

Effects of Fermented Mushroom Residue Feed on Growth Performance and Rumen Microorganisms of Goats

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Abstract

Background: The high content of fiber and macromolecular substances in mushroom residue limits its utilization potential. After we fermented seafood mushroom residue with microorganisms, we found that its palatability and utilization have been greatly improved. The purpose of this study was to investigate the effects of feed containing fermented seafood mushroom residue on the growth performance and rumen microorganisms of goats.

Methods: Forty-eight Jianyang big-ear goats with similar weight and good health were selected and randomly divided into 4 groups with 12 goats in each group. The control group (C) was fed a basic diet, and the experimental group 1 (L), 2 (M), and 3 (H) were fed diets supplemented with 20%, 30% and 40% fermented seafood mushroom residue.

Results: Incorporating 20% fermented mushroom residue significantly increased the average daily gain (ADG) and average daily feed intake (ADFI) and reduced feed to gain ratio (F/G). Incorporating 30% in the diet significantly increased both ADFI and F/G. Growth performance was reduced when greater than 30% fermented mushroom residue was added. The beta diversity of the rumen flora was significantly increased with the 20% fermented mushroom residue diet. The relative abundance of Veillonellaceae, Ruminococcaceae, and Prevotellaceae increased, and the KEGG pathways related to metabolism of cofactors and vitamins, lipid metabolism, and xenobiotics biodegradation and metabolism were enhanced. Forty-nine KEGG pathways significantly associated with ADG were identified. Twenty-six pathways, including protein kinases, mineral absorption, propanoate metabolism, and biosynthesis of unsaturated fatty acids, were positively correlated with ADG. The remaining pathways, including pathogenic *Escherichia coli* infection, phenylpropanoid biosynthesis, shigellosis, and cyanoamino acid metabolism, were negatively correlated with ADG.

Conclusions: These results show that adding 20% fermented seafood mushroom residue can improve the growth performance of goats and have a positive effect on goat rumen microorganisms.

Background

The shortage of animal feed raw materials and their cost have been important factors in restricting the development of animal husbandry. The development of unconventional feeds has become an urgent necessity for the animal husbandry industry [1]. Mushroom residue is the waste culture medium remaining after harvesting of edible fungi — its main components are cottonseed shell, straw, corn cob, bagasse, and a variety of agricultural by-products and industrial waste [2,3]. Many reports have shown that mushroom residue contains a large amount of crude protein, minerals, amino acids, and other active nutrients. It can be considered a potential high-quality, unconventional raw material for livestock and poultry feed [4,5]. At present, most mushroom residue is burned for power generation or discarded, while only a small proportion is processed for animal feed, wasting a precious biological resource and causing serious ecological damage to the environment [6]. Development and utilization of mushroom residue can,

therefore, save resources, reduce environmental pollution, alleviate the shortage of feed materials, reduce the cost of animal production, and provide economic benefits.

While the edible components of mushroom residue are composed mainly of cottonseed shell, straw, and other high-fiber materials, these have poor palatability and are difficult to digest. It is necessary to carry out fermentation treatment to improve palatability, nutritional value, and utilization [7]. Mushroom residue can be treated by microbial fermentation where enzymes degrade cellulose and hemicellulose into sugars, amino acids, vitamins, and other nutrients that are easily digested and absorbed by the animal [8].

Ruminant animals have many microorganisms in the rumen which help the body digest and absorb nutrients, and enhance the animal's resistance to pathogens [9]. Many studies have shown that *Bacteroidetes* and *Firmicutes* are the dominant phyla of bacteria in the rumen [10,11]. *Fibrobacter*, *Ruminococcus* and *Butyrivibrio* (*Firmicutes*) have a high capacity to decompose cellulose. Among the *Bacteroidetes*, *Prevotella* can decompose proteins, starch, and polysaccharides – crucial for promoting digestion and metabolism, and improving animal performance. This study investigates the use of *Bacillus subtilis*, *lactic acid bacteria*, and *Saccharomyces cerevisiae* composite inoculants for the fermentation of seafood mushroom residue in order to improve palatability and digestibility. High-throughput sequencing technology is employed to study the effect on rumen microorganisms of adding different quantities of fermented mushroom residue to goat feed, in order to promote utilization of mushroom residue and improve the rumen microbial ecology.

Materials And Methods

Materials

Mushroom residue was obtained from Fujian Shennong Mushroom Industry Co. Ltd. Fresh mushroom residue without mildew was selected for this study which employed fermentation with inoculation. Fermentation agent (number of live bacteria: *Bacillus subtilis* $\geq 100 \times 10^6$ CFU/g, *lactic acid bacteria* $\geq 10 \times 10^6$ CFU/g, *Saccharomyces cerevisiae* $\geq 1 \times 10^6$ CFU/g, total bacteria $\geq 110 \times 10^6$ CFU/g), was provided by Fujian Academy of Agricultural Sciences. The starter agent was activated for 40 minutes before use by mixing 50 kg of warm water, 10 kg of brown sugar, and 1 kg of starter. The activated liquid was added at a concentration of 0.1% to inoculate the fermentation of the mushroom residue which proceeded anaerobically for 21 d at room temperature. After fermentation was complete, the conventional analysis and the method of Van Soest [12] were used to determine the nutrient content of the residue (Table 1).

Table 1
Nutrition components of Seafood mushroom residue before and after
fermentation

project	Before the fermentation	After the fermentation
Dry matter / %	48.7	38.9
Crude protein / %	10.7	12.2
Crude fat / %	2.37	2.23
Neutral washing fiber /%	52.3	51.5
Acid washing fiber /%	44.0	43.8
Calcium / %	1.33	1.3
Phosphorus / %	0.37	0.39

Test Design

Forty-eight Jianyang big-ear goats (initial weight 17.0 ± 0.5 kg) were randomly divided into four groups of twelve goats. Each animal was numbered, dewormed, immunized, and raised in a well-ventilated goat house by trained staff. Goats were fed daily at fixed times (9:00 and 16:00) and drinking water was freely available. Goats in control group C were fed a basic diet while groups L, M, and H were fed diets supplemented with 20%, 30%, and 40% fermented seafood mushroom residue, respectively. The diets were formulated according to National Scientific Research Council nutritional requirements and dietary composition and nutritional levels are shown in Table 2. The feeding trial lasted for 130d with pre-feeding 10 d and trial 120 d.

Table 2
Composition and nutrient levels of experiment diets (Dry matter basis) %

Items	Groups			
	C	L	M	H
Seafood mushroom residue fermented feed	0	20	30	40
Peanut vine	46.00	31.30	24.00	16.23
Corn	26.00	22.00	20.00	18.00
Bran	13.50	13.00	13.05	13.00
Soybean meal	12.00	11.00	10.20	9.93
Limestone	0.05	0.20	0.35	0.55
Dicalcium Phosphate	0.95	1.00	0.90	0.80
Sodium chloride	0.50	0.50	0.50	0.50
Premix ¹⁾	1.00	1.00	1.00	1.00
Total	100	100	100	100
Nutrient levels ²⁾				
Crude protein	14.18	14.17	14.05	14.12
Calcium	1.49	1.45	1.43	1.40
Phosphorus	0.52	0.57	0.57	0.58
Metabolizable energy(MJ/Kg)	10.11	10.01	9.98	9.95
¹⁾ Each A Thousand of Premix Contains: VA 240000 IU, VE 160 IU, VD 50000 IU, Mn 60 mg, Zn 50 mg, Cu 12 mg, Fe 40 mg, I 1 mg, Co 0.15 mg, Se 0.05 mg. ²⁾ The metabolic energy was calculated, and the rest were measured.				

Sample collection

At the end of the feeding period, three goats were randomly selected from each group for slaughter. Before slaughter the goats fasted for 24 h and water was withdrawn for 12 h. After slaughter the rumen was removed and the contents collected and quickly filtered through four layers of sterile, degreasing gauze. Filtrate was placed in 5 ml tubes and stored at 80°C for DNA extraction and 16S rRNA high-throughput sequencing.

DNA extraction and PCR amplification

Rumen content samples were thawed and microorganism DNA extracted by the CTAB method. Concentration and purification of DNA were carried out by 1% agarose gel electrophoresis for 40 min (voltage 100 V).

V3 and V4 regions of bacterial 16S rRNA genes were amplified using the primer sequence(5'-3')341F:CCTAYGGGRBGCASCAG.806R: GGACTACNNGGGTATCTAAT. High-efficiency, high-fidelity PCR enzymes and Phusion® High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs) were used to maximize PCR amplification efficiency and accuracy. Equal amounts of PCR reagents and samples were thoroughly mixed and PCR products detected by 2% agarose gel electrophoresis. Target strips were recovered using a Qiagen gel recovery kit and a TruSeq® DNA PCR-Free Sample Preparation Kit was used to construct the library. The library was quantified using Qubit and Q-PCR and sent to Beijing NovaSeq PE250 for 16S rRNA high-throughput sequencing.

