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Yeast cultures improve lactation performance by influencing rumen microbial composition in dairy goats

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Research Article

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Abstract

This study evaluated the effects of dietary yeast culture (YC) supplementation on rumen microbiota and lactation performance in dairy goats. Twenty mid-lactation dairy goats were selected and divided into two groups: the control (CON) group was fed a basal diet; the YC group was supplemented with 10 g of YC in 1 kg of basal diet. The administration of YC was associated with a significant increase in dry matter intake, milk yield, milk protein yield, and milk lactose yield in dairy goats ($P < 0.05$). Additionally, serum total protein, albumin, creatinine, glucose, superoxide dismutase, and catalase levels were increased ($P < 0.05$). Furthermore, there was an increase in rumen pH and NH_3-N levels ($P < 0.05$), while volatile fatty acid levels were observed to decrease ($P < 0.05$). The study found no significant difference in the α-diversity of bacteria and fungi between the YC and CON groups ($P > 0.05$). However, 15 bacterial genera and 13 fungal genera were upregulated ($P < 0.05$), while 4 bacterial genera and 11 fungal genera were downregulated ($P < 0.05$) in the YC group. The relative abundance of pathogenic fungi *Dipodascus* and *Gibberella* decreased ($P < 0.05$). Correlation analysis revealed that the bacterial genera were not significantly correlated with lactation performance ($P > 0.05$), whereas fungal genera *Dipodascus* and *Gibberella* were significantly ($P < 0.05$) correlated with lactation performance. In conclusion, the study demonstrated that YC can influence the rumen microbial composition, reduce the abundance of harmful fungi in the rumen, and improve lactation performance in dairy goats, suggesting that the addition of YC to dairy goat diets has good application prospects.

Introduction

In recent years, the market demand for goat milk products has increased, and there has been a growing focus on how to improve the lactation performance of dairy goats. Farmers use feed additives to enhance the performance of their livestock. Yeast culture (YC) is a safe and reliable new feed additive that has been the focus of much attention in recent years. YC is a natural yeast fermentation product containing various bioactive substances such as yeast cells, vitamins, peptides, amino acids, proteins, organic acids, and oligosaccharides (Jensen et al. 2008). Dietary YC supplementation affects the performance by altering gastrointestinal microbial composition, which has been reported in dairy calves (Magalhaes et al. 2008), pigs (Lin et al. 2022), poultry (Zhang et al. 2020), and fattening lambs (Song et al. 2021). Moreover, supplementation of YC in dairy cows' diets can also increase milk yield and improve milk quality (Halfen et al. 2021). However, the effect of YC is variable, and its mechanism in dairy goat production remains unclear. Therefore, the relationship between YC and lactation performance requires further exploration.

The rumen, as the bioreactor of ruminants, utilizes rumen microbes to degrade feed nutrients and produce volatile fatty acids (VFAs), peptides, and ammonia that directly or indirectly affect the lactation performance of the host (Russell et al. 2001; Xue et al. 2020). Thus, in complex digestive and metabolic processes, rumen microorganisms play a critical role. Diet is the main factor that affects the composition of the rumen microbiota (Ghaffari et al. 2014). In studies on the effects of YC on rumen microbial composition, more attention has been paid to bacteria (Jiang et al. 2020) and less to fungi. Fungi are the least characterized group of rumen microorganisms, but they are essential for plant digestion in the rumen (Wang et al. 2022), and it has also been shown that rumen anaerobic fungi, which ferment cell wall carbohydrates (cellulose and hemicellulose) to produce VFAs, provide energy for the host animal and affect animal performance (Rabee et al. 2019). A substantial degree of fiber degradation is achieved by species of Piromyces, Neocallimastix, Orpinomyces, and

Table 1. Nutrient level of yeast cultures

Items	CP[1]	EE[2]	CF[3]	Water	Ash
Percentage (%)	≥18	≥1.5	≤12	≤12	≤3.0

[1]CP, crude protein;
[2]EE, ether extract;
[3]CF, crude fiber.

Ruminomyces (Wubah 2004). Both bacteria and fungi play an important role in the fermentation process in the rumen (Langda et al. 2020). Therefore, the effect of YC on the composition of bacteria and fungi in rumen of lactating dairy goats deserves further attention.

Therefore, the effects of YC on the lactation performance of dairy goats during lactation were investigated in this experiment. The effects of YC on the composition of rumen bacteria and fungi were revealed by 16S and ITS (Internal Transcribed Spacer) assays, and the relationships between microbial changes and rumen environment, serum indices, and lactation performance were analyzed and discussed. This study provides a theoretical basis for clarifying the application value of YC in dairy goat production.

