R-cluster enriched bacteria and P-cluster enriched bacteria. There are various ecological relationships that exist in microbial communities, ranging from mutualism to competition, while two taxa with similar niches tend to exclude each other for limited food and living space (Faust and Raes, 2012). Faust et al. (2012) also suggested that the patterns of microbial co-occurrence and exclusion are determined by both their evolutionary relatedness and functional similarity. In particular, taxa with close evolutionary relationships tended to positively associate with each other, while distantly related taxa with functional similarities tended to compete (Faust et al., 2012). Future studies

investigating the active microbial functions and taxa using culture-based technologies are required to confirm the function of those cluster related species on a deeper mechanistic level.

5. Conclusion

In the present study, we applied the enterotype concept to the rumen microbiome and found that the rumen microbiome in young goats can be classified into two clusters, which were apparently associated with the differences in rumen fermentation and the growth rate of goats. Some specific bacterial taxa and their associations with other bacteria in different bacterial clusters may have an influence on rumen fermentation, and thus drive the formation of host phenotype. The presence of rumen microbiome clustering and its association with rumen fermentation and host productivity could shed a light on modulating the rumen microbiome in early life to improve the growth performance of ruminant animals.

Author contributions

Dangdang Wang, **Yangchun Cao**, and **Junhu Yao** conceived and designed the experiments; **Dangdang Wang**, **Guangfu Tang**, **Yannan Wang**, **Junjian Yu**, **Luyu Chen**, **Jie Chen**, **Yanbo Wu**, and **Yuanjie Zhang** primarily performed the experiments; **Dangdang Wang** and **Guangfu Tang** analyzed the data; **Junhu Yao** and **Yangchun Cao** contributed reagents/materials/analysis tools; **Dangdang Wang**, **Guangfu Tang**, **Yangchun Cao**, and **Junhu Yao** wrote and revised the manuscript. **Junhu Yao** had primary responsibility for the final content.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Data availability statement

The data sets supporting the results of this article are included within the article and its additional supplemental material. The raw sequence data were submitted to NCBI Sequence Read Archive (SRA) with accession number PRJNA774483.

Appendix Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2023.05.013.