Data Analysis

Raw reads from the Illumina Nova sequencing platform were spliced according to overlap while sequence quality was controlled and filtered. After filtering, sequences with high similarity ($\geq 97\%$) were grouped into a classification operation unit (OTU), sequences were compared and analyzed, and species classification was applied using RDP Classifier 2.2, the taxonomic information of OTU was obtained. According to the above taxonomic information, the sample dilution curve [13] was drawn and ACE index, Chao1 index, Shannon index and Simpson index were calculated. SPSS 25.0 statistical software was used for single factor analysis of variance. Duncan's method was used for multiple comparisons, $p < 0.05$ being regarded as significant, and data were expressed as mean \pm standard error.

Results

Effects of fermented mushroom residue feed on goat growth performance

Body weight (BW) and average daily gain (ADG) in group L were significantly higher than in the other three groups ($p < 0.05$), average daily feed intake (ADFI) in group L were significantly higher than groups C and H ($p < 0.05$). Feed to gain ratio (F/G) in group L was lower than control group C, but not significantly ($p > 0.05$), and was significantly lower than groups M and H ($p < 0.05$). There was no significant difference between ADG in groups M and C ($p > 0.05$), which were both significantly higher than group H ($p < 0.05$). ADFI in group M was significantly higher than groups C and H ($p < 0.05$). F/G in group M was significantly higher than groups C and L ($p < 0.05$). In group H, BW and ADG were significantly lower than group C ($p < 0.05$), while ADFI and F/G were significantly higher ($p < 0.05$) (Fig. 1, Table 3).

Table 3
The effect of mushroom residue fermented feed on goat growth performance

Items	Groups				<i>P</i> -value
	C	L	M	H	
BW(Kg)	33.40 ± 0.26 ^b	35.90 ± 0.31 ^a	33.17 ± 0.74 ^{bc}	31.50 ± 0.64 ^c	0.003
ADG(g)	129.17 ± 0.96 ^b	152.78 ± 1.94 ^a	129.44 ± 2.17 ^b	116.39 ± 4.09 ^c	0.00
ADFI(Kg)	1.50 ± 0.02 ^c	1.67 ± 0.01 ^a	1.64 ± 0.01 ^a	1.57 ± 0.01 ^b	0.00
F/G	11.64 ± 0.11 ^b	10.94 ± 0.20 ^b	12.65 ± 0.17 ^a	13.53 ± 0.53 ^a	0.001
Note: Different lowercase letters in the same line significant differences (<i>P</i> < 0.05). The table below is the same					

Effects of fermented mushroom residue feed on goat rumen microbial diversity

Sequencing and OTU analysis

Paired ends were sequenced on the Illumina Nova platform and sequences with 97% consistency (identity) were clustered into a total of 1309 OTUs. Venn diagrams intuitively illustrate the common and unique OTUs among sample groups to show the degree of similarity of microbial communities. Groups C, L, M, and H had a total of 1056 core OTUs (accounting for 80.67% of all OTUs) with 7, 9, 6, and 10 OTUs being unique to the four respective groups (Fig. 2a). By drawing a sparse curve of the observed species index (rarefaction curve) the depth of sequencing of the samples was determined to be sufficient to reflect the microbial diversity in the community samples. The curves gradually plateau, indicating that the volume of sequencing data is reasonable and has sufficient depth (Fig. 2b).

Alpha diversity analysis

Chao1 and ACE indices represent species richness while Shannon and Simpson indices represent the structural diversity of flora. These indices showed no significant differences among the treatment groups (*p* > 0.05), but the highest mean values for Simpson and Shannon indices were seen in group H, and for Chao1 and ACE indices in group L (Fig. 3, Table 4).

Table 4
Alpha diversity index

Items	Groups				<i>P</i> -value
	C	L	M	H	
Shannon index	7.53 ± 0.14	7.53 ± 0.20	7.62 ± 0.10	7.65 ± 0.22	0.942
Simpson index	0.98 ± 0.00	0.98 ± 0.01	0.98 ± 0.01	0.98 ± 0.01	0.949
Chao1 index	1049.72 ± 26.83	1068 ± 29.49	1053.58 ± 19.91	1051.25 ± 28.23	0.957
ACE index	1053.75 ± 23.14	1065.69 ± 30.30	1049.19 ± 12.87	1044.54 ± 20.76	0.920

Beta diversity analysis

Changes in rumen microbial community structure in the treatment groups were detected by Principal coordinates analysis (PCoA). The contribution rates of the first and second principal components were 21.05% and 14.55%, respectively. Group L samples were distributed to the left of the PCoA plot while other groups were distributed to the right, indicating differences in rumen microbial communities between groups (Fig. 4). Figure 5 shows that the beta diversity index of groups L and H were significantly higher than group C ($p < 0.05$), further demonstrating that the structure of the rumen flora changed.

Relative abundance of rumen microorganisms

Phylum level abundance

Seventeen different phyla were detected in the rumen samples. *Firmicutes* and *Bacteroidetes* dominated, accounting for over 90% of the total bacteria (Fig. 6, Table 5). The abundance of these phyla differed between treatment groups, the highest abundance of *Firmicutes* being in group L (55.34%) and the highest *Bacteroidetes* in group H (49.40%). Group H had the highest relative abundances of *Synergistetes* (2.15%), *Spirochaetes* (0.42%), and *Elusimicrobia* (0.12%).

Table 5
Relative abundance of phylum horizontal microorganisms (%)

Items	Groups				P-value
	C	L	M	H	
Firmicutes	49.14 ± 2.07	55.34 ± 4.8	42.6 ± 3.09	43.9 ± 4.44	0.15
Bacteroidetes	46.77 ± 2.24	39.49 ± 4.34	49.37 ± 2.94	49.4 ± 3.47	0.20
Tenericutes	1.08 ± 0.12	1.45 ± 0.27	2.92 ± 1.09	1.15 ± 0.23	0.16
Proteobacteria	1.68 ± 0.16	2.07 ± 0.86	2.13 ± 0.45	2.11 ± 0.08	0.91
Synergistetes	0.41 ± 0.08 ^b	0.42 ± 0.21 ^b	0.67 ± 0.36 ^b	2.15 ± 0.72 ^a	0.05
Euryarchaeota	0.03 ± 0.01	0.17 ± 0.17	1.07 ± 0.62	0.12 ± 0.07	0.16
Spirochaetes	0.14 ± 0.03 ^b	0.2 ± 0.05 ^{ab}	0.11 ± 0.01 ^b	0.42 ± 0.14 ^a	0.07
unidentified Bacteria	0.09 ± 0.03	0.14 ± 0.06	0.11 ± 0.02	0.16 ± 0.08	0.82
Elusimicrobia	0.08 ± 0.02 ^{ab}	0.02 ± 0.01 ^b	0.05 ± 0.01 ^{ab}	0.12 ± 0.04 ^a	0.09
Kiritimatiellaeota	0.05 ± 0.02	0.11 ± 0.05	0.03 ± 0.01	0.07 ± 0.02	0.27

Family level abundance

At the family level, sequences were assigned to 52 different families. The most abundant families in the four groups were identified to understand which bacteria were likely to be the most important (Fig. 7 and Table 6). *Ruminococcaceae*, *Prevotellaceae*, *Rikenellaceae*, *Lachnospiraceae*, and *Christensenellaceae* were the most abundant families. In Group L, *Ruminococcaceae* abundance reached 20.79%, which was significantly higher than group H ($p < 0.05$). Abundance of *Veillonellaceae* in Group L was significantly higher than control group C ($p < 0.05$). The abundance of *Prevotellaceae* increased in line with the amount of fermented mushroom residue in the diets, reaching 18.36% in Group H.

