Dynamic alterations in rumen bacterial community and metabolome characteristics in response to feed nutrient levels of cashmere goats

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Research

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Abstract

Background: Dietary energy and protein play important roles in rumen fermentation. However, the comprehensive impacts of dietary energy and protein on rumen bacterial composition and ruminal metabolites were largely unknown. Therefore, the objective of the current study was to investigate the changes in rumen bacterial community and metabolites in response to the diets with simultaneous changes of dietary energy and protein levels in Shaanbei white cashmere goats (SWCG).

Methods: A total of 12 ruminal samples were collected from SWCG, which were divided into two groups, including high-energy and high-protein (Group H; crude protein, CP: 9.37% in dry matter; metabolic energy, ME: 9.24 MJ/kg) or control (Group C; CP: 8.73%; ME:8.60 MJ/kg) groups. The experiment lasted for 65 days, including 10 days for adaptation. 16S rRNA gene sequencing and quantitative real-time polymerase chain reaction (qRT-PCR) were performed to identify the rumen bacterial community. Metabolomics analysis was done to investigate the rumen metabolites and the related metabolic pathways in Groups C and H.

Results: The study observed 539 genera belonging to 30 phyla, which were distributed throughout the rumen samples. The high-energy and high-protein diets increased the relative abundance of phylum *Bacteroidetes* and genera *Prevotella*_1 and *Succiniclasticum*, while decreased the number of phylum *Proteobacteria* ($p<0.05$). Among the 24 differential metabolites (VIP>1.0, $p<0.05$) detected in this study, the dominant differential metabolites were amino acids, peptides and analogs. Tyrosine metabolism played an important role among the 9 main metabolic pathways. Correlation analysis revealed that *Prevotella*_1 showed strong positive correlation with 5-methoxyindole-3-acetic acid ($r=0.601$, $p<0.05$) and catechol ($r=0.608$, $p<0.05$). *Succiniclasticum* was positively correlated with 2-ketoadipate ($r=0.741$, $p<0.01$).

Conclusions: Our findings revealed that the diets with high energy and protein in Group H significantly altered the composition of ruminal bacteria and metabolites, which could help to improve the dietary energy and protein use efficiency in goats.

Background

Shaanbei white cashmere goat (SWCG) is a local breed in the northern Shaanxi province of China and the total population of SWCG exceeds 10 million. SWCG is well-known for cashmere wool and meat, which are the most important economic sources of the local farmers [1, 2]. Traditional grazing management is mainly dependent on natural pastures, which are limited in the extremely harsh winter. Hence, nutritional management, especially the choice of dietary nutrient levels, is important to promote the growth of goats [3].

The rumen is a complex microbial ecosystem in ruminants. It can ferment feedstuffs to volatile fatty acids (VFAs), microbial proteins and vitamins, which play important roles in animal health and production [4–6]. Among the microbiota, bacteria are the most abundant, diverse and metabolically active species in

the rumen [7, 8]. Bacterial community in the rumen are linked to various factors, such as animal diet, breed, age health and geographic region [9, 10]. Diet is the major determinant of the microbial composition in the rumen [11].

The functions of the rumen microbiota make ruminants highly adaptable to various diets [12]. The energy and protein levels in the diets are the most restrictive factors for ruminal microbial growth [13, 14]. Dietary protein is utilized to synthesize microbial protein (MCP) for host utilization [15–17]. However, protein overfeeding increases the excreted nitrogen from urine and feces, which causes environmental pollution [18] and economic losses [19]. Previous studies have reported that dietary energy can promote protein to synthesize MCP [20–22]. The effective way to improve average daily weight gain (ADG) and production performance in cattle is increasing dietary energy levels under the same concentration of forage ratio [5].

Previous studies showed that the phenotypic traits of ruminants were affected by rumen microbiota, whose functions could be reflected by the ruminal metabolites [23–25]. However, most studies have only focused on the single change of dietary protein/energy and few reports studied the comprehensive effects of dietary energy and protein on the rumen bacterial composition and rumen metabolites [3, 16, 18, 22, 26, 27].

In our previous study, we detected that high levels of dietary energy (metabolic energy, ME: 9.24 MJ/kg) and protein (crude protein, CP: 9.37% in dry matter (DM)) could significantly enhance the ADG, dressing percentage and eye muscle area of SWCG [28]. The primary objective of this study was to investigate the changes in the rumen bacterial diversity by 16S rRNA gene sequencing and qRT-PCR, and the metabolites and key metabolic pathways by Gas Chromatography Tandem Time-of-Flight Mass Spectrometry (GC-TOFMS)-based metabolomics by increasing dietary energy and protein. Furthermore, we explored the comprehensive relationships between ruminal bacterial communities and rumen metabolites to improve dietary energy and protein use efficiency in SWCG.

Methods

Animals and sampling

A total of 12 SWCG (aged 8 months, an average initial body weight of 24.5 ± 1.87 kg, six males and six females) were selected and fed at Diqingyuan farm (37.6 °N, 108.79 °E) located at Yulin, Shannxi Province, China. Based on their diet types, all goats were randomly allocated into two groups, including high-energy and high-protein (Group H; crude protein, CP: 9.37% in dry matter; metabolic energy, ME: 9.24 MJ/kg) and control (Group C; CP: 8.73%; ME:8.60 MJ/kg) groups (n = 6 per group). The experimental diets were formulated based on the Feeding Standard of Meat-Producing Sheep and Goats (NY/T816-2004, China). In addition, the ratio of dietary energy to protein and the ratio of dietary forage to concentrate in the two groups were not changed. (See Additional file 1: Table S1).

All animals were fed at 9:00 am and 4:00 pm twice daily. The experiment was terminated on the 65th day (November to January), including a 10-day of pre-feeding period. Rumen contents were collected after

slaughtering the goats. The samples were immediately frozen in liquid nitrogen and stored at -80 °C for further studies.

Dna Extraction, 16s Rrna Gene Amplicon And Sequencing

Bacterial genomic DNA was extracted from rumen fluid samples using a stool DNA kit (OMEGA Bio-Tek, Norcross, GA, USA). Total DNA from rumen content samples was used to construct 16S rRNA libraries, targeting the V3-V4 region of bacteria. PCR products were mixed with equimolar ratios and purified by Qiagen Gel Extraction Kit (Qiagen, Germany). Finally, Illumina HiSeq platform for Paired-End sequencing at the Novogene (Beijing, China) was used for sequencing.

Sequence Processing

The raw data was spliced and filtered to obtain the clean data by the QIIME 1.9.1 software package [29]. Operational taxonomic units (OTUs) were clustered with 97% similarity cut-off using UCLUST [30]. The indices of Alpha diversity were analyzed by MOTHUR (version v.1.30.2) [31]. Beta diversity was calculated by weighted UniFrac distance. Rarefaction curves, Venn diagram, bar and heatmap graphs were visualized by R software (version 3.1.2). Significant interactions between rumen bacteria were showed using Networkx. The raw reads obtained in the current study have been deposited in the NCBI Sequence Read Archive (SRA) database under accession number SRP258202.