Materials and methods

Ethics approval

The Animal Care and Utilization Committee of Northwest Agriculture and Forestry University (Yangling, Shaanxi, China) approved all experimental protocols used in this study, including dairy goat feeding tests, rumen fluid collection, blood collection, and fecal collection (protocol no. DK2022008). The protocols conformed to the university's guidelines for animal research.

Animals and experimental design

Twenty mid-lactation Saanen dairy goats (lactation period around 150 days) with similar health status and body weight, all of which in their second lactation, were selected from Xinlongmen Goat Farm, Lantian County, Xi'an, Shaanxi Province. They were randomly divided into two groups of ten animals each. The experimental groups were as follows: the control (CON) group was treated with a basal diet (concentrate: forage ratio of 4:6); the YC group was fed the basic diet supplemented with 10 g/kg YC. YC used in this study was provided by Xi'an Xinhanbao Biotechnology Co., Ltd. Table 1 presents the nutritional composition of YC, respectively. Table 2 shows the composition of the basal diet. Dairy goats were fed twice daily (8:00 am and 4:00 pm) and had free access to water in a clean and hygienic environment. After a 10-day adaption, daily feed intake and milk production were continuously recorded for 8 weeks.

Milk production and composition

Milk production was recorded daily during the experiment. Milk samples were collected in the morning and afternoon on days 0, 14, 28, 42, and 56. The samples were mixed in a 3:2 ratio, treated with preservatives, and sent to the nearest Xi'an Animal Husbandry Station for milk composition and somatic cell count (SCC) analysis.

Feed sampling, fecal sampling, and chemical analysis

Feed intake was measured and recorded daily at the beginning of the study. During the last 3 days of the experiment, the remaining feed and fecal samples were collected continuously. Fecal samples up to 10 g were collected with Polyethylene (PE) gloves and stored at −20°C. The samples were dried in an oven at 105°C ± 2°C under atmospheric pressure to constant weight and the dry matter content was determined. Crude protein (CP) was determined according to AOAC Official Method 988.05 Protein (Crude) in Animal Feed and Pet Food, and crude fat (EE) was determined according to the standard method (ISO 6492:1999). Neutral detergent fiber (NDF) was determined according to AOAC Official Method 2002.04, acid detergent fiber (ADF) was determined according to AOAC Official Method 973.18, and ash insoluble in hydrochloric acid was used as a marker in the digestibility study and determined by reference to ISO (5985:2002), and then the apparent digestibility of CP, NDF, and ADF was calculated using the following formula:

$$\text{Apparent digestibility } (\%) = \left(1 - \frac{\text{a } (\%)}{\text{c } (\%)} \times \frac{\text{b } (\%)}{\text{d } (\%)}\right) \times 100$$

The letters in the formula mean that a = fecal nutrients, b = dietary acid insoluble ash, c = fecal acid insoluble ash, and d = dietary nutrients.

Table 2. Ingredients and nutrient levels in the basal diet, % (as-fed basis)

Items	Content
Ingredients	
Whole corn silage	22.02
Chinese wildrye	14.77
Maize	32.88
Wheat bran	7.66
Soybean meal	5.53
Barley	15.21
$CaHPO_4$	0.32
NaCl	0.32
$NaHCO_3$	0.97
Premix[1]	0.32
Total	100
Nutrient levels[2]	
NEmf/(MJ/kg)	5.88
CP	11.04
NDF	35.79
ADF	21.59
Ca	0.40
Total phosphorus	0.26

[1]The premix provided the following per kilogram of the diet; Vitamin A 3 000 IU, Vitamin D 300 IU, Vitamin E 15 IU, Fe 30 mg, Cu 8 mg, Zn 30 mg, Mn 40 mg, I 0.25 mg, Se 0.1 mg, Co 0.1 mg.
[2]Nutrient levels were calculated values; NEm. was a calculated value according to ingredient composition, while the others were measured values.

Serum indices

On the 56th day of the test, 10 mL of blood was collected from the jugular vein using a coagulant tube, and the serum was separated by centrifugation at 3,000 × *g* for 10 min at 4°C and stored at −20°C until analysis. Total protein (TP), albumin (ALB), creatinine (CR), blood urea nitrogen (BUN), γ-glutamyl transferase

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(GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (Glu), triglyceride (TG), and total cholesterol (TCHO) were detected by a fully automatic biochemical analyzer (Roche cobasc800). Kits for the detection of superoxide dismutase (SOD), malondialdehyde (MDA), total antioxidant capacity (T-AOC), glutathione peroxidase (GSH), catalase (CAT), immunoglobulin A (IgA), immunoglobulin M (IgM), and immunoglobulin G (IgG) were purchased from Shanghai Preferred Bioscience & Technology Co. Ltd in China. Kits for the determination of estrogen (E) and prolactin (PRL) were purchased from Nanjing Jiancheng Reagent Company in China. All experimental methods and steps were performed according to the instructions provided with the kits.