Table 6
Relative abundance of level microorganisms in families (%)

Items	Groups				<i>P</i> -value
	C	L	M	H	
Ruminococcaceae	15.87 ± 1.02 ^{ab}	20.79 ± 4.67 ^a	13.4 ± 0.41 ^{ab}	11.33 ± 1.21 ^b	0.12
Prevotellaceae	7.16 ± 1.01	8.78 ± 1.17	11.64 ± 1.45	18.36 ± 6.46	0.18
Rikenellaceae	19.67 ± 2.56 ^a	11.00 ± 2.74 ^b	15.33 ± 1.69 ^{ab}	13.24 ± 1.83 ^{ab}	0.12
unidentified Bacteroidales	2.05 ± 0.57	3.58 ± 0.72	7.28 ± 4.51	3.00 ± 0.66	0.45
Lachnospiraceae	11.46 ± 0.39	11.24 ± 1.37	10.59 ± 0.90	11.33 ± 2.38	0.98
Christensenellaceae	10.82 ± 1.66	9.82 ± 2.23	11.05 ± 1.83	8.63 ± 1.87	0.80
Erysipelotrichaceae	6.03 ± 3.74	3.43 ± 2.17	1.52 ± 0.55	6.27 ± 2.15	0.50
Veillonellaceae	1.85 ± 0.14 ^b	5.6 ± 1.41 ^a	2.42 ± 0.81 ^{ab}	3.22 ± 0.97 ^{ab}	0.09
Acidaminococcaceae	1.25 ± 0.17	2.35 ± 1.20	1.60 ± 0.18	1.36 ± 0.39	0.64
Mycoplasmataceae	0.59 ± 0.08 ^b	0.81 ± 0.21 ^b	2.55 ± 1.02 ^a	0.63 ± 0.09 ^b	0.08

Genus level abundance

At the genus level, 79 different genera were detected in the rumen. Among these, *unidentified_Bacteroidales*, *unidentified_Lachnospiraceae*, and *unidentified_Ruminococcaceae* were the dominant bacteria (Fig. 8 and Table 7). The abundance of *unidentified_Ruminococcaceae* in group H was significantly lower than in groups C and M ($p < 0.05$). The abundance of *unidentified_Prevotellaceae* in group H (1.59%) was significantly higher than in group C ($p < 0.05$).

Table 7
Relative abundance of horizontal microorganisms (%)

Items	Groups				P-value
	C	L	M	H	
unidentified Bacteroidales	2.05 ± 0.57	3.58 ± 0.72	7.28 ± 4.51	3.00 ± 0.66	0.45
unidentified Lachnospiraceae	4.32 ± 0.36	4.17 ± 0.93	4.15 ± 0.54	4.29 ± 0.97	0.10
unidentified Ruminococcaceae	4.00 ± 0.49 ^a	3.28 ± 0.30 ^{ab}	3.98 ± 0.06 ^a	2.74 ± 0.41 ^b	0.10
Succiniclasticum	1.23 ± 0.17	2.30 ± 1.22	1.58 ± 0.19	1.34 ± 0.39	0.66
Mycoplasma	0.59 ± 0.08 ^b	0.81 ± 0.21 ^b	2.55 ± 1.02 ^a	0.63 ± 0.09 ^b	0.08
unidentified Prevotellaceae	0.66 ± 0.13 ^b	0.82 ± 0.12 ^{ab}	1.15 ± 0.07 ^{ab}	1.59 ± 0.46 ^a	0.12
Fretibacterium	0.39 ± 0.08 ^b	0.41 ± 0.20 ^b	0.64 ± 0.35 ^b	2.14 ± 0.71 ^a	0.05
unidentified Rikenellaceae	1.79 ± 0.07	1.23 ± 0.16	2.36 ± 0.41	2.25 ± 0.55	0.18
Saccharofermentans	1.81 ± 0.38	1.60 ± 0.34	1.52 ± 0.42	1.57 ± 0.25	0.94
Bacteroides	0.27 ± 0.04	0.89 ± 0.4	0.33 ± 0.08	0.40 ± 0.05	0.21

Inter-group species differences and KEGG pathway analysis

Linear discriminant analysis effect size (LEfSe) analysis (LDA score > 4) was performed on the relative abundance of species of intestinal flora in group pairs C and L, and L and M, to identify species with significantly differing abundances. In the analysis of groups C and L, the microorganisms labeled in group L at the family level were *Veillonellaceae* (Fig. 9a). In the analysis of groups L and M, *Firmicutes* and *Ruminococcaceae* were labeled at the phylum and family levels in group L (Fig. 9b).

KEGG pathways were predicted based on 16S rRNA gene sequencing data using Tax4Fun to study changes in intestinal microbial function in various groups of samples. Twenty-seven KEGG pathways were identified as varying between groups C and L (Fig. 10a). The pathways related to metabolism of cofactors and vitamins, lipid metabolism, and xenobiotics biodegradation and metabolism were more abundant in group L. Twelve KEGG pathways were identified with differential enrichment in groups L and H (Fig. 10b). Group L showed significant enrichment in replication, recombination and repair proteins, and RNA polymerase pathways. There were 14 KEGG pathways differing between groups L and M, those associated with sporulation, biosynthesis of proteins, and kinases being more abundant in group L (Fig. 10c).

Correlation analysis between KEGG pathways and ADG

To identify potential functional capacities correlated with ADG, Pearson correlation analysis was performed on the relative abundance of KEGG pathways and ADG phenotypic values (Fig. 11). Of the 49 KEGG pathways significantly correlated with ADG ($p < 0.05$), 26, including protein kinases, mineral absorption, propanoate metabolism, and biosynthesis of unsaturated fatty acids, were positively correlated with ADG, while the remaining 23, including the pathogenic *Escherichia coli* infection, phenylpropanoid biosynthesis, shigellosis, and cyanoamino acid metabolism, were negatively correlated.

Discussion

Effects of fermented mushroom residue feed on goat growth performance

Previous studies have shown that adding fermented mushroom residue to the diet can improve growth performance of livestock animals. Fazaeli et al. [14] found that adding 20% fermented *Agaricus bisporus* residue to the diet of fattening sheep significantly increased ADG and ADFI ($p < 0.05$) and reduced F/G. However, when the additive exceeded 20%, feed intake and digestibility were significantly reduced ($p < 0.05$). Song et al. [15] fed Berkshire fattening pigs a basic control diet and added 30%, 50%, and 70% fermented *Pleurotus ostreatus* residue. In the supplemented groups ADFI and feed to gain ratio were significantly elevated ($p < 0.05$). Addition of 30% fermented *Pleurotus ostreatus* residue to experimental pigs diets did not significantly affect ADG ($p > 0.05$), but ADG was significantly higher in the 50% and 70% groups ($p < 0.05$). Kim et al. [16] added high cellulose-decomposing bacteria to *Pleurotus eryngii* residue to promote anaerobic fermentation before feeding to Han Yu bulls. Dry matter intake and BW of the experimental group increased by 15% and 12%, respectively, compared with the control group.

In the current study, fermented mushroom residue had significant effects on BW, ADG, ADFI, and F/G of fattening goats. BW, ADG, and ADFI initially increased then decreased, while F/G initially decreased before increasing in line with the amount of dietary fermented mushroom residue. The beneficial effects were greatest with 20% dietary residue, which is generally consistent with previously published studies. The reason for the improvement in growth performance is likely to be that mushroom residue contains a large amount of crude protein and is rich in minerals, amino acids, and other nutrients that promote animal growth [17,18]. Fermentation of mushroom residue degrades cellulose, hemicellulose and other macromolecular substances to sugars, amino acids, vitamins, and other nutrients which are easily digested and absorbed by ruminants, improving the beneficial feed properties of this material [19,20].

Effects of fermented mushroom residue feed on goat rumen microorganisms

Alpha diversity analysis focused on rumen microbial community richness and diversity. Adding fermented mushroom residue to goat diets improved Shannon, Chao1, and ACE indices, but not significantly. Chao1 and ACE indices were highest in group L, indicating that fermented mushroom residue could increase the richness and diversity of the rumen microbial communities. This is not consistent with the results of Jami et al. [21]. This may be because the composition of gastrointestinal

flora is influenced by many factors, such as dietary nutrition level, heredity, and stress, which lead to differences in microbial community composition [22]. The beta diversity indexes of groups L and H were also significantly higher than control group C ($p < 0.05$), which further indicates that the addition of fermented mushroom residue changes the diversity of microbial communities. This is in agreement with the results of Rey et al. [23]. Following the addition of fermented mushroom residue, the rumen flora in the goats gradually tended to a stable state and reached a dynamic balance.

Gastrointestinal microorganisms can promote the ontogenetic process of animals and participate in animal metabolism. Qin et al. [10] and Ley et al. [24] Studies have consistently shown that *Firmicutes* and *Bacteroidetes* are the dominant bacterial phyla in ruminants. *Firmicutes* are mainly involved in the energy absorption process and *Bacteroidetes* in the carbohydrate metabolism process. When the ratio of *Firmicutes* to *Bacteroidetes* (F/B) in the intestinal tract is relatively high it can be easier to promote the absorption and storage of energy by the host. In this study the main microorganisms in the control and experimental groups were *Firmicutes* and *Bacteroidetes* but their relative abundance varied between groups. Since the F/B ratio was highest in group L (1.46), it can be speculated that 20% dietary fermented mushroom residue promoted the storage of fat in fattening goats. This result is consistent with that of Kittelmann et al. [25] *Firmicutes* is the main phylum responsible for fiber decomposition and contains a large number of cellulose-decomposing fungi [26]. This study showed that the relative abundance of *Ruminococcaceae* (*Firmicutes*) was highest when 20% fermented mushroom residue was incorporated (group L). *Ruminococcaceae* is the major family of fiber-decomposing bacteria in the rumen and can degrade cellulose, hemicellulose, and other macromolecules into nutrients which are easily digested and absorbed by the animal, thus promoting growth [27]. In addition, LEfSe analysis showed that *Veillonellaceae* abundance in group L was significantly higher than in control group C. *Veillonellaceae* metabolize lactic acid into short chain fatty acids (SCFAs), acetate, and propionate via the methylmalonyl-CoA pathway [28]. Scheiman et al. [29] found that lactic acid generated after continuous exercise could be absorbed by *Veillonellaceae* and converted into SCFAs to improve exercise performance. High propionate content is beneficial to ruminant production, its major role being as the main substrate of gluconeogenesis which improves production performance [30].