Prediction Of Rumen Bacterial Function

The function of detected rumen bacteria was predicted via phylogenetic analysis of communities by reconstruction of unobserved states (PICRUSt2)[32].The predicted sequences were aligned to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [33].

Quantitative Real-time Polymerase Chain Reaction (qrt-pcr) Analysis

Changes in phylum *Bacteroidetes* and genus *Prevotella* between different treatments were verified by absolute qRT-PCR using iCycle thermos cycler (Bio-Read-CFX, CA, USA). The primers and annealing temperature were shown in Additional file 1: Table S2. The external standard curves were constructed by 10-fold serial dilution of plasmid DNA containing the cloned marker loci. All standard curves met the required efficient standards ($R^2 > 0.99$, 90%>E > 120%). The reaction mixture and conditions were according to our previous study [34].

Metabolomics Analysis By Gc-tofms

The rumen samples were used for metabolomics analysis. For each sample, 200 μL rumen fluid sample, 200 μL liquid methanol and 20 μL L-2-Chlorophenylalanine (CAS#: 103616-89-3, ≥ 98%) (1 mg/mL in H_2O) as an internal standard were sequentially added to the 1.5 mL Eppendorf (EP) tubes. The mixture was vortexed for 10 s and then centrifuged at 13000 rpm for 15 min at 4 °C. After centrifugation, 370 μL of the supernatant was transferred to a 2 mL GC/MS glass vial and dried in a vacuum concentrator without heating. After evaporation, 80 μL of methoxy amination hydrochloride (20 mg/mL in pyridine) was added to the sample and incubated for 30 min at 80 °C. Meanwhile, 100 μL of the N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) were added to the sample aliquots and the mixture was incubated at 70 °C for 1.5 h.

Derivatized samples were analyzed using the Agilent 7890B gas chromatograph system (Agilent, USA) coupled with the LECO Chroma TOF PEGASUS HT (LECO, USA) [35]. Injecting 1 μL aliquot of the analyte into splitless mode, helium was used as the carrier gas. The injection, transfer line and ion source temperatures were 280, 270 and 220 °C, respectively. The mass spectrometry data were performed in full-scan mode with 50–500 m/z at 20 scans/s after a solvent delay of 6.1 min.

The extraction of raw peaks, filtering and calibration of the baseline data, peak alignment, deconvolution analysis, peak identification and peak area integration were performed by Chroma TOF 4.3X software [36]. The content of each component was calculated by the peak area normalization method. Principal component analysis (PCA) and orthogonal correction partial least squares discriminant analysis (OPLS-DA) were conducted using SIMCA software (V14.1).

Statistical analysis

Statistical differences were performed by Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA). Data were analyzed using one-way analysis of variance (ANOVA). Significant differences between Groups C and H were calculated using Student's t-test. For the GC-TOFMS data, differential metabolites between two groups were identified combining variable importance in the projection (VIP) > 1 and $p < 0.05$. Significant correlations between rumen bacteria and metabolite variables were assessed by Spearman correlation analysis, if the correlation coefficients (r, in absolute values) were above 0.55. The statistical significance was set at $p < 0.05$.

Results

Diversity, richness and similarity of the ruminal bacterial communities

A total of 826,727 16S rRNA gene sequences were obtained from 12 different samples with 61,658 rarefied sequencing reads per sample. Group C exhibited the highest number of unique sequences (667 OTUs), followed by Group H (35 OTUs). Approximately 71% of the total OTUs (1704 OTUs) were shared among two groups (Fig. 1A). The rarefaction curves (Fig. 1B) reached the saturation plateau and the

indices of Good's coverage were above 0.99 (See Additional file 1: Table S3), indicating that the sequencing depth was reasonable. ACE (Fig. 1C) and Chao (Fig. 1D) indices were significantly decreased when the goats were fed with high energy and protein diets in Group H ($p < 0.05$), while Shannon and Simpson indices had no significant effects ($p > 0.05$) (See Additional file 1: Table S3).

ANOSIM showed significant differences in rumen bacterial community structures at phylum ($R = 0.315$, $p = 0.037$), genus ($R = 0.452$, $p = 0.009$) and OTU levels ($R = 0.426$, $p = 0.014$), suggesting that the statistical differences in the bacterial community between the groups (Table 1).

Table 1
Analysis of similarities (ANOSIM) for rumen microbial composition at the phylum, genus and OTU level.

Items	*R*			*p* value		
	Phylum	Genus	OTU	Phylum	Genus	OTU
Groups (H and C)	0.315	0.452	0.426	0.037	0.009	0.014

Composition And Differences Of Ruminal Bacterial Communities

A total of 30 phyla were detected by taxonomic analysis. The top 5 prominent phyla in Groups H and C were *Bacteroidetes* (abundances of 63.76% and 54.46%, respectively), *Firmicutes* (21.20% and 19.04%), *Proteobacteria* (8.40% and 19.13%), *Fibrobacteres* (2.76% and 2.29%) and *Kiritimatiellaeota* (1.24% and 1.90%), which are accounted for more than 96% (Fig. 2A, Additional file 1: Table S4). With the increase of energy and protein levels in diets, the abundance of *Bacteroidetes* increased significantly ($p < 0.05$), while the abundance of *Proteobacteria* significantly decreased ($p < 0.05$) (Fig. 2B, Additional file 1: Table S4).

When sequences were analyzed at a lower taxonomical level, more detailed information about rumen bacteria was found. A total of 539 bacterial genera were detected. Within Group C, the most abundant sequences were those related to *Prevotella*_1 (the abundance of 25.17%), norank_f__*Succinivibrionaceae* (10.35%), norank_f__*Bacteroidales*_RF16_group (5.33%), unclassified_f__*Prevotellaceae* (4.85%), norank_f__*F082* (4.31%) and *Succinivibrionaceae*_UCG-002 (3.84%). Within Group H, the dominant taxa were associated with *Prevotella*_1 (35.36%), unclassified_f__*Prevotellaceae* (4.53%) *Succinivibrionaceae*_UCG-002 (3.94%), norank_f__*Bacteroidales*_RF16_group (3.79%), norank_f__*F082* (3.64%) and *Rikenellaceae*_RC9_gut_group (3.36%) (Fig. 2C, Additional file 1: Table S5). In addition, the relative abundances of genera *Prevotella*_1 and *Succiniclasticum* were significantly increased when energy and protein levels in diets were increased ($p < 0.5$) (Fig. 2D, Additional file 1: Table S5).

Quantitative Real-time Pcr Analysis

According to 16S rRNA gene sequencing data, the differences in the number of *Bacteroidetes* (phylum level) and *Prevotella* (genus level) between Groups C and H were further verified by absolute qRT-PCR. As shown in Table 2, the number of *Prevotella* and *Bacteroidetes* in the rumen of Group H was significantly increased ($p < 0.05$) compared with Group C.

Table 2
Influence of different nutrient levels in the diets on the number of bacteria[a].