Rumen fermentation

On the 56th day of the experiment, 50 mL of rumen fluid was collected from each goat through a rumen catheter, and its pH was immediately determined using a pH-3B high precision pH meter. NH_3-N was determined by the phenol-sodium hypochlorite colorimetric method (Mendoza et al. 2011), and VFA was determined by gas chromatography (Agilent 7890A, USA). The remaining rumen fluid samples were placed in liquid nitrogen and finally stored at −80°C for subsequent analysis of rumen microbial composition.

16S rRNA and ITS gene sequencing

To determine rumen microbiota, rumen fluid samples were thawed overnight at 4°C. Rumen fluid DNA from the test dairy goats was extracted using the TIANamp Stool DNA Kit. DNA quality was detected by electrophoresis and NanoDrop 2000 spectrophotometer. After quality control, the DNA was divided into two parts for the determination of 16S and ITS, respectively. Amplification primers used for 16S and ITS sequencing were different, 341F and 806R for 16S rRNA (V3–V4 region) and ITS1-1F and ITS1-1R for ITS (ITS1 region), and specific primer information was listed in Table S1. The library was then constructed using the TruSeq® DNA PCR-Free Sample Preparation Kit, the NovaSeq6000 was used for onboard sequencing, and 250 bp paired-end reads were generated. Next, the data were subjected to paired-end reads assembly and quality control, including Data split, Sequence assembly (Mago et al. 2011), Data filtration (Bokulich et al. 2013; Caporaso et al. 2010), and Chimera removal (Edgar et al. 2011), to obtain effective tags. Operational taxonomic units (OTUs) were clustered with a 97% identity and analyzed for species classification based on the clean data. According to the clustering results of OTUs, the representative sequences of each OTU were annotated with species, and the corresponding species information and species-based abundance distribution were obtained, which were used for the calculation and analysis of α-diversity, β-diversity, and principal coordinate analysis (PCoA). Alpha diversity indices in our samples were calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2. 15.3). Beta diversity were calculated by QIIME software (Version 1.9. 1). PCoA analysis was displayed by ade4 package and ggplot2 package in R software (Version 2. 15.3).

Statistical analysis

Differential microbial composition was analyzed by Wilcoxon rank-sum test. Spearman correlation analysis was conducted to generate bacterial–fungal interaction network. SPSS was used to process and analyze the experimental data. The effects of YC on lactation performance indices, serum indices, and rumen fermentation parameters were analyzed by *t*-test. GraphPad Prism 8.0 was used to perform Pearson correlation analysis to study the relationship between microorganisms and lactation performance, rumen environment, and other factors.

Results

Lactation performance

Lactation performance was measured, and the results showed that feeding YC could improve dry matter intake (DMI), milk yield, and milk lactose yield but decreased milk protein rate ($P < 0.05$). There were no significant differences in milk fat rate, milk fat yield, milk protein yield, and milk lactose rate (Fig. 1A, $P > 0.05$). There were no significant differences in SCC, CP, ADF, and NDF of dairy goats (Table S2).

Serum indices

Compared with the CON group, dairy goats fed with YC had a higher levels of TP, ALB, CR, Glu, SOD, and CAT (Fig. 2A, $P < 0.05$), whereas no significant differences for BUN, GGT, ALT, AST, TG, TCHO, MDA, T-AOC, GSH, E, PRL, IgA, IgM, and IgG were observed among the two groups (Table S3).

Rumen fermentation parameters

To investigate the impact of changes in rumen microbial composition on the rumen environment, we measured the pH, NH_3-N, and VFA content of rumen fluid. The results showed that the pH and NH_3-N levels in the YC group were significantly higher than those in the CON group (Fig. 3A, $P < 0.05$), and concentrations of total VFA, acetate, propionate, and butyrate in YC group were significantly reduced (Fig. 3B, $P < 0.05$), while the levels of iso-butyrate, valerate, iso-valerate, and acetate:propionate were not significantly different (Table S4, $P > 0.05$).

Microbial composition of rumen of dairy goats

To investigate the effect of YC on the rumen environment of dairy goats, 16S sequencing and ITS sequencing were conducted. As shown in Fig. 4A and 4B, the rank abundance curves of bacteria and fungi leveled off, indicating sufficient and feasible sequencing data with more than 99.5% coverage of bacteria and fungi (Table 4). The α-diversity indices, including observed_species, Shannon, Simpson, Chao1, and ACE indices, indicated no significant difference between the YC and CON groups ($P > 0.05$) (Table 3). PCoA showed that there was no significant separation between the YC and CON groups based on the bacterial results obtained by 16S sequencing (Fig. 4C) and the fungal results obtained by ITS sequencing (Fig. 4D). At the phylum level, Firmicutes (average of two groups, 47.66%), Bacteroidota (32.89%), and Euryarchaeota (7.90%) were the dominant bacterial communities in the rumen of dairy goats (Fig. 4E). At the genus level, the dominant bacterial genera were *Rikenellaceae_RC9_gut_group* (11.57%), followed by *Prevotella* (11.47%), *Ruminococcus* (8.02%), and *Methanobrevibacter* (7.83%) (Fig. 4F). At the phylum level of fungi, the dominant microbial community was Basidiomycota