References

Accetto T, Avguštin G. The diverse and extensive plant polysaccharide degradative apparatuses of the rumen and hindgut prevotella species: a factor in their ubiquity? Syst Appl Microbiol 2019;42:107–16.
Anderson CL, Fernando SC. Insights into rumen microbial biosynthetic gene cluster diversity through genome-resolved metagenomics. Commun Biol 2021;4:818.
Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature 2011;473:174–80.
Auffret MD, Stewart RD, Dewhurst RJ, Duthie C-A, Watson M, Roehe R. Identification of microbial genetic capacities and potential mechanisms within the rumen microbiome explaining differences in beef cattle feed efficiency. Front Microbiol 2020;11:1229.
Baldwin RL, Connor EE. Rumen function and development. Vet Clin North Am Food Anim Pract 2017;33:427–39.
Bandarupalli VVK, St-Pierre B. Identification of a candidate starch utilizing strain of from bovine rumen. Microorganisms 2020;8.
Cao Y, Yao J, Sun X, Liu S, Martin GB. Amino acids in the nutrition and production of sheep and goats. Adv Exp Med Biol 2021;1285:63–79.
Carberry CA, Kenny DA, Han S, Mccabe MS, Waters SM. Effect of phenotypic residual feed intake and dietary forage content on the rumen microbial community of beef cattle. Appl Environ Microbiol 2012;78:4949–58.
Chen J, Lei XJ, Wang L, Zhang YL, Wang DD, Zhao LC, et al. Effects of rumen-protected leucine on production performance and starch digestion in the small intestine of lactating goats. Anim Feed Sci Technol 2022;287:115270.
Christensen L, Roager HM, Astrup A, Hjorth MF. Microbial enterotypes in personalized nutrition and obesity management. Am J Clin Nutr 2018;108:645–51.
Christensen L, Vuholm S, Roager HM, Nielsen DS, Krych L, Kristensen M, et al. Prevotella abundance predicts weight loss success in healthy, overweight adults consuming a whole-grain diet ad libitum: a post hoc analysis of a 6-wk randomized controlled trial. J Nutr 2019;149:2174–81.
Clark S, Mora García MB. A 100-year review: advances in goat milk research. J Dairy Sci 2017;100:10026–44.
Costea PI, Hildebrand F, Arumugam M, Bäckhed F, Blaser MJ, Bushman FD, et al. Enterotypes in the landscape of gut microbial community composition. Nat Microbiol 2018;3.
Dai Q, Ma J, Cao G, Hu R, Zhu Y, Li G, et al. Comparative study of growth performance, nutrient digestibility, and ruminal and fecal bacterial community between yaks and cattle-yaks raised by stall-feeding. Amb Express 2021;11:98.
Deng Y, Jiang Y-H, Yang Y, He Z, Luo F, Zhou J. Molecular ecological network analyses. BMC Bioinf 2012;13:113.
Dinsmoor AM, Aguilar-Lopez M, Khan NA, Donovan SM. A systematic review of dietary influences on fecal microbiota composition and function among healthy humans 1-20 years of age. Adv Nutr 2021;12:1734–50.
Emerson EL, Weimer PJ. Fermentation of model hemicelluloses by prevotella strains and butyrivibrio fibrisolvens in pure culture and in ruminal enrichment cultures. Appl Microbiol Biotechnol 2017;101:4269–78.
Faust K, Raes J. Microbial interactions: from networks to models. Nat Rev Microbiol 2012;10:538–50.
Faust K, Sathirapongsasuti JF, Izard J, Segata N, Gevers D, Raes J, et al. Microbial co-occurrence relationships in the human microbiome. PLoS Comput Biol 2012;8:e1002606.
Guo N, Wu Q, Shi F, Niu J, Zhang T, Degen AA, et al. Seasonal dynamics of diet-gut microbiota interaction in adaptation of yaks to life at high altitude. NPJ Biofilms and Microbiomes 2021;7:38.
Hills RD, Pontefract BA, Mishcon HR, Black CA, Sutton SC, Theberge CR. Gut microbiome: profound implications for diet and disease. Nutrients 2019;11.
Hjorth MF, Christensen L, Larsen TM, Roager HM, Krych L, Kot W, et al. Pretreatment prevotella-to-bacteroides ratio and salivary amylase gene copy number as prognostic markers for dietary weight loss. Am J Clin Nutr 2020;111:1079–86.
Jiang Q, Lin L, Xie F, Jin W, Zhu W, Wang M, et al. Metagenomic insights into the microbe-mediated b and k vitamin biosynthesis in the gastrointestinal microbiome of ruminants. Microbiome 2022;10:109.
Li F, Li Z, Li S, Ferguson JD, Cao Y, Yao J, et al. Effect of dietary physically effective fiber on ruminal fermentation and the fatty acid profile of milk in dairy goats. J Dairy Sci 2014;97:2281–90.
Li J, Zhao F, Wang Y, Chen J, Tao J, Tian G, et al. Gut microbiota dysbiosis contributes to the development of hypertension. Microbiome 2017;5:14.
Liu K, Zhang Y, Yu Z, Xu Q, Zheng N, Zhao S, et al. Ruminal microbiota-host interaction and its effect on nutrient metabolism. Anim Nutr 2021;7:49–55.
Low L, Suleiman K, Shamdas M, Bassilious K, Poonit N, Rossiter AE, et al. Gut dysbiosis in ocular mucous membrane pemphigoid. Front Cell Infect Microbiol 2022;12:780354.
Molinaro A, Bel Lassen P, Henricsson M, Wu H, Adriouch S, Belda E, et al. Imidazole propionate is increased in diabetes and associated with dietary patterns and altered microbial ecology. Nat Commun 2020;11:5881.
Morand-Fehr P, Boutonnet J, Devendra C, Dubeuf J, Haenlein G, Holst P, et al. Strategy for goat farming in the 21st century. Small Rumin Res 2004;51:175–83.
Nichols K, Dijkstra J, Breuer MJH, Lemosquet S, Gerrits WJJ, Bannink A. Essential amino acid profile of supplemental metabolizable protein affects mammary gland metabolism and whole-body glucose kinetics in dairy cattle. J Dairy Sci 2022;105:7354–72.
Ramayo-Caldas Y, Mach N, Lepage P, Levenez F, Denis C, Lemonnier G, et al. Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. ISME J 2016;10:2973–7.
Reichardt N, Duncan SH, Young P, Belenguer A, Mcwilliam Leitch C, Scott KP, et al. Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. ISME J 2014;8:1323–35.
Richardson CR, Hatfield EE. The limiting amino acids in growing cattle. J Anim Sci 1978;46:740–5.
Rodríguez-Daza M-C, Roquim M, Dudonné S, Pilon G, Levy E, Marette A, et al. Berry polyphenols and fibers modulate distinct microbial metabolic functions and gut microbiota enterotype-like clustering in obese mice. Front Microbiol 2020;11:2032.