	Groups		SEM	*p* value
	C	H		
Bacteroidetes	6.71	7.60	0.359	0.004
Prevotella	6.12	6.88	0.266	0.006

[a] The number of bacteria was shown by the logarithm of the values for gene copies per 10 ng DNA

Functional Predictions Of Rumen Bacteria

The potential functions of the bacterial community in the rumen of SWCG were predicted by the PICRUSt2 based on 16S rRNA gene sequencing data. At KEGG level 1, metabolism-related pathways had the highest abundance (> 50%). Compared with Group C, the rumen bacteria of Group H were predicted to have significantly higher capability of influencing metabolism and genetic information processing and lower capability of influencing environmental information processing, cellular processes and human diseases ($p < 0.05$) (See Additional file 1: Table S6). At KEGG level 2, the highest relative abundance was carbohydrate metabolism. In addition, the abundances of genes belonged to carbohydrate metabolism, energy metabolism, nucleotide metabolism, glycan biosynthesis and metabolism, biosynthesis of other secondary metabolites, translation, and replication and repair were significantly higher in Group H than Group C. The abundances of genes involved in lipid metabolism, membrane transport and signal transduction were significantly higher in Group C compared with Group H (Fig. 3, See Additional file 1: Table S7).

Metabolic Pathways Of Differential Metabolites

In order to provide a comprehensive view of the differential metabolites between Groups C and H, pathway analysis was visualized in Fig. 5. The varied rumen microbial metabolites between Groups C and H were identified to be mainly involved in the 9 main metabolic pathways, including beta-alanine metabolism; tyrosine metabolism; pantothenate and CoA biosynthesis; sphingolipid metabolism; glutathione metabolism; glycerophospholipid metabolism; pyrimidine metabolism; tryptophan metabolism; and arginine and proline metabolism. These pathways are mainly involved in amino acids metabolism, lipid metabolism and nucleotide metabolism. Additionally, among these metabolic pathways, tyrosine metabolism has the largest impact.

Correlation Analysis Between Rumen Bacteria And Rumen Metabolites

Based on Spearman correlation analysis ($|r| > 0.55$ and $p < 0.05$), we constructed the correlation networks between the bacterial genera in Groups C and H, respectively. As shown in Additional file 2: Figure S2A and Figure S2B, 171 and 79 edges were observed in Group C and Group H, respectively, which indicated that the relationships between the bacterial genera in Group C were more complex than those in Group H. The comprehensive relationships between ruminal bacterial genera were observed in this study (See Additional file 3:Table S9). Among them, *Prevotella*_1 was positively correlated with *Succiniclasticum* ($r = 0.580$, $p < 0.05$) and *Ruminococcus*_2 ($r = 0.651$, $p < 0.05$). *Selenomonas*_1 was positively correlated with *Prevotellaceae*_UCG-004 ($r = 0.78$, $p < 0.01$)

We determined the relationships between the differential metabolites and the top 50 bacterial communities at the genus level (Fig. 6 and Additional file 4:Table S10). *Prevotella*_1 was positively correlated with 5-methoxyindole-3-acetic acid ($r = 0.601$, $p < 0.05$) and catechol ($r = 0.608$, $p < 0.05$), but negatively correlated with aconitic acid (r=-0.594, $p < 0.05$), 4-hydroxyphenylacetic acid (r= -0.643, $p < 0.05$) and phosphate (r= -0.720, $p < 0.01$). *Succiniclasticum* had strong positive correlation with 2-ketoadipate ($r = 0.741$, $p < 0.01$), while was negatively correlated with phosphate (r= -0.62, $p < 0.05$) and 2,8-dihydroxyquinoline (r= -0.65, $p < 0.05$). *Ruminococcus*_2 was strong positively correlated with uracil ($r = 0.578$, $p < 0.05$), catechol ($r = 0.613$, $p < 0.05$) and itaconic acid ($r = 0.578$, $p < 0.05$), while negatively correlated with 4-hydroxyphenylacetic acid (r=-0.75, $p < 0.01$). In addition, 5-oxoproline had high positive correlation with *Lachnospiraceae*_ND3007_group ($r = 0.608$, $p < 0.05$). Also, spermidine was positively correlated with *Selenomonas*_1 ($r = 0.678$, $p < 0.05$), *Ruminococcaceae*_NK4A214_group ($r = 0.629$, $p < 0.05$), *Lachnospiraceae*_NK3A20_group ($r = 0.722$, $p < 0.01$), *Prevotellaceae*_UCG-004 (r = 0.615, $p < 0.05$) and *Prevotellaceae*_NK3B31_group ($r = 0.615$, $p < 0.05$), while was negatively correlated with norank_*Gastranaerophilales*(r=-0.657, $p < 0.05$), norank_*Clostridiales*_vadinBB60_group (r=-0.601, $p < 0.05$), norank_WCHB1-41 (r=-0.706, $p < 0.05$) and *Ruminococcaceae*_UCG-002 (r=-0.650, $p < 0.05$). Both *Butyrivibrio*_2 and norank_*Lachnospiraceae* were negatively correlated with L-noradrenaline (r=-0.694, $p < 0.05$; r=-0.615, $p < 0.05$) and 5-methoxyindole-3-acetic acid (r=-0.606, $p < 0.05$; r=-0.685, $p < 0.05$), respectively.

Metabolite		RT[a]	Mass	Similarity	VIP	*p* value	FC[b]
Pyridine							
uracil		11.41	241	889	1.8272	0.0398	2.044
Amino acids, peptides, and analogs							
5-oxoproline		13.80	156	802	1.6496	0.0479	0.500
N,N-dimethylarginine		19.94	342	349	1.5252	0.0440	0.143
Fatty acids and conjugates							
Aconitic acid		16.39	229	639	1.9677	0.0146	0.563
3,4-dihydroxybenzoic acid		17.17	193	633	1.2278	0.0406	0.435
4-hydroxyphenylacetic acid		15.20	179	589	1.2501	0.0022	0.332
itaconic acid		11.33	247	478	1.9730	0.0187	5.630
1-hexadecanol		18.63	299	344	1.4496	0.0414	0.074
5-methoxyindole-3-acetic acid		20.62	290	276	1.9622	0.0320	88.955
2,4-diaminobutyric acid		15.08	200	261	1.6349	0.0209	0.223
2-keto-isovaleric acid		8.22	172	224	1.8180	0.0447	0.008
Lipids and lipid-like molecules							
O-phosphoethanolamine		16.75	172	648	1.9346	0.0183	0.437
2-ketoadipate		10.14	89	471	1.9160	0.0233	4.709
methyl *trans*-cinnamate		12.43	56	274	1.7464	0.0167	3.317
Sugars							
6-deoxy-D-glucose		16.10	318	458	1.3621	0.0216	0.199
galactose		17.8	156	375	2.0195	0.0483	0.001
Sugar Acids and Derivatives							
3-phosphoglycerate		16.99	227	546	2.0770	0.0331	0.009
Amines							
spermidine		20.85	174	577	1.2013	0.0384	2.758
Others	phosphate	10.48	84	758	2.0119	0.0282	0.068
	pyrophosphate	15.39	451	629	1.9063	0.0072	0.260
	catechol	11.16	254	472	2.0167	0.0021	6.021
	2,8-dihydroxyquinoline	17.46	290	422	1.1676	0.0417	0.098
	noradrenaline	20.49	174	370	1.3603	0.0363	5.943
	dehydroascorbic acid	17.44	61	307	2.0687	0.0436	0.001

[a]retention time; [b]fold change, FC>1 means that this metabolite is higher in Group H than in the Group C.