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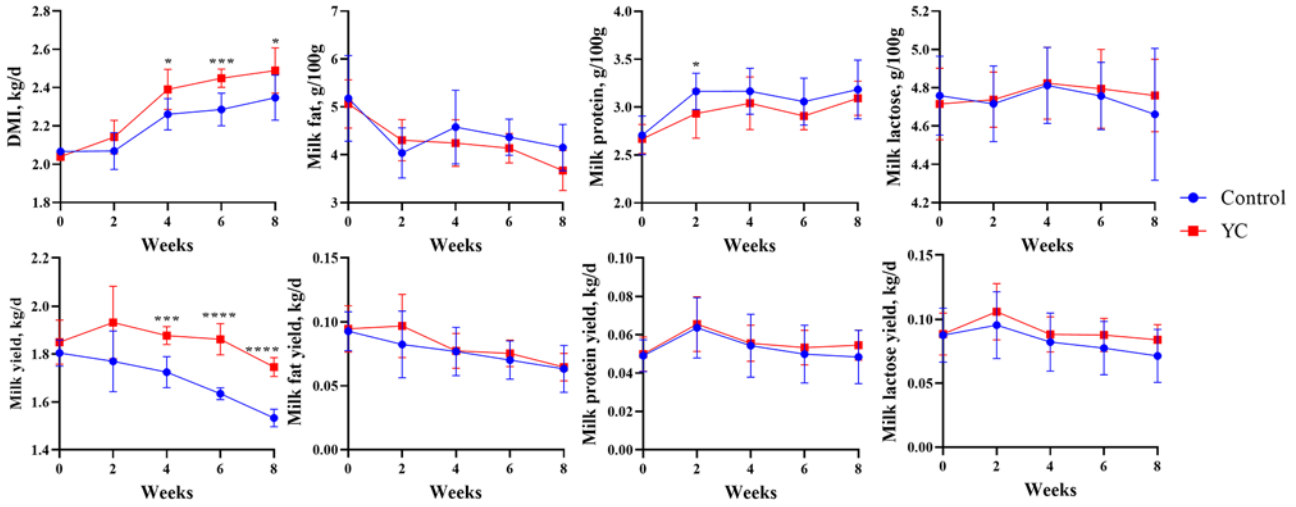

Figure 1. Effect of yeast cultures on lactation performance of dairy goat. Changes in indicators related to lactation performance at weeks 0, 2, 4, 6, and 8 mean value for each group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3. Effect of YC on α-diversity of bacteria and fungi in rumen

	Bacteria				Fungi			
Item	CON	YC	SEM	*p*-value	CON	YC	SEM	*p*-value
Observed	1703.333	1862.166	54.554	0.153	1219.500	1129.666	99.958	0.675
Shannon	8.278	8.323	0.120	0.861	5.001	5.073	0.157	0.830
Simpson	0.987	0.987	0.001	0.936	0.893	0.896	0.006	0.803
Chao1	1821.306	2006.127	59.941	0.128	1366.269	1234.143	111.458	0.578
ACE	1829.046	2014.685	60.765	0.132	1394.323	1256.327	112.616	0.565
Goods	0.995	0.995	0.0001	0.209	0.996	0.997	0.0003	0.473

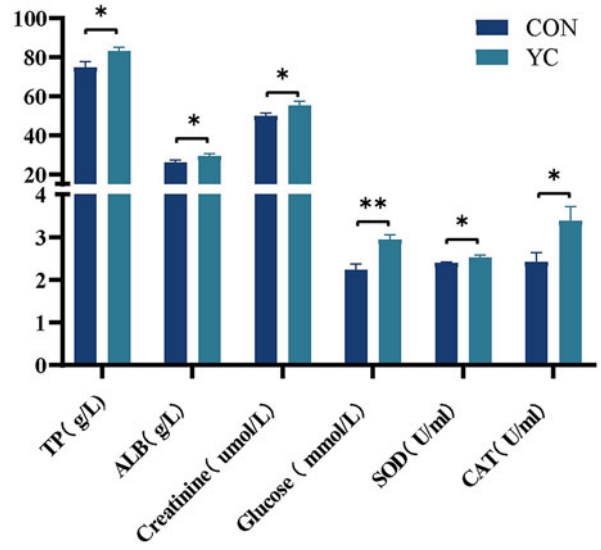

Figure 2. Serum parameter difference and correlation analysis. Serum indicators of differences between YC and CON. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

(54.17%) and Ascomycota (11.22%) (Fig. 4G), and at the genus level, the dominant fungi were *Wallemia* (52.87%), followed by *Dipodascus* (2.21%) and *Aspergillus* (1.49%) (Fig. 4H).