Bacteroidetes, as one of the dominant phyla in the gastrointestinal tract of animals, accounts for 10–50% of total rumen microorganisms. It is mainly involved in the degradation of non-fibrous carbohydrates and the metabolism of polysaccharides, and plays an important role in animal growth and health [31,32]. In this study, the abundances of *Bacteroides* and *Prevotellaceae* were higher in the experimental groups than control group. The relative abundance of *Prevotellaceae* increased in line with the amount of dietary fermented mushroom residue, reaching 18.36%. This is consistent with studies in dairy cattle [33], beef cattle [34] and sheep [35]. The high nutrient content of fermented mushroom residue promoted *Prevotellaceae* growth until it became the dominant bacterial family in the rumen of the goats. *Prevotellaceae* plays a role in rumen digestion and absorption of carbohydrates through direct and indirect pathways, its abundance in the rumen indirectly reflecting the level of carbohydrate digestion [36,37]. A higher abundance of *Prevotellaceae* in the rumen can also improve protein degradation and utilization [38,39]. Fraga et al. [40] also found that *Prevotellaceae* is involved in the synthesis of SCFAs

and can ferment sugars and lactate through succinic acid and acrylic acid pathways to produce propionic acid, thus improving the rumen environment.

The comparative analysis of intestinal microbial function showed that KEGG pathways related to metabolism of cofactors and vitamins, lipid metabolism, and xenobiotic biodegradation and metabolism were more abundant in group L (20% mushroom residue). The main participant in metabolism of cofactors, vitamins, and pigments such as porphyrin and chlorophyll, is *Bacteroides* [41]. Some studies have shown that *Ruminococcaceae* contribute to lipid metabolism, including synthesis and degradation of ketone bodies. The effect of *Ruminococcaceae* on the lipid metabolism of the host can be mediated via metabolites (such as SCFAs, secondary bile acids, and trimethylamine) produced by the rumen flora and by pro-inflammatory bacterial-derived factors such as fats and polysaccharides [42].

Forty-nine KEGG pathways were identified that were significantly correlated with ADG. Twenty-six pathways, including protein kinases, propanoate metabolism, and biosynthesis of unsaturated fatty acids, were positively correlated with ADG. Shchemelinin et al. [43] found that protein kinases are involved in various pathological processes including malignant tumors. Protein kinase activities are known to fall in chronic myeloid leukemia, gastrointestinal stromal tumors, and various other sarcomas and cancers, as well as non-malignant diseases. They, therefore, function to maintain intestinal health and enhance disease resistance in animals. Propanoate metabolism mediated by rumen flora activates gastrointestinal gluconeogenesis through the enteroencephalic circuit, which is beneficial to promoting body weight gain and controlling blood glucose metabolism [44]. Many unsaturated fatty acids are hydrogenated by rumen microorganisms to produce stearic acid which, when oxidized, provides an energy source for metabolism and can also improve the composition of fatty acids in ruminant meat [45,46]. Twenty-three KEGG pathways, including pathogenic *Escherichia coli* infection and shigellosis, were negatively correlated with ADG. Pathogenic *Escherichia coli* infection can cause diarrhea, acute gastroenteritis, and result in weight loss [47]. *Shigella* adhere to intestinal epithelial cells, destroying the intestinal mucosa and causing ulceration which can lead to intestinal obstruction and diarrhea, adversely affecting the health and growth of the host [48,49].

Conclusion

This study shows that adding 20% fermented seafood mushroom residue to the diet of goats improved growth performance and altered the structure and composition of the rumen flora. Dynamic changes in the flora at phylum, family, and genus levels were observed. Various KEGG pathways were enhanced by the inclusion of dietary mushroom residue. In particular, protein kinases, mineral absorption, propanoate metabolism, and biosynthesis of unsaturated fatty acids played important roles in promoting ADG in goats.

Abbreviations

BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; F/G: feed to gain ratio; OTU: operational taxonomic unit; PCoA: Principal coordinates analysis; LEfSe: linear discriminant analysis effect size; LDA: linear discriminant analysis; KEGG: kyoto encyclopedia of genes and genomes; SCFA: short chain fatty acid.

Declarations

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Authors' contributions

QH conceived and designed the experiments, supervised the experiment progress, revised the manuscript; XH designed the experiments, analyzed the data, wrote and revised the manuscript; YZ and MD performed the experiments, analyzed the data, and wrote the manuscript; XC, and XH performed the experiments. All authors read and approved the final manuscript.

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Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All procedures involving animals were performed in accordance with the guidelines for the care and use of experimental animals approved by the State Council of the People's Republic of China. The project was specially approved by Animal Care and Use Committee in Fujian Academy of Agricultural Sciences.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interest.

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Figures

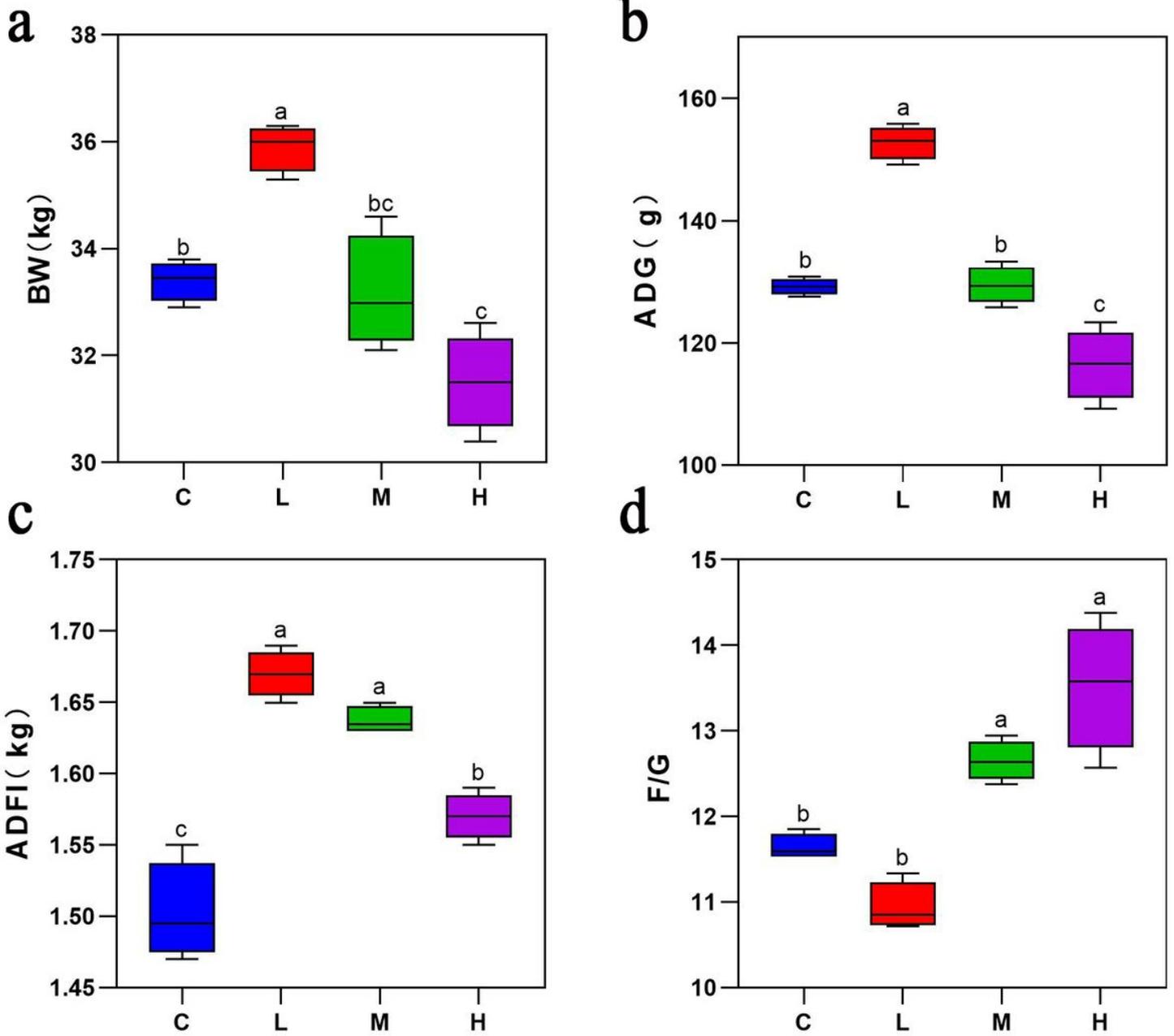


Figure 1

Effect of fermented mushroom residue feed on goat growth performance. (a) BW:body weight; (b) ADG: average daily gain; (c) ADFI: average daily feed intake; (d) F/G: feed to gain ratio. Different lowercase letters in the picture indicate significant differences. C: control group; L: experimental group1; M: experimental group2; H: experimental group 3.

