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

**CURRENT STATUS:** POSTED

Yaoyue Wang
Northwest Agriculture and Forestry University

Peng Tang
Northwest Agriculture and Forestry University

Yafei Xiao
Northwest Agriculture and Forestry University

Jianming Liu
Northwest Agriculture and Forestry University

Yulin Chen
Northwest Agriculture and Forestry University

Yuxin Yang
Northwest Agriculture and Forestry University

yangyuxin2002@126.com Corresponding Author
ORCiD: https://orcid.org/0000-0002-1742-2328

10.21203/rs.3.rs-26868/v1

**SUBJECT AREAS**
Animal Science

**KEYWORDS**
dietary energy; protein; rumen bacterial composition; 16S rRNA gene sequencing; metabolomics
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-keto adipate \((r=0.741, p<0.01)\).

**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**
Schaanbei 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
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$_2$O) 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).

| Items                        | R value | p value |
|------------------------------|---------|---------|
| Groups (H and C)             |         |         |
| Phylum                      | 0.315   | 0.452   |
| Genus                       | 0.426   | 0.037   |
| OTU                         | 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

| Groups | SEM  | \(p\) value |
|--------|------|-------------|
| C      |      |             |
| H      |      |             |
| **Bacteroidetes** | 6.71 | 0.359 | 0.004 |
| **Prevotella** | 6.12 | 0.266 | 0.006 |

*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-ketoisocaproate ($r = 0.741$, $p < 0.01$), 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                              | RTa | Mass | Similarity | VIP | $p$ value | FCb |
|-----------------------------------------|-----|------|------------|-----|-----------|-----|


| Component                          | m/z | ionization | ion peak | S/N ratio | 1/S ratio |
|-----------------------------------|-----|------------|----------|-----------|-----------|
| 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 |
| Metabolite                  | a | b | c | d | e | f |
|----------------------------|---|---|---|---|---|---|
| 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 |

<sup>a</sup>retention time; <sup>b</sup>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 cm², 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.

Succinlasticum 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 Succinlasticum significantly increased in the Group H. Similarly, the number of Succinlasticum 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- *Succinivibrio* 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 concentration 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.

**Funding**

This work was supported by Agriculture Research System of China [CARS-39-12] and National Key Research and Development Program [2018YFD0501905].

**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.

**Acknowledgments**

We would like to thank Wang Guojun for helping us to feed the animals and Diqingyuan farm for providing experiment conditions.

**References**

1. Han X, Li B, Wang X, Chen Y, Yang Y. Effect of dietary concentrate to forage ratios on ruminal bacterial and anaerobic fungal populations of cashmere goats. Anaerobe.
2. Wang S, Ge W, Luo Z, Guo Y, Jiao B, Qu L, et al. Integrated analysis of coding genes and non-coding RNAs during hair follicle cycle of cashmere goat (Capra hircus). BMC Genomics. 2017; 18:1-13.

3. Wang H, He Y, Li H, Wu F, Qiu Q, Niu W, et al. Rumen fermentation, intramuscular fatty acid profiles and related rumen bacterial populations of Holstein bulls fed diets with different energy levels. Appl Microbiol Biot. 2019;103: 4931-42.

4. Jami E, Israel A, Kotser A, Mizrahi I. Exploring the bovine rumen bacterial community from birth to adulthood. ISME J. 2013;7:1069-79.

5. Bi Y, Zeng S, Zhang R, Diao Q, Tu Y. Effects of dietary energy levels on rumen bacterial community composition in Holstein heifers under the same forage to concentrate ratio condition. BMC Microbiol. 2018;18(1):69.

6. Wang W, Li C, Li F, Wang X, Zhang X, Liu T, et al. Effects of early feeding on the host rumen transcriptome and bacterial diversity in lambs. Sci Rep. 2016; 6:32479.

7. Pitta DW, Indugu N, Kumar S, Vecchiarelli B, Sinha R, Baker LD, et al. Metagenomic assessment of the functional potential of the rumen microbiome in Holstein dairy cows. Anaerobe. 2016; 38:50-60.