Differences in rumen microbial composition

To compare the effect of YC on the microbial composition of the rumen, Wilcoxon rank-sum test analysis revealed that, at the genus level, 19 bacterial genera differed by relative abundance. The YC group showed an increase in 15 bacterial genera and a decrease in 4 bacterial genera compared to the CON group (Fig. 5A, $P < 0.05$). At the genus level, 24 distinct fungal genera were found, with the YC increasing the relative abundance of 13 fungal genera and decreasing the relative abundance of 11 fungal genera (Fig. 5B, $P < 0.05$).

Microbial function prediction

Through PICRUSt2 function prediction and differential pathway enrichment analysis (Fig. S1), we focused on 22 differential metabolic pathways (Fig. 6A, $P < 0.05$). We noted that in the YC group, the flavone and flavonol biosynthesis, valine, leucine and isoleucine degradation, betalain biosynthesis, limonene and pinene degradation, fatty acid degradation, butanoate metabolism, benzoate degradation, folate biosynthesis, beta-alanine metabolism, ascorbate and aldarate metabolism, glutathione metabolism, phenylalanine metabolism, metabolism of xenobiotics by cytochrome P450, tyrosine metabolism, and inositol phosphate metabolism metabolic pathways were upregulated, and carbon fixation in photosynthetic organisms, terpenoid backbone biosynthesis, biosynthesis of various secondary metabolites – part

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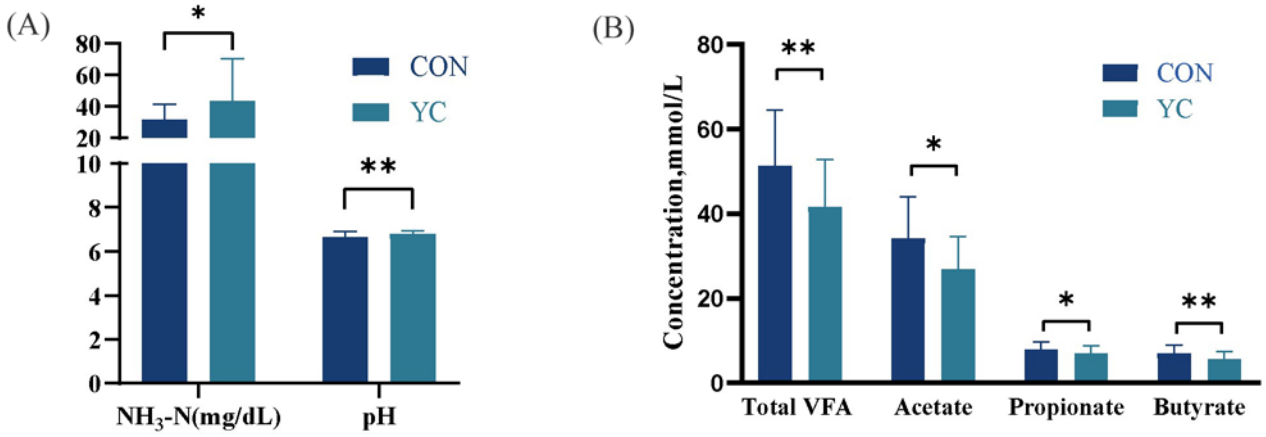

Figure 3. Rumen fermentation parameters and correlation analysis. (A) NH_3-N and pH levels in the rumen of CON and YC. (B) Concentration of volatile fatty acids in the rumen.

1, biosynthesis of ansamycins, D-alanine metabolism, biosynthesis of secondary metabolites, and peptidoglycan biosynthesis were downregulated. With respect to FunGuild functional prediction of fungi, the results of *t*-test showed that compared with the CON group, the enrichment of plant-undefined-saprotroph and plant-pathogen in the guild classification and pathotroph in the mode classification were significantly reduced (Fig. 6B, $P < 0.05$).