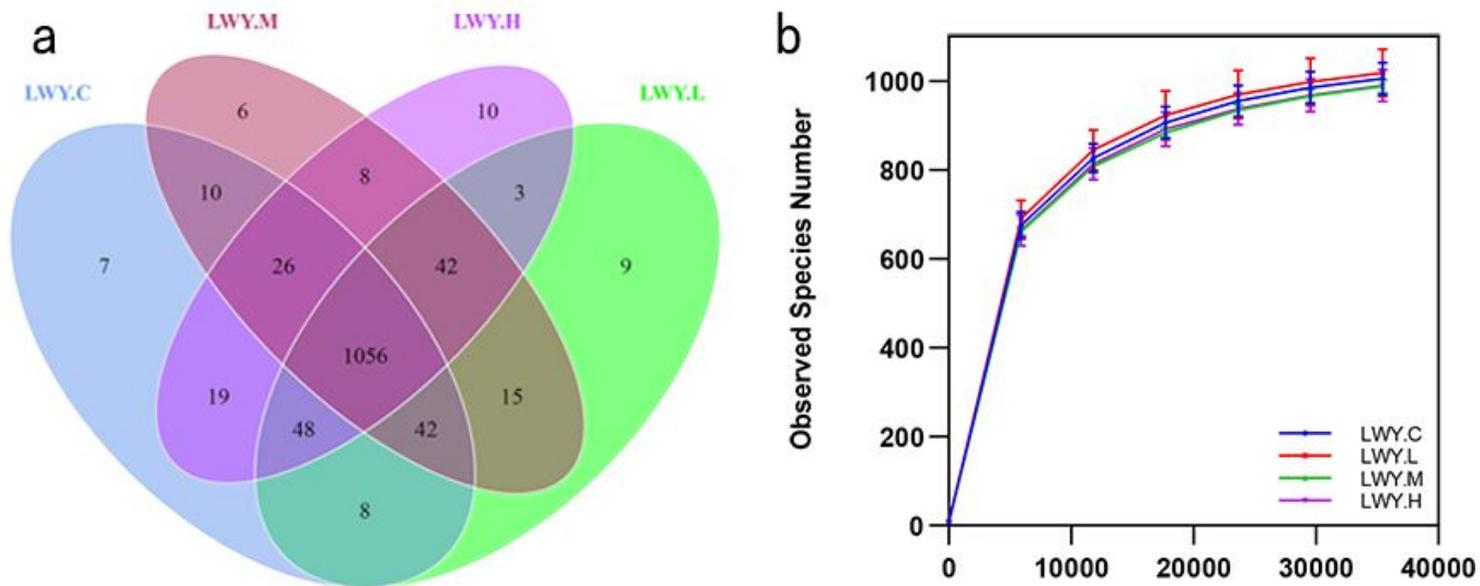


Figure 2

(a) Venn diagram showing the degree of overlap of bacterial OUTs between treatment groups. (b) Sparse curves of the observed species index. LWY C, LWY L, LWY M, and LWY H are rumen fluid samples in groups C, L, M, and H, respectively