8. Deusch S, Camarinha-Silva A, Conrad J, Beifuss U, Rodehutscord M, Seifert J. A structural and functional elucidation of the rumen microbiome influenced by various diets and microenvironments. Front Microbiol. 2017; 8:1605.

9. Prajapati VS, Purohit HJ, Raje DV, Parmar N, Patel AB, Jones OAH, et al. The effect of a high-roughage diet on the metabolism of aromatic compounds by rumen microbes: a metagenomic study using Mehsani buffalo (Bubalus bubalis). Appl Microbiol Biot. 2016;100:1319-31.

10. Li H, Zhou R, Zhu J, Huang X, Qu J. Environmental filtering increases with elevation
for the assembly of gut microbiota in wild pikas. Microb biotechnol. 2019;12:976-92.

11. Petri RM, Schwaiger T, Penner GB, Beauchemin KA, Forster RJ, McKinnon JJ, et al. Characterization of the core rumen microbiome in cattle during transition from forage to concentrate as well as during and after an acidotic challenge. PLoS One. 2013;8:e83424.

12. Morgavi DP, Kelly WJ, Janssen PH, Attwood GT. Rumen microbial (meta) genomics and its application to ruminant production. Animal. 2013;7(s1):184-201.

13. Lv X, Cui K, Qi M, Wang S, Diao Q, Zhang N. Ruminal microbiota and fermentation in response to dietary protein and energy levels in weaned lambs. Animals. 2020;10:109.

14. Clark JH, Davis CL. Some aspects of feeding high producing dairy cows. J Dairy Sci. 1980; 63:873-85.

15. Wang P, Tan Z, Guan L, Tang S, Zhou C, Han X, et al. Ammonia and amino acids modulates enzymes associated with ammonia assimilation pathway by ruminal microbiota in vitro. Livest Sci. 2015; 178: 130-9.

16. He Y, Yu Z, Qiu Q, Shao T, Niu W, Xia C, et al. Effects of dietary protein levels and calcium salts of long-chain fatty acids on nitrogen mobilization, rumen microbiota and plasma fatty acid composition in Holstein bulls. Anim Feed Sci Tech. 2018; 246:1-10.

17. de Melo Amaral P, Mariz LDS, Benedeti PDB, Da Silva LG, de Paula EM, Monteiro HF, et al. Effects of static or oscillating dietary crude protein levels on fermentation dynamics of beef cattle diets using a dual-flow continuous culture system. PLoS One. 2016;11: e0169170.

18. Liu Q, Wang C, Li HQ, Guo G, Huo WJ, Pei CX, et al. Effects of dietary protein levels and rumen-protected pantothenate on ruminal fermentation, microbial enzyme
activity and bacteria population in Blonde d’Aquitaine× Simmental beef steers. Anim Feed Sci Tech. 2017; 232:31-9.

19. Broderick GA. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J Dairy Sci. 2003;86:1370-81.

20. Fiems LO, De Boever JL, Ampe B, Vanacker JM, De Campeneere S, Sonck B. Effect of energy restriction and dietary protein level during the re-alimentation period on feed efficiency in double-muscled Belgian Blue cows. Livest Sci. 2015; 174:46-52.

21. Cutrim DO, Alves KS, Dos Santos RDC, Da Mata VJV, Oliveira LRS, Gomes DÍ, et al. Body composition and energy and protein nutritional requirements for weight gain in Santa Ines crossbred sheep. Trop Anim Health Pro. 2016; 48:683-8.

22. Lu Z, Xu Z, Shen Z, Tian Y, Shen H. Dietary energy level promotes rumen microbial protein synthesis by improving the energy productivity of the ruminal microbiome. Front Microbiol. 2019;10: 847.

23. Hua C, Tian J, Tian P, Cong R, Luo Y, Geng Y, et al. Feeding a high concentration diet induces Unhealthy Alterations in the composition and metabolism of ruminal microbiota and host response in a goat model. Front Microbiol. 2017;8: 138.

24. Schären M, Frahm J, Kersten S, Meyer U, Hummel J, Breves G, et al. Interrelations between the rumen microbiota and production, behavioral, rumen fermentation, metabolic, and immunological attributes of dairy cows. J Dairy Sci. 2018;101:4615-37.