Table 3

Significant differential metabolites between Groups C and H (VIP>1.0; $p<0.05$).

Discussion

The effects of feed nutrient levels on growth performance, carcass characteristics and serum biochemical indices of SWCG have been reported in our previous study [28]. In brief, compared with Group C, ADG (95.37 vs. 81.06 g; $p < 0.05$), dressing percentage (48.79% vs. 44.04%; $p < 0.05$) and eye muscle area (21.72 vs. 19.55 cm^2, $p < 0.05$) were significantly increased in Group H, which indicated that the higher dietary energy and protein levels remarkably improved the growth performance and carcass characteristics of goats in our previous study. In addition, other studies reported that the serum biochemical parameters were sensitive indicators of health status of animals [37–39]. In our previous study, few differences in serum biochemical indices between Groups C and H ($p > 0.05$) suggested the similar healthy status of goats in the two groups [28].

Comparison of the composition and differences of ruminal bacterial communities

As mentioned above, diets with high energy and protein levels in Group H effectively promoted the growth performance and carcass characteristics of goats. Meanwhile, the digestion and absorption of these diets were closely related to the rumen bacteria [40–42]. A previous study reported that the changes in the ruminal microbiota could help promote the ADG of goats [5]. Therefore, we determined the differences of rumen bacterial communities of SWCG with simultaneous changes of dietary energy and protein levels in this study.

In this study, 16S rRNA gene sequencing was used to assess the rumen bacterial community in SWCG. Liu et al. [43] and Tapio et al. [44] reported that the richness of the bacterial community were influenced by diets. Our results also revealed low bacterial richness (ACE and Chao indices) with the increasing levels of dietary energy and protein, while no significant changes were detected in the bacterial diversity (Shannon and Simpson indices). In line with the previous studies [45, 46], this study revealed that *Bacteroidetes*, *Firmicutes* and *Proteobacteria* were the most dominant phyla in the two groups. These bacterial phyla were the core microbiota in the rumen and their structural compositions were unchanged regardless of feeding different types of diets [47]. Among the thirty phyla detected in this study, the abundance of *Bacteroidetes* increased significantly in Group H, which might be related to the protein degradation function of this phylum [48].

The effects of dietary nutrient levels on the bacterial population at the genus level were also detected in this study. Similar to the results of decreased bacterial richness in Group H, the high energy and protein levels of diets reduced the complexity of rumen bacterial interactions. Among the genera detected in the present study, *Prevotella*_1, belonging to the *Bacteroidetes*, was the most abundant bacteria in both the rumens of goats fed with different diets, which was consistent with the previous reports [3, 5]. In addition, the population of *Prevotella*_1 was significantly increased in Group H. The difference of abundances between the groups might be related to bacterial functions. The genus *Prevotella* is mainly involved in protein metabolism [3, 5, 49]. Wang et al. [3] reported that the number of *Prevotella*_1 was increased when the animals fed with the high protein diet. *Succiniclasticum* mainly participates in fermenting succinate

to propionate, which is the most precursor of glucose in ruminants [50, 51]. In our study, the relative abundance of *Succiniclasticum* significantly increased in the Group H. Similarly, the number of *Succiniclasticum* increased in the high-concentrate diet/high-energy diet group in previous reports [5, 11]. *Bacteroidetes* and *Prevotella* were selected in this study to verify the differences between two groups by absolute qRT-PCR and the results agreed with those by 16S rRNA gene amplicon sequencing. Furthermore, the previous studies have reported that the level of dietary protein were positively correlated with the relative abundance of *Prevotella* [26, 52] and the increasing levels of protein could promote the growth of cellulolytic bacteria [18]. In this context, the cellulolytic bacteria-*Succiniclasticum* and *Ruminococcus*_2 [27, 53] were positively associated with *Prevotella*_1 in our study.

Functional Prediction Of The Ruminal Bacteria In Swcg

Whether the changes in the bacterial community structures would lead to functional differences were detected by PICRUST2. Consistent with Miao et al. [54] and He et al. [55], we found that the abundances of metabolism were the highest in the rumen at KEGG level 1. The differences of diet affected the KEGG pathways of bacteria[56], hence, the integrated results demonstrated an overall increase in genetic information processing and metabolism, and an decrease in human diseases when goats fed with high energy and protein diets. Liu et al. [57] also reported that the KEGG pathways involved in carbohydrate metabolism were highly enriched in the microbiota of individuals fed with high energy diets, which was consistent with our study. Furthermore, the increasing number of *Prevotella*_1 in Group H of this study and the involvement of the genus in energy metabolism, nucleic acid metabolism and glycan biosynthesis and metabolism [52] might explain the increase of the above pathways in Group H. Although PICRUSt2 approach was utilized to predict the rumen bacterial functions, this method did not accurately detect the related function due to the limited number of sequencing studies in ruminants [58].

Comparison Of The Composition And Differences Of Ruminal Metabolites

Microbiota interacts with numerous physiological functions in the host through its metabolic products [59]. Thus, we used the GC-TOFMS analysis to explore the metabolic functions of ruminal microbiota. The main metabolites were amino acids, peptides and analogues in this study, which was consistent with the previous reports [43, 60]. Amino acids in the rumen are the key precursors for protein and polypeptides synthesis and are mainly obtained from the dietary proteins and microproteins [61].

According to the OPLS-DA results, a clear difference of ruminal metabolites was demonstrated between Groups C and H. These results confirmed that ruminal metabolites were closely related to the composition of diets [62].

Previous studies reported that uracil concentration in the rumen was increased with high concentrate diets [63, 64], which was consistent with our study. In addition, correlation analysis revealed that uracil

was positively correlated with *Ruminococcus*_2. The increased concentration of uracil in the rumen of SWCG in Group H might reflect that some bacterial nucleic acids were rapidly degraded to uracil by *Ruminococcus*_2 [65]. 5-oxoproline (pyroglutamic acid) could be the intermediate product in the glutathione cycle and its concertation was negatively correlated with the concentration of antioxidant-glutathione [63, 66, 67]. Based on the correlation analysis, *Lachnospiraceae*_ND3007_group might decrease the concentration of 5-oxoproline and more glutathione was produced in Group H to promote antioxidative capacity. Similarly, previous study also found that the concentration of 4-hydroxyphenylacetic acid was decreased when cows were fed with high-quality forage [68]. Microbiota degrade dietary protein to tryptophan, which could be later converted into melatonin [69]. Melatonin (N-acetyl-5-methoxytryptamine) as an effective antioxidant [70] could be ultimately oxidized to 5-methoxyindole-3-acetic acid [71]. Hence, the level of 5-methoxyindole-3-acetic acid in Group H with higher protein diets was significantly higher than that in Group C. In this study, catechol as an antioxidant [72, 73] was positively related to the relative abundances of *Ruminococcus*_2 and *Prevotella*_1, which might imply that high energy and protein levels in Group H could enhance the catechol concentration by these two genera. Xue et al. [74] reported that the content of spermidine increased in the group of high concentrate diets compared with moderate concentration of diets, which is in line with our study. Furthermore, correlation analysis in this study revealed that spermidine had high positive relationships with *Selenomonas*_1, *Ruminococcaceae*_NK4A214_group, *Lachnospiraceae*_NK3A20_group, *Prevotellaceae*_UCG-004 and *Prevotellaceae*_NK3B31_group. Spermidine is an organic compound widely used as an antioxidant [75]. The upregulation of spermidine observed in Group H of this study might enhance the antioxidative capacity in the rumen of this group by the above genera. These data implied that the diets with high energy and protein levels could improve the ruminal antioxidative capacity.