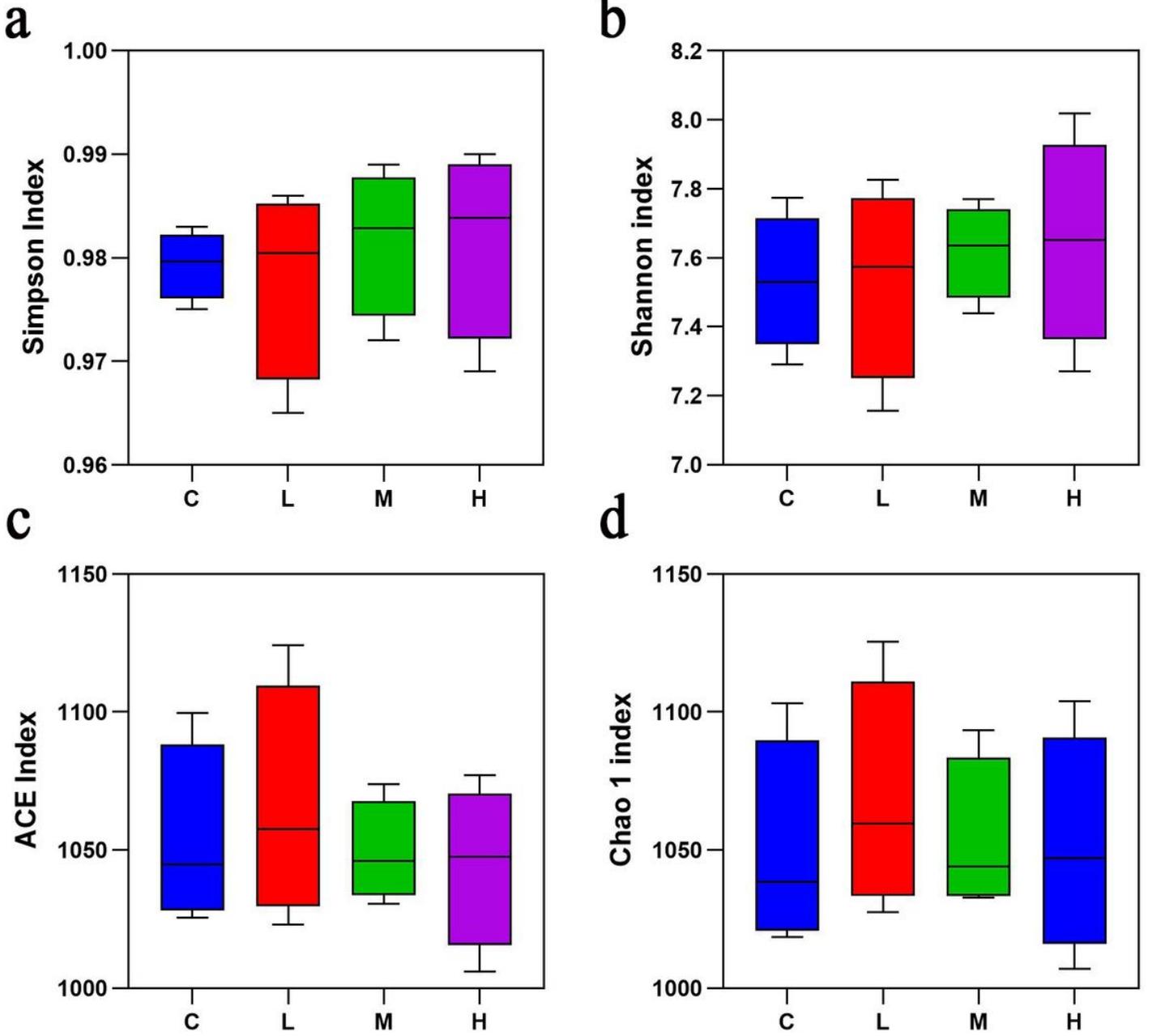


Figure 3

Box diagrams of alpha diversity indices

PCoA - PC1 vs PC2

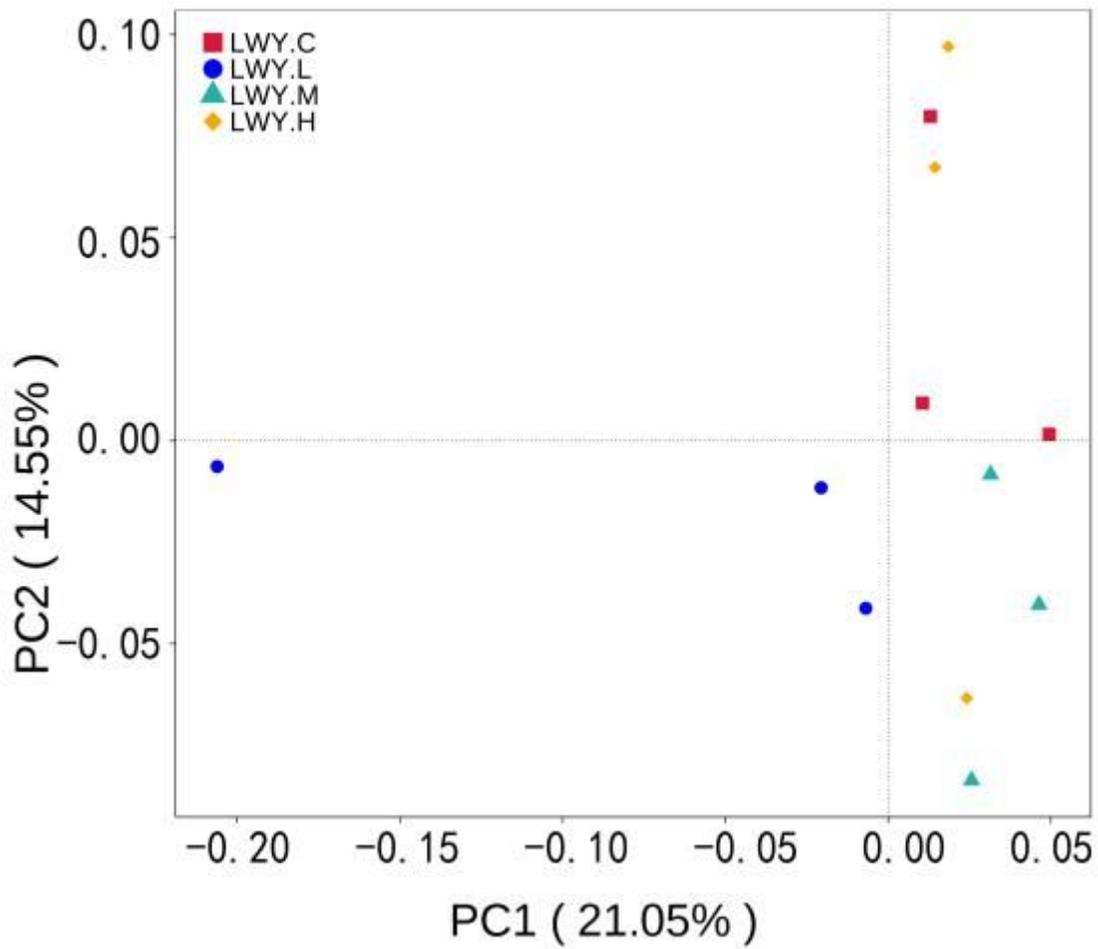


Figure 4

Principal coordinates analysis of rumen microbial community structures based on the unweighted UniFrac distance metric

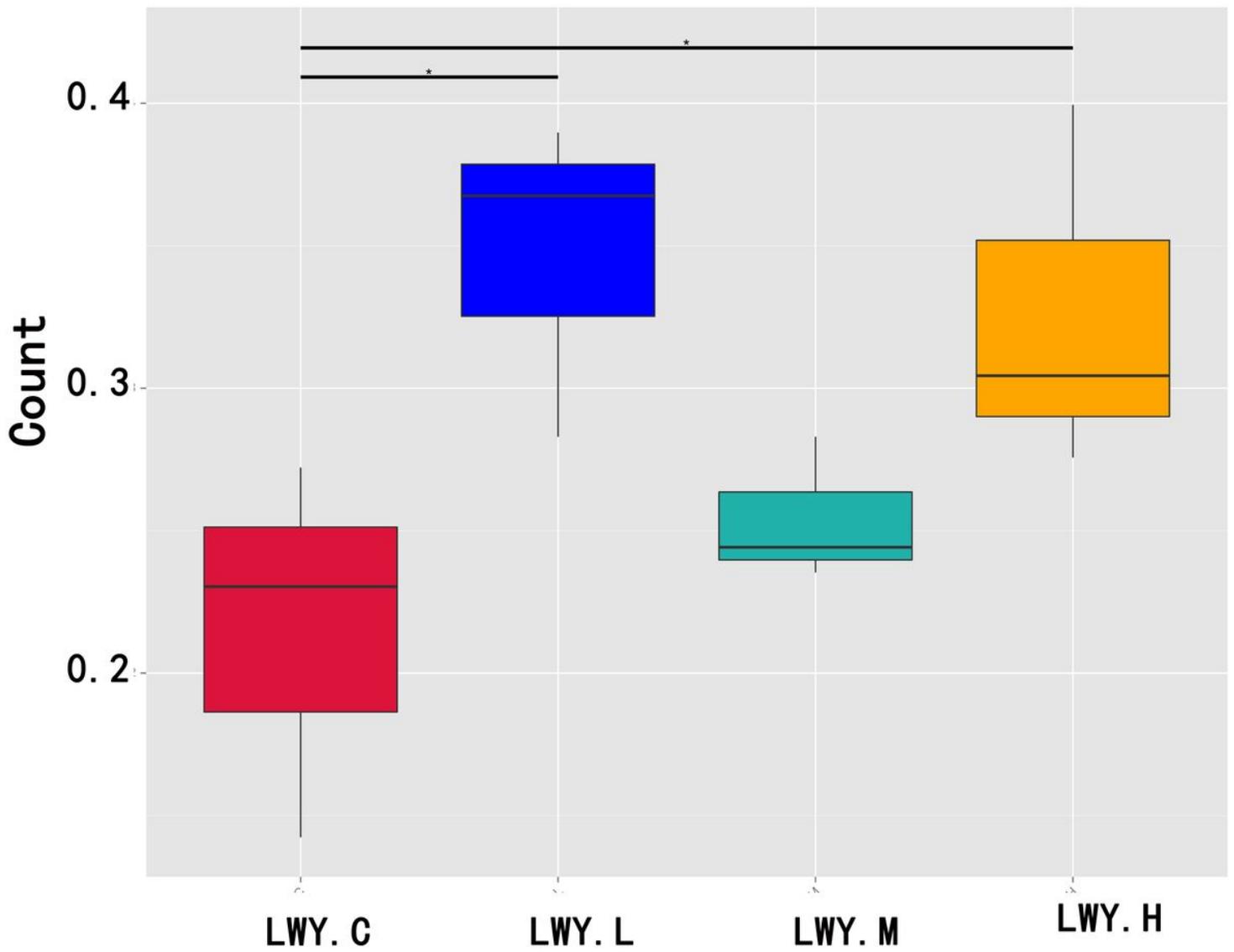


Figure 5

Beta diversity box diagram of rumen microbial community structures based on the weighted UniFrac metric