25. Tang Z, Chen G, Hong Q, Huang S, Smith HM, Shah RD, et al. Multi-Omic Analysis of the Microbiome and Metabolome in Healthy Subjects Reveals Microbiome-Dependent Relationships Between Diet and Metabolites. Front Genet. 2019;10:454.

26. Du C, Nan X, Wang K, Zhao Y, Xiong B. Evaluation of the digestibility of steam-exploded wheat straw by ruminal fermentation, sugar yield and microbial structure in
vitro. RSC ADV. 2019;9:41775-82.

27. Wang L, Liu K, Wang Z, Bai X, Peng Q, Jin L. Bacterial community diversity associated with different utilization efficiencies of nitrogen in the gastrointestinal tract of goats. Front microbiol. 2019;10:239.

28. Tang P, Wang Y, Wang G, Chen Y, Yang Y. Effects of dietary energy and protein levels on growth performance, serum biochemical indexes, slaughter performance and meat quality of white cashmere goats in Shaanbei white cashmere goats. Chinese journal of animal nutrition. 2018;30: 2194-2201(in Chinese with English abstract).

29. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335-6.

30. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010; 26:2460-1.

31. Amato KR, Yeoman CJ, Kent A, Righini N, Carbonero F, Estrada A, et al. Habitat degradation impacts black howler monkey (Alouatta pigra) gastrointestinal microbiomes. ISME J. 2013;7:1344-53.

32. Douglas GM, Maffei V, Zaneveld J, Yurgel SN, Brown JR, Taylor CM, et al. PICRUSt2: An improved and extensible approach for metagenome inference. bioRxiv. 2019:672295.

33. Fu L, Jiang B, Liu J, Zhao X, Liu Q, Hu X. Genome sequence analysis of a flocculant-producing bacterium, Paenibacillus shenyangensis. Biotechnol Lett. 2016;38:447-53.

34. Wang Y, Cao P, Wang L, Zhao Z, Chen Y, Yang Y. Bacterial community diversity associated with different levels of dietary nutrition in the rumen of sheep. Appl Microbiol Biot. 2017;101:3717-28.

35. Li Q, Kang J, Ma Z, Li X, Liu L, Hu X. Microbial succession and metabolite changes during traditional serofluid dish fermentation. LWT-Food Sci Technol. 2017;84:771-9.
36. Kind T, Wohlgemuth G, Lee DY, Lu Y, Palazoglu M, Shahbaz S, et al. FiehnLib: mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. Anal Chem. 2009;81:10038-48.

37. Adeyemi KD, Sabow AB, Aghwan ZA, Ebrahimi M, Samsudin AA, Alimon AR, et al. Serum fatty acids, biochemical indices and antioxidant status in goats fed canola oil and palm oil blend. Journal of Animal Science & Technology. 2016;58:6.

38. Dinu M, Whittaker A, Pagliai G, Benedettelli S, Sofi F. Ancient wheat species and human health: biochemical and clinical implications. J Nutr Biochem. 2018;52: 1-9.

39. Otomaru K, Wataya K, Uto T, Kasai K. Blood biochemical values in Japanese Black calves in Kagoshima Prefecture, Japan. J Vet Med Sci. 2015;78:301-3.

40. Clark JH, Klusmeyer TH, Cameron MR. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy Cows1. J Dairy Sci. 1992;75:2304-23.

41. Han X, Yang Y, Yan H, Wang X, Qu L, Chen Y. Rumen Bacterial Diversity of 80 to 110-Day-Old Goats Using 16S rRNA Sequencing. PLoS One. 2015;10:e117811.

42. Petri RM, Neubauer V, Humer E, Kröger I, Reisinger N, Zebeli Q. Feed additives differentially impact the epimural microbiota and host epithelial gene expression of the bovine rumen fed diets rich in concentrates. Front Microbiol. 2020;11:119.

43. Liu C, Wu H, Liu S, Chai S, Meng Q, Zhou Z. Dynamic Alterations in yak rumen bacteria community and metabolome characteristics in response to feed type. Front Microbiol. 2019;10:1116.

44. Tapio I, Fischer D, Blasco L, Tapio M, Wallace RJ, Bayat AR, et al. Taxon abundance, diversity, co-occurrence and network analysis of the ruminal microbiota in response to dietary changes in dairy cows. PLoS One. 2017;12: e0180260.