Based on the metabolomic analysis, we found that significantly different metabolites were involved in lipid metabolism and nucleotide metabolism, and this result was also identified by PICRUSt2 analysis. Tyrosine metabolism played an important role among those 9 main metabolic pathways in this study, which was also detected by Ferguson et al. [76]. Furthermore, the enriched abundances of beta-alanine, arginine and proline metabolism in this study were related to their functions. Beta-alanine could be metabolized into acetic acid and its concentration is positively associated with the amount of starch and readily available carbohydrate [43]. Arginine and proline involved in RNA synthesis and protein glycosylation are necessary for cellular function [75]. Additionally, we observed that the changes in the concentrations of differential metabolites were correlated with pyrimidine metabolism, which was associated with the dietary protein. Dietary nitrogen from protein could be degraded and reused by the microbiota in order to synthesize microbial nucleic acids [43, 77].

Conclusions

In this study, 16S rRNA gene sequencing and GC-TOFMS-based metabolomics were used to investigate the changes in rumen bacteria and metabolites in response to the diets with simultaneous changes of dietary energy and protein levels in SWCG. We observed that the bacterial richness was significantly reduced and the rumen bacterial composition was significantly altered with the increasing levels of

dietary energy and protein. Metabolomics analysis revealed that the dominant differential metabolites were amino acids, peptides and analogs. Besides, some metabolites could enhance the ruminal antioxidative capacity in Group H, which might modulate the antioxidant activity in the host. Integrative information on the relationships between the bacterial genera and differential metabolites in the rumen of SWCG can provide a better understanding of ruminal bacterial functions and metabolites that contribute to the development of SWCG husbandry. However, the potential mechanisms involved in the ruminal antioxidative capacity and the interactions between ruminal bacteria and metabolites are needed to be studied in the future research.

Abbreviations

SWCG: Shaanbei white cashmere goat; VFAs: volatile fatty acids; MCP: microbial protein; ADG: average daily weight gain; GC-TOFMS: Gas Chromatography Tandem Time-of-Flight Mass Spectrometry; ME: metabolic energy; OTUs: Operational taxonomic units; SRA: Sequence Read Archive; PICRUSt2: phylogenetic analysis of communities by reconstruction of unobserved states; KEGG: Kyoto Encyclopedia of Genes and Genomes; qRT-PCR: quantitative real-time polymerase chain reaction; TMCS: trimethylchlorosilane; PCA: principal component analysis; OPLS-DA: orthogonal correction partial least squares discriminant analysis; SPSS: Statistical Package for the Social Sciences; ANOVA: one-way analysis of variance; VIP: variable importance in the projection.

Declarations

Ethics approval and consent to participate

The use of animals and all experimental protocols (protocol number: 100403) were authorized by the Institutional Animal Care and Use Committee of Northwest A&F University (Yangling, Shaanxi, China).

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed are available from the corresponding author on request.

Competing interests

The authors declare no competing financial interests.

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Authors' contributions

YY and CY conceived and designed the experiments. TP and LJ managed goats. WY and TP collected samples. WY, TP and XY performed bacterial and metabolic analysis. WY performed statistical analysis of all data and wrote the manuscript. All authors read and approved the final version of the manuscript.

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Additional Files

Additional file 1: Table S1-S8.

Table S1. Composition and nutrient contents of the experimental diets (DM basis).

Table S2. Real time-PCR primers used in this study.

Table S3. Number of diversity estimates based on the 16S rRNA gene libraries from the sequencing analysis.

Table S4. The phyla in the rumen bacteria of goats.

Table S5. Distribution of genera in different groups.

Table S6. Predicted functions at level 1 of the rumen bacterial microbiota.

Table S7. Predicted functions at level 2 of the rumen bacterial microbiota.

Table S8. The mean of relative quantitative values of differential metabolites (VIP>1.0; $p<0.05$).

Additional file 2: Figures S1-S2

Figure S1. Metabolic phenotype profile of rumen. (A) GC-TOF/MS total ion current chromatograms of rumen contents from Groups C and H; (B) PCA plot of ruminal metabolites of Groups C and H.

Figure S2. Correlation analysis of the top 50 bacterial genera in Group C (A.) or H (B.), respectively. Nodes represent bacterial genera, and edges represent significant interactions among nodes (the absolute Spearman coefficients were above 0.55). The node color corresponds to the phylum taxonomic classification. The edge color represents positive (red) and negative (green) correlations.

Additional file 3: Table S9. sheet 1. The correlation between bacterial genera; sheet 2. The *p*-value between bacterial genera.

Additional file 4: Table S10. sheet 1. The correlation between bacterial genera and differential metabolites; sheet 2. The *p*-value among bacterial genera and differential metabolites