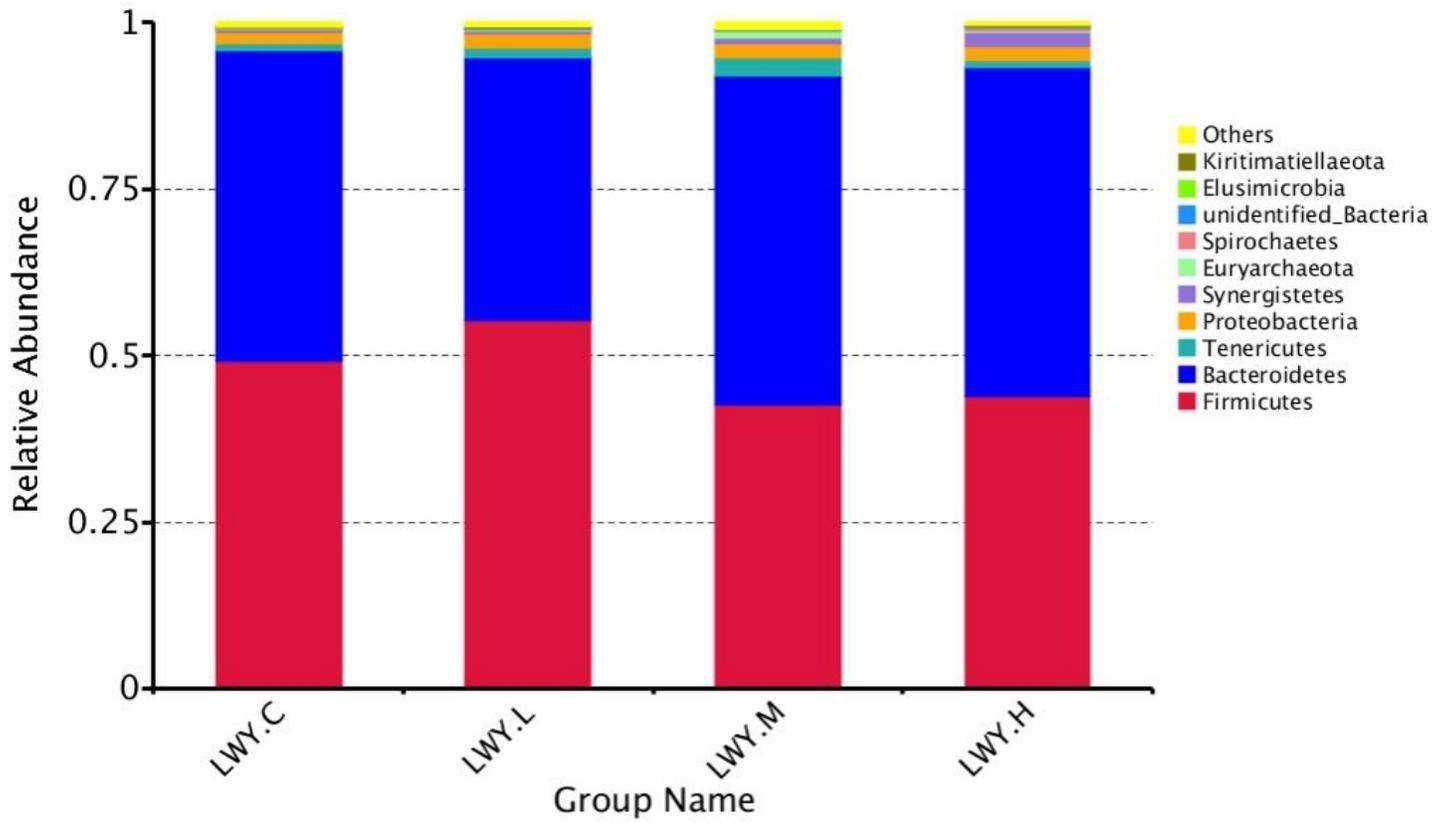


Figure 6

Species detected in rumen contents at phylum level

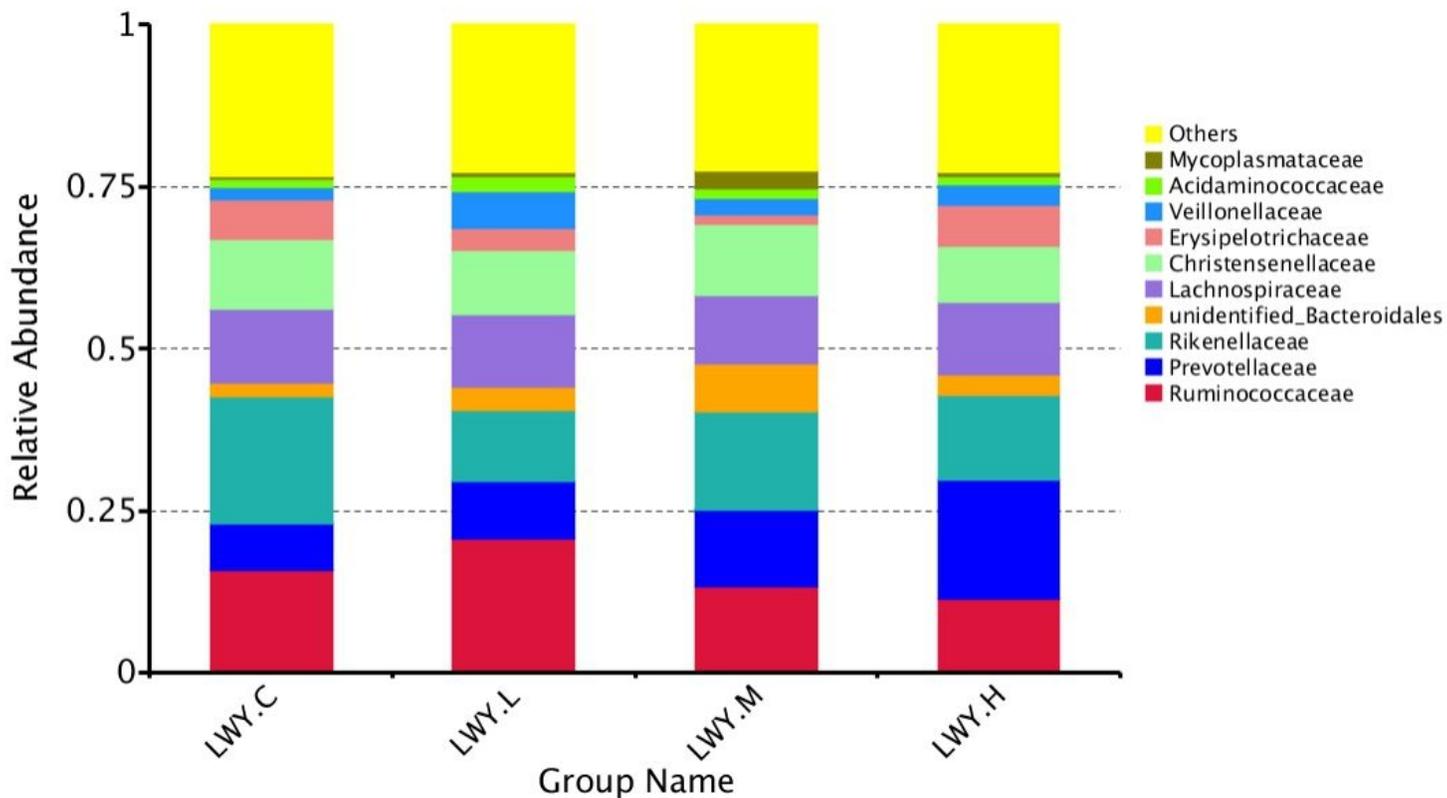


Figure 7

Species detected in rumen contents at family level

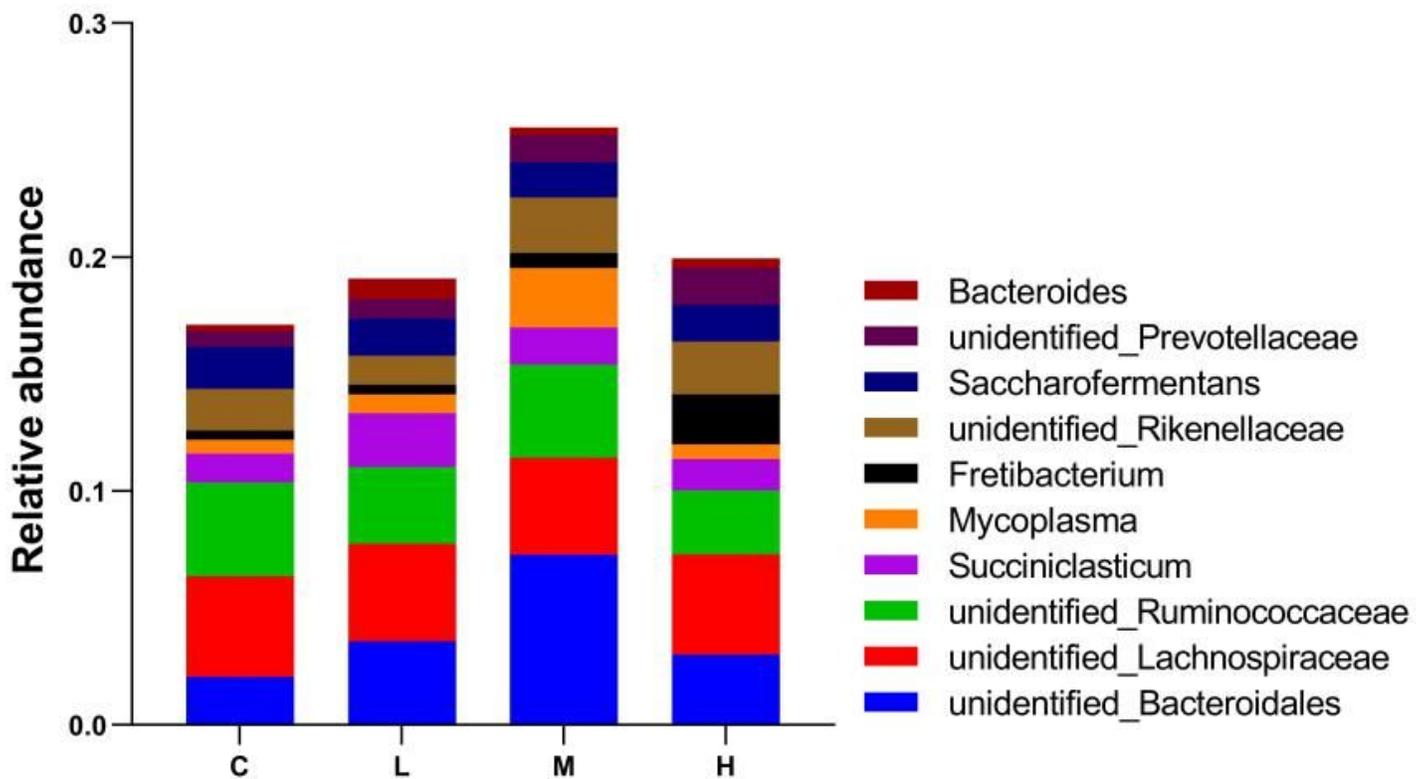


Figure 8

Species detected in rumen contents at genus level

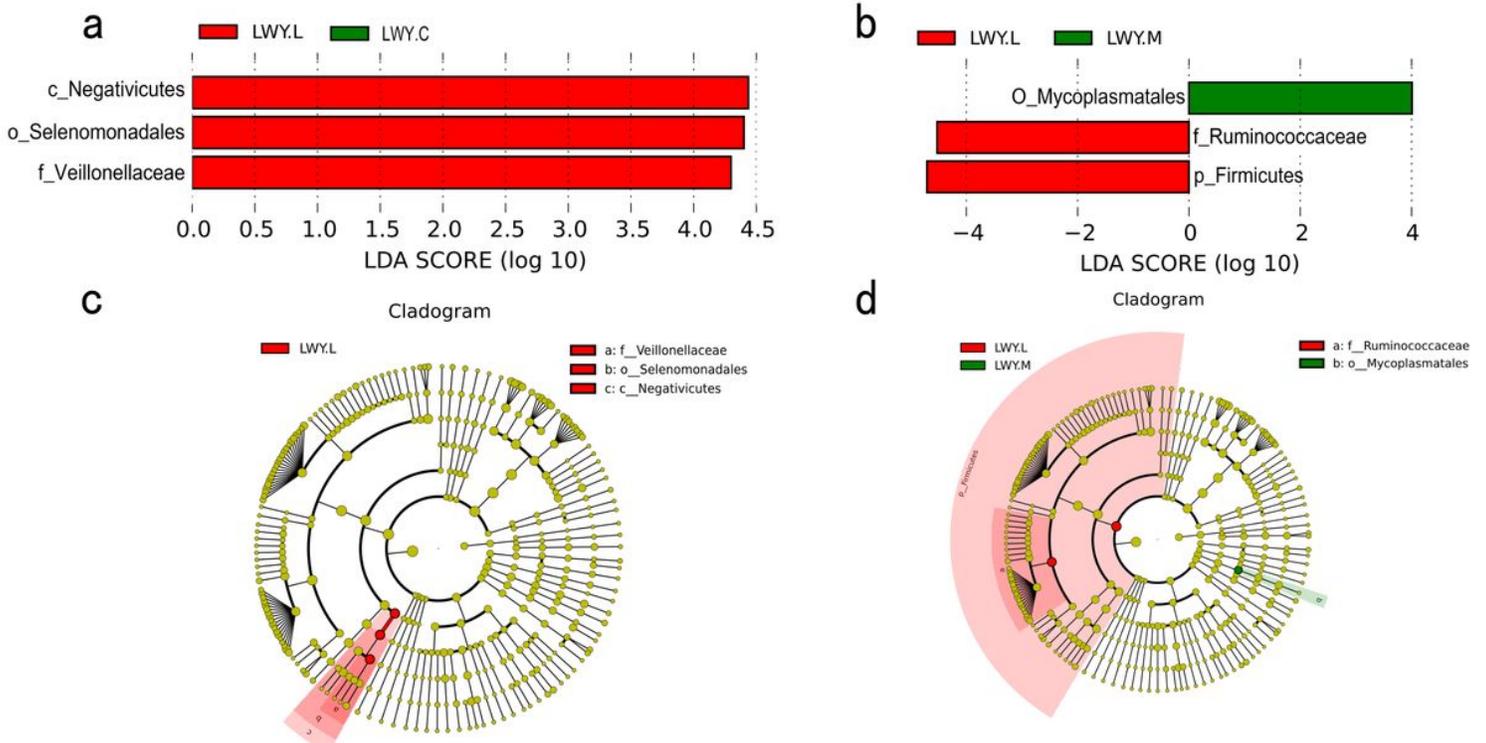


Figure 9

LefSe analysis of goat rumen flora. (a) and (b) Histograms of LDA scores for taxa with differences in abundance between groups C and L, and groups L and M, respectively. LDA score indicates the effect size and ranking of each indicative taxon. (c) and (d) Cladograms of enriched taxa in the