45. Zhang R, Ye H, Liu J, Mao S. High-grain diets altered rumen fermentation and
epithelial bacterial community and resulted in rumen epithelial injuries of goats. Appl Microbiol Biot. 2017;101:6981-92.

46. Zeng H, Guo C, Sun D, Seddik H, Mao S. The Ruminal Microbiome and Metabolome Alterations Associated with Diet-Induced Milk Fat Depression in Dairy Cows. Metabolites. 2019 2019-07-23;9:154.

47. Henderson G, Cox F, Ganesh S, Jonker A, Young W, Abecia L, et al. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. SCI REP. 2015;5:14567.

48. Solden LM, Hoyt DW, Collins WB, Plank JE, Daly RA, Hildebrand E, et al. New roles in hemicellulosic sugar fermentation for the uncultivated Bacteroidetes family BS11. ISME journal. 2017;11:691-703.

49. Oliphant K, Allen-Vercoe E. Macronutrient metabolism by the human gut microbiome: major fermentation by-products and their impact on host health. Microbiome. 2019;7:91.

50. Pope PB, Smith W, Denman SE, Tringe SG, Barry K, Hugenholtz P, et al. Isolation of Succinivibrionaceae implicated in low methane emissions from Tammar wallabies. Science. 2011;333:646-8.

51. Sean MCM, Paul C, Kate K, Aaron OC, Eoin OH, Alejandro PR, et al. Illumina MiSeq phylogenetic amplicon sequencing shows a large reduction of an uncharacterised Succinivibrionaceae and an increase of the Methanobrevibacter gottschalkii clade in feed restricted cattle. PLoS One. 2015;10:e133234.

52. Wang L, Jin L, Xue B, Wang Z, Peng Q. Characterizing the bacterial community across the gastrointestinal tract of goats: Composition and potential function. Microbiologyopen. 2019;8: e820.

53. Uyeno Y, Shigemori S, Shimosato T. Effect of probiotics/prebiotics on cattle health
and productivity. Microbes environ. 2015;30:126-32.

54. Miao L, Wang P, Hou J, Yao Y, Liu Z, Liu S. Low concentrations of copper oxide nanoparticles alter microbial community structure and function of sediment biofilms. Sci Total Environ. 2019;653:705-13.

55. He J, Yi L, Hai L, Ming L, Gao W, Ji R. Characterizing the bacterial microbiota in different gastrointestinal tract segments of the Bactrian camel. Sci Rep. 2018;8: 654.

56. Quan J, Cai G, Yang M, Zeng Z, Ding R, Wang X, et al. Exploring the fecal microbial composition and metagenomic functional capacities associated with feed efficiency in commercial DLY pigs. Front Microbiol. 2019;10:52.

57. Liu R, Hong J, Xu X, Feng Q, Zhang D, Gu Y, et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. Nat Med. 2017;23:859-68.

58. Meale SJ, Li SC, Azevedo P, Derakhshani H, DeVries TJ, Plaizier JC, et al. Weaning age influences the severity of gastrointestinal microbiome shifts in dairy calves. Sci Rep. 2017;7: 198.

59. Ren W, Wang P, Yan J, Liu G, Yin Y. Melatonin alleviates weanling stress in mice: Involvement of intestinal microbiota. J Pineal Res. 2017;64:e12448.

60. Alarcon P, Hidalgo AI, Manosalva C, Cristi R, Teuber S, Hidalgo MA, et al. Metabolic disturbances in synovial fluid are involved in the onset of synovitis in heifers with acute ruminal acidosis. Sci Rep. 2019;9: 5452.

61. Mariz LDS, Amaral PM, Valadares Filho SC, Santos SA, Detmann E, Marcondes MI, et al. Dietary protein reduction on microbial protein, amino acid digestibility, and body retention in beef cattle: 2. Amino acid intestinal absorption and their efficiency for whole-body deposition. J Anim Sci. 2018;96:670-83.

62. Simon D, Amélia CS, Jürgen C, Uwe B, Markus R, Jana S. A structural and functional
elucidation of the rumen microbiome influenced by various diets and microenvironments. Front Microbiol. 2017;8:1605.