Figures

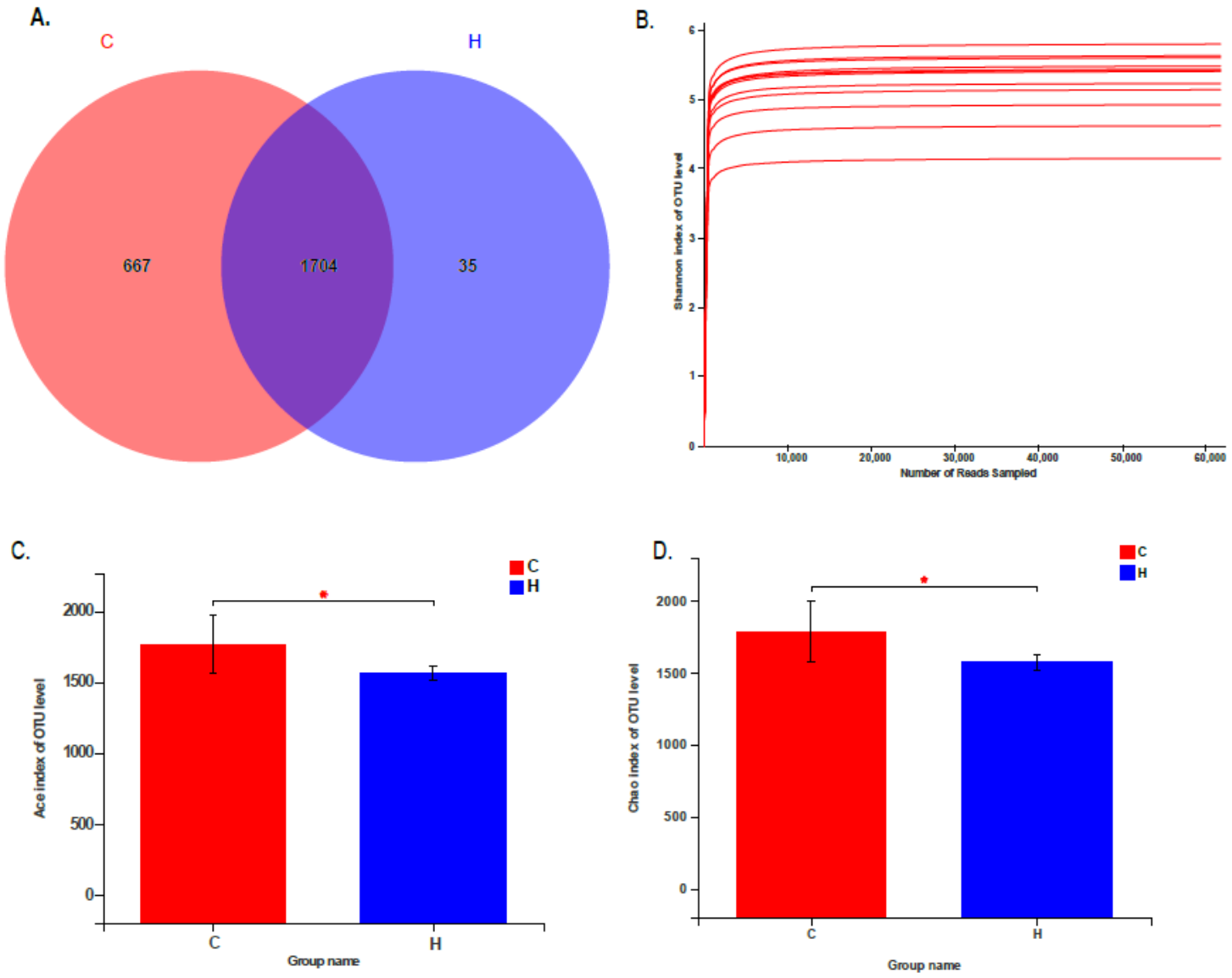

Figure 1

16S rRNA gene sequences in different dietary groups. A Venn diagram illustrating the overlap of bacterial OTUs at a 3% dissimilarity level for Groups C and H. (A). The samples of Group C included goats that were fed with a typical total mixed ration (TMR) and the samples of Group H included goats that were fed with the high-energy and high-protein diets. Rarefaction analysis of different samples (B). Differences in ACE indices (C) and Chao indices (D) between Groups C and H.

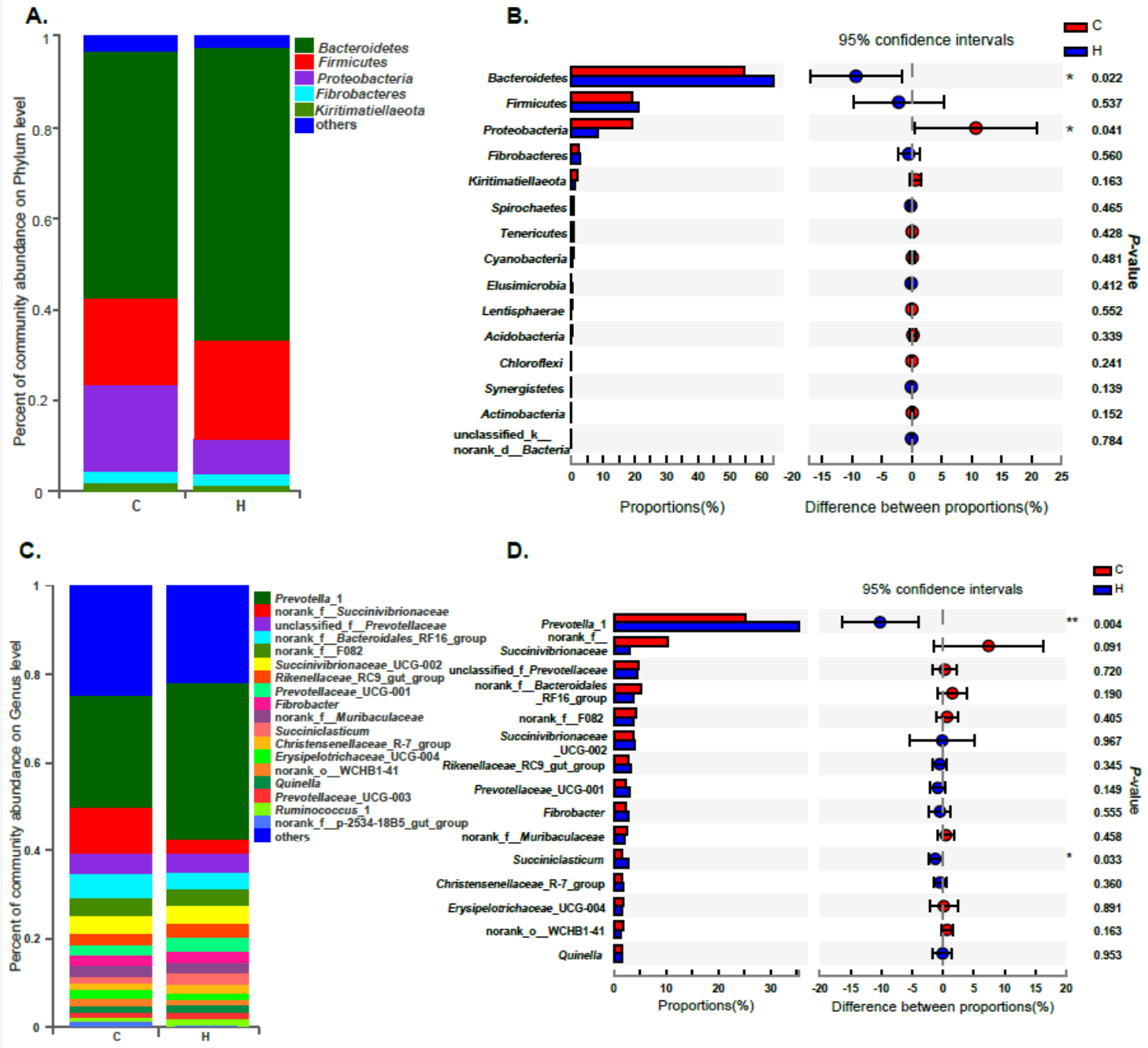

Figure 2

Distribution of bacteria in different groups. The color-coded bar plots represent the average distribution of bacterial phyla (A) and genera (C), respectively. Only the dominant bacteria (with a relative abundance ≥1%) among rumen bacteria are shown. Extended error bar plots illustrate the mean proportions and differences in the phyla (B) or genera (D) in rumen samples. * indicates p<0.05.