Microbial network

To assess the differential microbes in the center, a network of bacteria, fungi, and bacteria–fungi correlations was constructed by using Spearman correlation analysis ($|R| > 0.6$, $P < 0.05$). The analysis showed that in the bacterial network, *Woeseia, Roseivivax, Leisingera, Rubitalea, Cyanobium_PCC-6307, and Vibrio* were located at the core position (degree = 13) (Fig. 7A). The network results for fungi indicated that the core fungal genera were *Sordaria* (degree = 19) and *Xeromyces* (degree = 17) (Fig. 7B). Next, we further focus on the major bacterial genera correlated with fungi (Fig. 7C) found that *Vibrio* (degree = 13) and *UCG* (degree = 18) have a strong correlation with fungi. On the contrary, fungi *Galactomyces* (degree = 17), *Neocollimasix* (degree = 15), *Byssochlamys* (degree = 14), *Gibberella* (degree = 13), and *Dipodascus* (degree = 14) have a strong correlation with bacteria (Fig. 7C).

Correlation analysis

Next, we wanted to investigate the relationship between rumen microbial composition and rumen fermentation parameters. Correlation analyses showed that *Xeromyces* was positively correlated with pH and negatively correlated with propionate concentration ($P < 0.05$). *Roseivivax, Leisingera*, and *Xeromyces* were negatively correlated with acetate ($P < 0.05$). *Vibrio, Cyanobium_PCC_6307, Xeromyces,* and *Galactomyces* were negatively correlated with total VFA ($P < 0.05$). *Xeromyces* is negatively correlated with butyrate ($P < 0.05$). *Gibberella* was positively correlated with total VFA and butyrate, and *Galactomyces* was positively correlated with pH (Fig. 8A, $P < 0.05$). Redundancy analysis (RDA) showed that bacteria contributed significantly to pH, while fungi contributed significantly to VFA and NH_3-N ($|R| > 0.6$, $P < 0.05$) (Fig. 8B). Correlation analysis of microorganisms with blood parameters and lactating performance reveals that milk fat rate was positively correlated with *Gibberella* level. Milk protein yield was negatively correlated with *Dipodascus*. CP and ADF were negatively correlated with *Neocallimastix*, and NDF was negatively correlated with *Rubritalea* and *Cyanobium_PSC-6307* (Fig. 8C, $P < 0.05$). TP was negatively correlated with *Gibberella* ($P < 0.05$). Glucose was negatively correlated with *Gibberella* and *Dipodascus* and positively correlated with *Sordaria* ($P < 0.05$). There was a significant positive correlation between SOD and *Neocallimastix*, while CAT is positively associated with *Rubritalea, Vibrio, Cyanobium_PCC-6307, Galactomyces*, and *Byssochlamys* ($P < 0.05$).

Discussion

The composition of YC is primarily constituted by yeast extracellular metabolites, which are variable components of the culture medium following fermentation. Additionally, it comprises cell wall components, as well as a limited number of inactive yeast cells (Wang et al. 2023). YCs are employed as a source of protein in lieu of fishmeal and as an immunomodulator for fish (Xv et al. 2021). The effects of YC on rumen pH regulation, ruminal environmental stability, VFA absorption by ruminal epithelium, and the reduction of inflammatory responses associated with high-concentrate feeding have been confirmed in dairy cows (Li et al. 2016). Studies have shown that YC modulates rumen pH, alters fermentation patterns, and improves productivity (Mohamed et al. 2009; Ranjan et al. 2013), and Carpinelli et al. showed that YC affects rumen microbial composition and lactation performance in dairy cows (Carpinelli et al. 2021). Current studies on the effects of YC on rumen microorganisms have focused on bacteria, but little has been reported on fungi, so it is worthwhile to investigate the relationship between fungi and lactation performance. This study analyzed the effects of YC on rumen microbial composition in mid-lactation dairy goats using 16S and ITS sequencing. The results showed that feeding YC could decrease the relative abundance of *Dipodascus* and *Gibberella*, decrease VFA concentrations, increase serum antioxidant levels and increase milk yield. Correlation analyses showed that the rumen fungi *Dipodascus* and *Gibberella* were at the center of a network of differential microbial interactions, and their proportions were significantly negatively correlated with milk protein yield. The relationship between YC and lactation performance