Schwab CG, Broderick GA. A 100-year review: protein and amino acid nutrition in dairy cows. J Dairy Sci 2017;100:10094–112.
Shabat SKB, Sasson G, Doron-Faigenboim A, Durman T, Yaacoby S, Berg Miller ME, et al. Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants. ISME J 2016;10:2958–72.
Song E-J, Han K, Lim T-J, Lim S, Chung M-J, Nam MH, et al. Effect of probiotics on obesity-related markers per enterotype: a double-blind, placebo-controlled, randomized clinical trial. EPMA J 2020;11:31–51.
Sun G, Zhang H, Wei Q, Zhao C, Yang X, Wu X, et al. Comparative analyses of fecal microbiota in european mouflon (*Ovis orientalis musimon*) and blue sheep (*Pseudois nayaur*) living at low or high altitudes. Front Microbiol 2019;10:1735.
Sun X, Gibbs S. Diurnal variation in fatty acid profiles in rumen digesta from dairy cows grazing high-quality pasture. Anim Feed Sci Technol 2012;177:152–60.
Tröscher-Mußotter J, Saenz JS, Grindler S, Meyer J, Kononov SU, Mezger B, et al. Microbiome clusters disclose physiologic variances in dairy cows challenged by calving and lipopolysaccharides. mSystems 2021;6:e0085621.
Van Lingen HJ, Plugge CM, Fadel JG, Kebreab E, Bannink A, Dijkstra J. Thermodynamic driving force of hydrogen on rumen microbial metabolism: a theoretical investigation. PLoS One 2016;11:e0161362.
Vlaeminck B, Fievez V, Demeyer D, Dewhurst RJ. Effect of forage:Concentrate ratio on fatty acid composition of rumen bacteria isolated from ruminal and duodenal digesta. J Dairy Sci 2006;89:2668–78.
Wallace RJ, Sasson G, Garnsworthy PC, Tapio I, Gregson E, Bani P, et al. A heritable subset of the core rumen microbiome dictates dairy cow productivity and emissions. Sci Adv 2019;5:eaav8391.
Waters JL, Ley RE. The human gut bacteria christensenellaceae are widespread, heritable, and associated with health. BMC Biol 2019;17:83.
Webb LA, Sadri H, Schuh K, Egert S, Stehle P, Meyer I, et al. Branched-chain amino acids: abundance of their transporters and metabolizing enzymes in adipose tissue, skeletal muscle, and liver of dairy cows at high or normal body condition. J Dairy Sci 2020;103:2847–63.
Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y-Y, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011;334:105–8.
Xin H, Khan NA, Liu X, Jiang X, Sun F, Zhang S, et al. Profiles of odd- and branched-chain fatty acids and their correlations with rumen fermentation parameters, microbial protein synthesis, and bacterial populations based on pure carbohydrate incubation. Front Nutr 2021;8:733352.
Xu E, Yang H, Ren M, Wang Y, Xiao M, Tang Q, et al. Identification of enterotype and its effects on intestinal butyrate production in pigs. Animals 2021:11.
Xue M-Y, Sun H-Z, Wu X-H, Liu J-X, Guan LL. Multi-omics reveals that the rumen microbiome and its metabolome together with the host metabolome contribute to individualized dairy cow performance. Microbiome 2020;8:64.
Yuan Z, Yan W, Wen C, Zheng J, Yang N, Sun C. Enterotype identification and its influence on regulating the duodenum metabolism in chickens. Poultry Sci 2020;99:1515–27.
Zhang XM, Medrano RF, Wang M, Beauchemin KA, Ma ZY, Wang R, et al. Corn oil supplementation enhances hydrogen use for biohydrogenation, inhibits methanogenesis, and alters fermentation pathways and the microbial community in the rumen of goats. J Anim Sci 2019;97:4999–5008.
Zhou J, Deng Y, Luo F, He Z, Tu Q, Zhi X. Functional molecular ecological networks. mBio 2010;1.