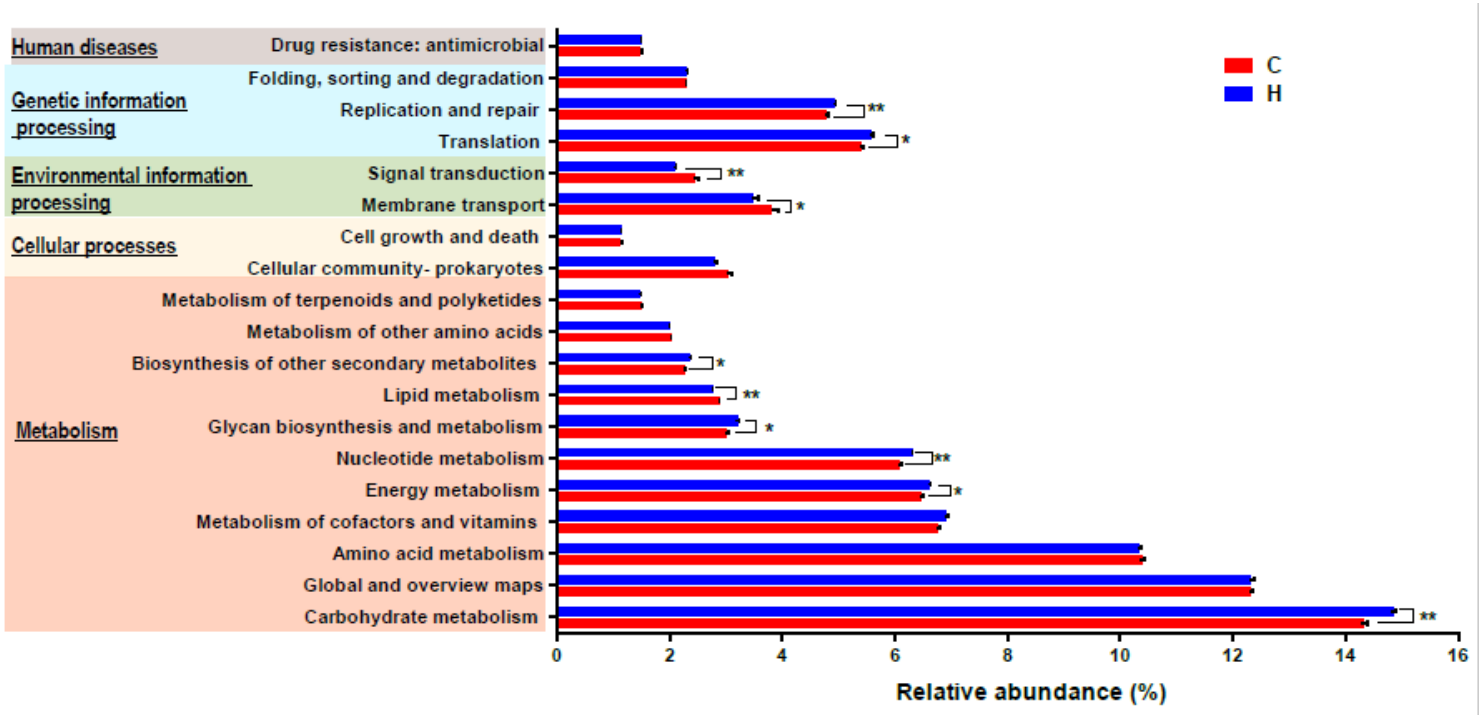

Figure 3

Differences in bacterial metabolism function at KEGG level 2 between Groups C and H using PICRUSt2.

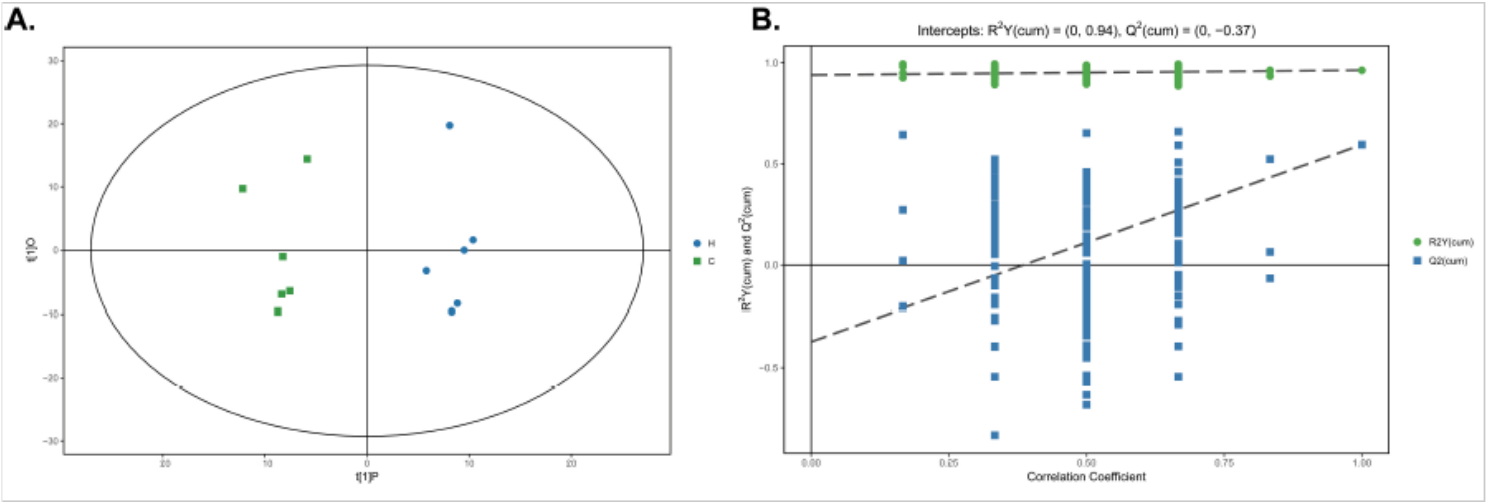

Figure 4

Orthogonal partial least squares discriminant analysis [(O)PLS-DA] plots of rumen metabolites between Groups C and H. Score scatter plot of OPLS-DA model for Group H versus C (A). Permutation test of OPLS-DA model for Group H versus C (B).