63. Zhang RY, Liu YJ, Yin YY, Jin W, Mao SY, Liu JH. Response of rumen microbiota, and metabolic profiles of rumen fluid, liver and serum of goats to high-grain diets. Animal. 2019;13:1855-64.

64. Ametaj BN, Zebeli Q, Saleem F, Psychogios N, Lewis MJ, Dunn SM, et al. Metabolomics reveals unhealthy alterations in rumen metabolism with increased proportion of cereal grain in the diet of dairy cows. Metabolomics. 2010;6:583-94.

65. Mcalian AB, Smith RH. Degradation of nucleic acids in the rumen. Br J Nutr. 1973;29:331-45.

66. Matsui R, Ferran B, Oh A, Croteau D, Shao D, Han J, et al. Redox regulation via glutaredoxin-1 and protein S-glutathionylation. Antioxid Redox Sign. 2020;32:677-700.

67. Saoi M, Li A, McGlory C, Stokes T, von Allmen MT, Phillips SM, et al. Metabolic perturbations from step reduction in older persons at risk for sarcopenia: plasma biomarkers of abrupt changes in physical activity. Metabolites. 2019;9:134.

68. Sun H, Wang B, Wang J, Liu H, Liu J. Biomarker and pathway analyses of urine metabolomics in dairy cows when corn stover replaces alfalfa hay. J Anim Sci biotechno. 2016;7: 49.

69. Tan D, Zheng X, Kong J, Manchester LC, Hardeland R, Kim SJ, et al. Fundamental issues related to the origin of melatonin and melatonin isomers during evolution: relation to their biological functions. Int J Mol Sci. 2014;15:15858-90.

70. Lu J, Sun Z, Fang Y, Zheng J, Xu S, Xu W, et al. Melatonin suppresses microglial necroptosis by regulating deubiquitinating enzyme A20 after intracerebral hemorrhage. Front Immunol. 2019;10:1360.
71. Lerner AB, Case JD, Takahashi Y. Isolation of melatonin and 5-methoxyindole-3-acetic acid from bovine pineal glands. J Biol Chem. 1960;235:1992-7.

72. Morawski FDM, Deon M, Nicolodi S, de Menezes EW, Costa TMH, Dias SLP, et al. Magnetic silica/titania xerogel applied as electrochemical biosensor for catechol and catecholamines. Electrochim Acta. 2018;264:319-28.

73. Nikolaevskaya EN, Kansuzyan AV, Filonova GE, Zelenova VA, Pechennikov VM, Krylova IV, et al. Germanium dioxide and the antioxidant properties of catechols. Eur J Inorg Chem. 2019; 2019:676-81.

74. Xue F, Pan X, Jiang L, Guo Y, Xiong B. GC-MS analysis of the ruminal metabolome response to thiamine supplementation during high grain feeding in dairy cows. Metabolomics. 2018;14(5):67.

75. Elolimy A, Alharthi A, Zeineldin M, Parys C, Loor JJ. Residual feed intake divergence during the preweaning period is associated with unique hindgut microbiome and metabolome profiles in neonatal Holstein heifer calves. J Anim Sci Biotechno. 2020;11:13.

76. Ferguson AA, Roy S, Kormanik KN, Kim Y, Dumas KJ, Ritov VB, et al. TATN-1 mutations reveal a novel role for tyrosine as a metabolic signal that influences developmental decisions and longevity in Caenorhabditis elegans. PLoS Genet. 2013;9:e1004020.

77. Stentoft C, Rojen BA, Jensen SK, Kristensen NB, Vestergaard M, Larsen M. Absorption and intermediary metabolism of purines and pyrimidines in lactating dairy cows. Br J Nutr. 2015;113:560-

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
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 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 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.
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 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 ≤ 0.01, respectively.
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
This is a list of supplementary files associated with this preprint. Click to download.

Additionalfile4.xlsx
Additionalfile2.docx
Additionalfile3.xlsx
Additionalfile1.docx
Additionalfile2.docx
Additionalfile3.xlsx
Additionalfile1.docx
Additionalfile4.xlsx