gut microbiome of groups C and L, and L and M, respectively. The central point represents the root of the taxonomic tree (Bacteria) with each ring representing the next lower taxon, the diameter representing relative abundance (phylum to genus: p, phylum; c, class; o, order; f, family; g, genus)

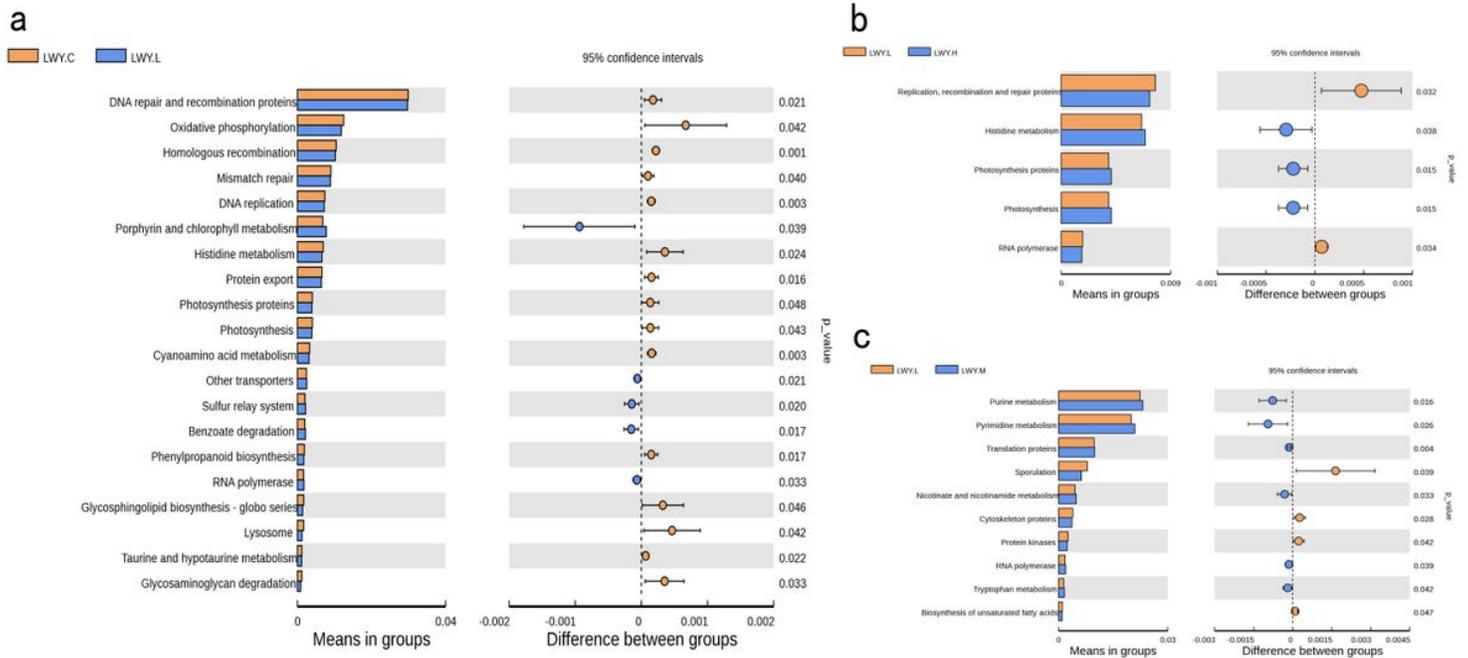


Figure 10

Predicted metagenome functions based on KEGG pathway analysis. Extended error bar plots show the significantly different KEGG pathways between groups C and L (a), L and H (b), and L and M (c)

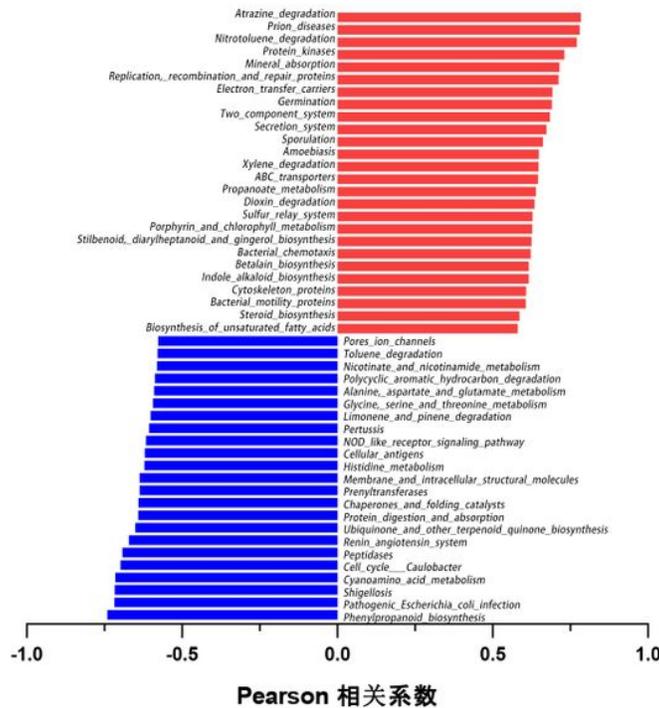


Figure 11

Correlation analysis of average daily gain and KEGG pathways