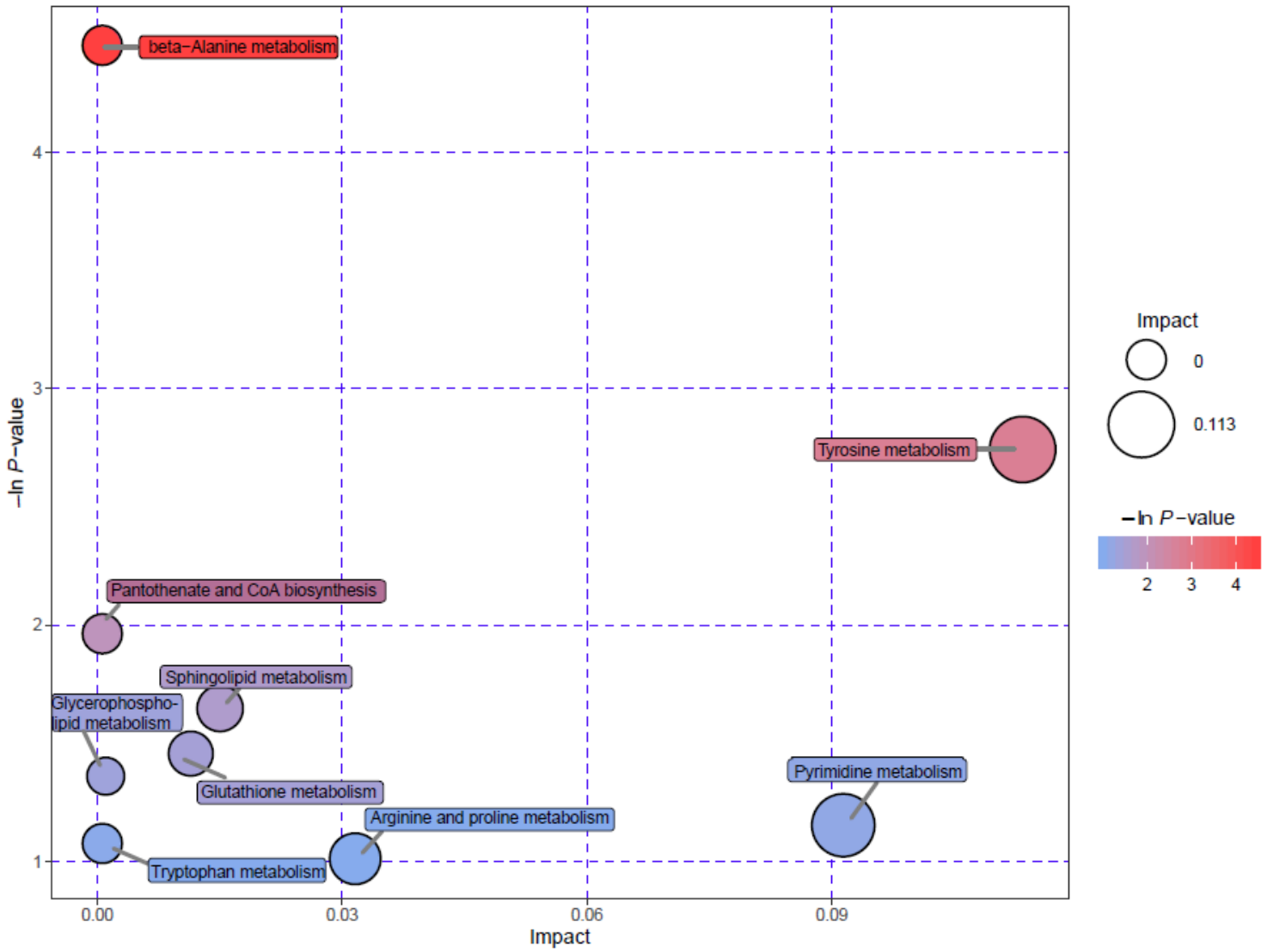

Figure 5

Metabolome view map of the deferential metabolites (VIP>1, p<0.05) identified in rumen from goats fed with the diets with different energy and protein levels. The large size indicates high pathway enrichment, and dark color indicates high pathway impact values.

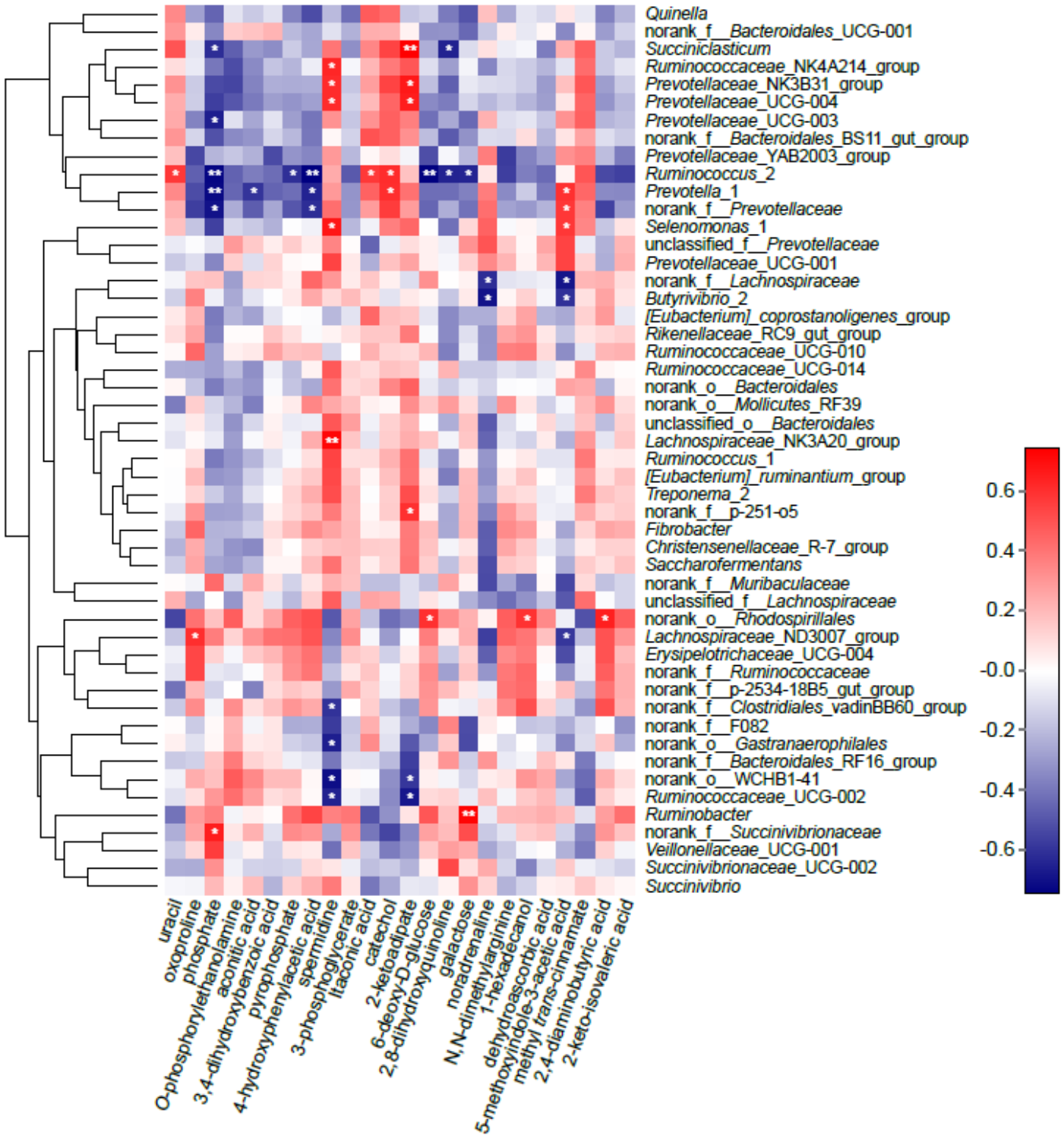

Figure 6

Correlation between bacterial genera and deferential metabolites affected by diets with different energy and protein levels. * and ** indicate $p < 0.05$ and $p \leq 0.01$, respectively.

Supplementary Files

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