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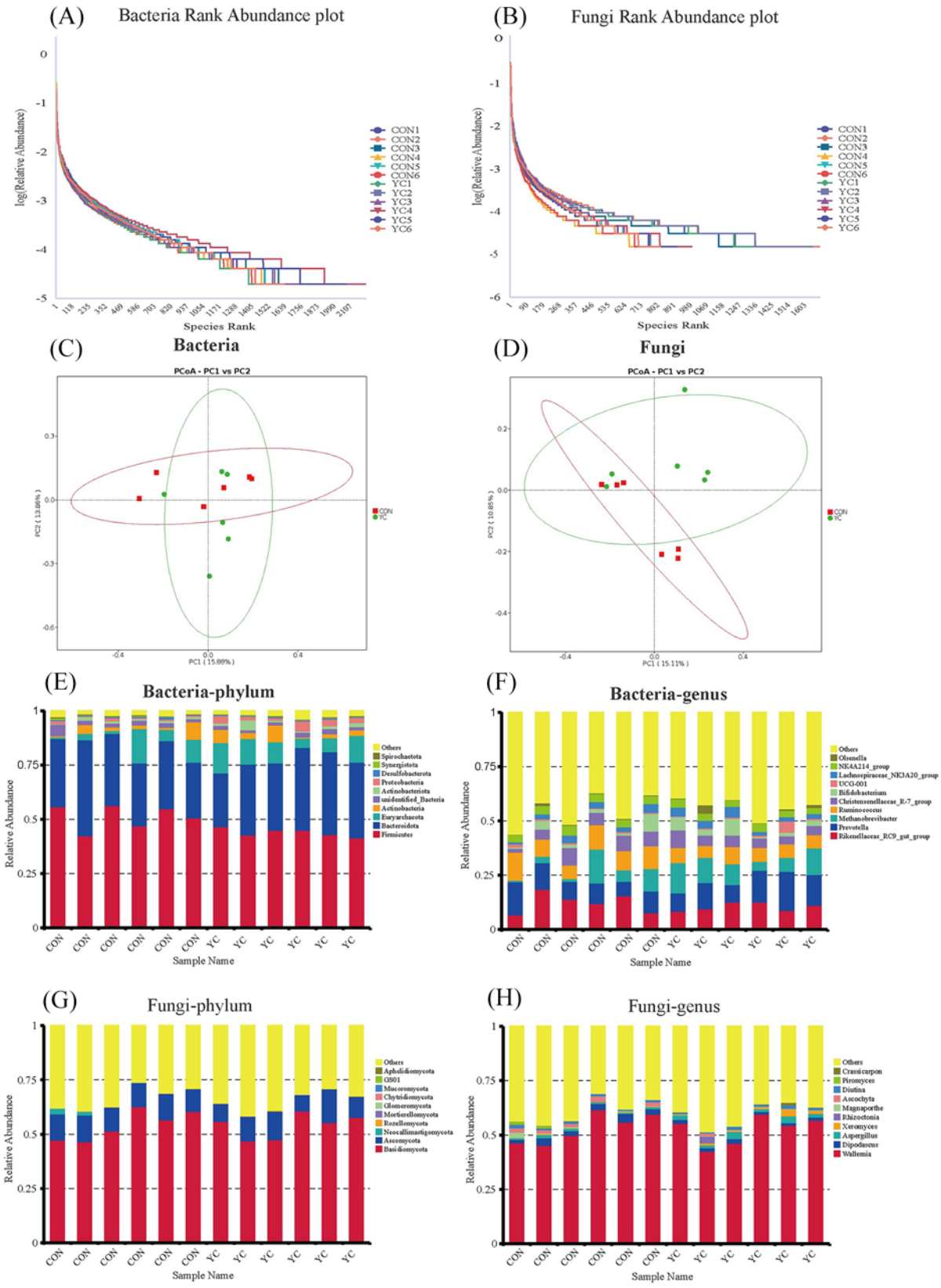

Figure 4. Effect of yeast cultures on the composition of rumen microbiota. (A) Rank abundance curves of bacteria. (B) Rank abundance curves of fungi. (C) PCOA results for bacteria. (D) PCoA results for fungi. (E) Taxonomic analysis of rumen bacterial groups at the phylum level. (F) Taxonomic analysis of rumen bacterial groups at the genus level. (G) Taxonomic analysis of rumen fungi groups at the phylum level. (H) Taxonomic analysis of rumen fungi groups at the genus level.

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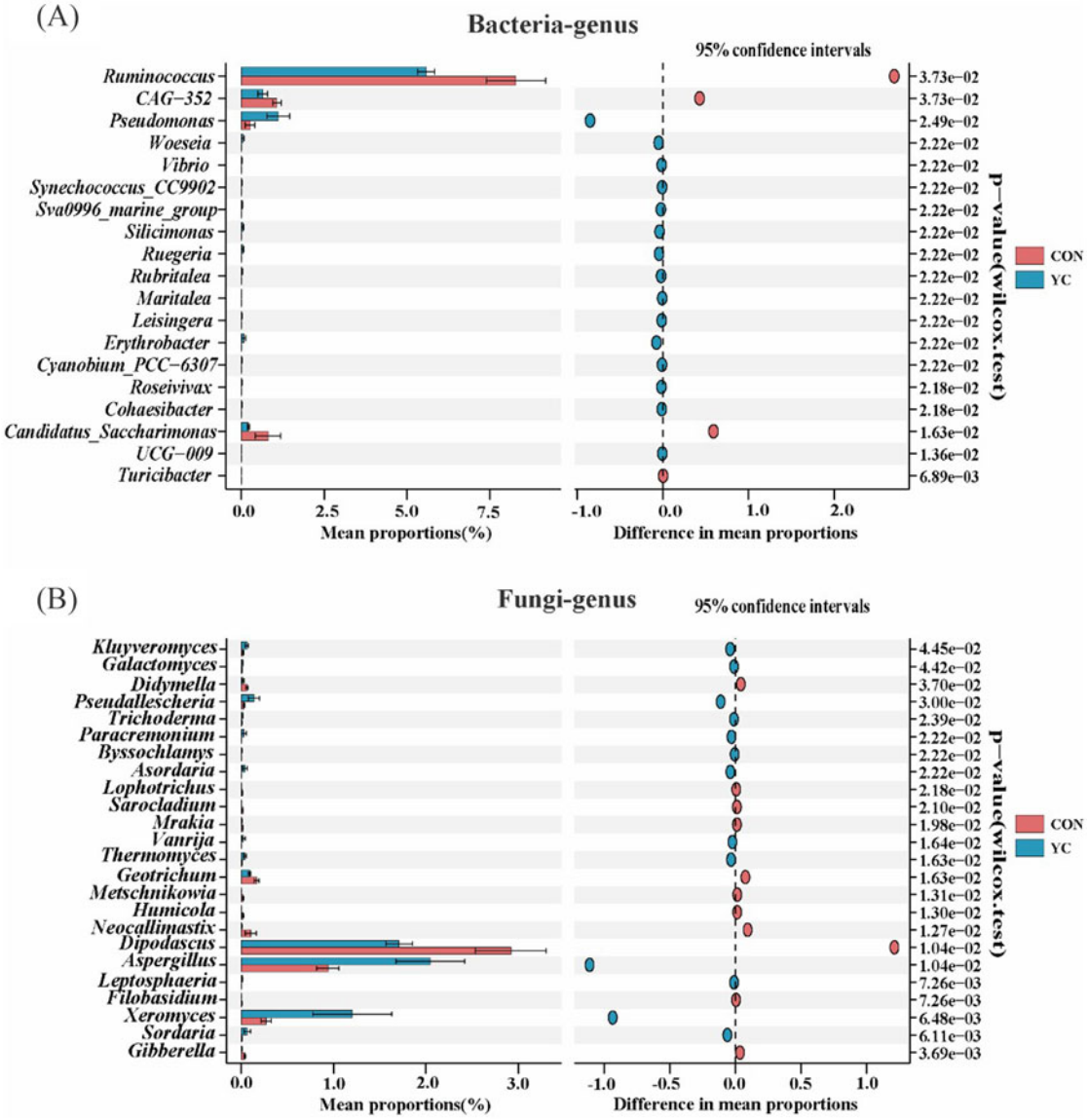

Figure 5. Differences at the genus level between fungi and bacteria. (A) Significantly different genus of bacteria, $P < 0.05$. (B) Significantly different genus of fungi, $P < 0.05$.

has been relatively understudied. The present study found that YC improves lactation performance in dairy goats, and alterations in rumen fungi appear to play an important role in this process.

The results revealed that YC altered the rumen microbial composition, and 19 microbial genera with different relative abundance were identified at the bacterial genus level. Moreover, analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment results revealed that YC significantly upregulated 15 metabolic pathways. Among these pathways, the flavone and flavonol biosynthesis metabolic pathway belongs to the phenylpropanoid metabolic pathway, which produces a large amount of phenolic acids, lignans, stilbenes, and other polyphenols (Ma et al. 2017). These compounds help to limit the proliferation of undesirable microorganisms (Xia et al. 2023). The upregulation of the valine, leucine, and isoleucine degradation pathway indicates an increase in rumen metabolism (Zhuang et al. 2023). Fatty acid degradation and butanoate metabolism can directly impact the concentrations of VFA in the rumen. Benzoate degradation pathway is associated with *Pseudomonas* abundance (Wang et al. 2011). Additionally, an increased folate biosynthesis pathway is associated with the improved production performance of ruminants (Abbasi et al. 2018; Cheng et al. 2020). Therefore, YC affects the microbial composition of the rumen, leading to changes in the intensity of metabolic pathways, among which the inhibition of undesirable microbial proliferation by flavone and flavonol biosynthesis pathway may be one of the reasons for improving production performance.

Despite representing only 20% of the rumen biomass, fungi are the most efficient contributors to fiber degradation (Rezaeian et al. 2004). Next, we focused on changes in the composition of fungi in the rumen. At the fungal level, 24 different genera were identified. Of these, the reduced *Dipodascus* and *Gibberella* induced by YC were the center of a network of interactions. The presence of pathogenic species in *Dipodascus* (Kulesza et al. 2021) can lead to fungal infections in some patients with low immunity (Lakshmi et al. 2023). *Gibberella* is also a genus of pathogenic fungi

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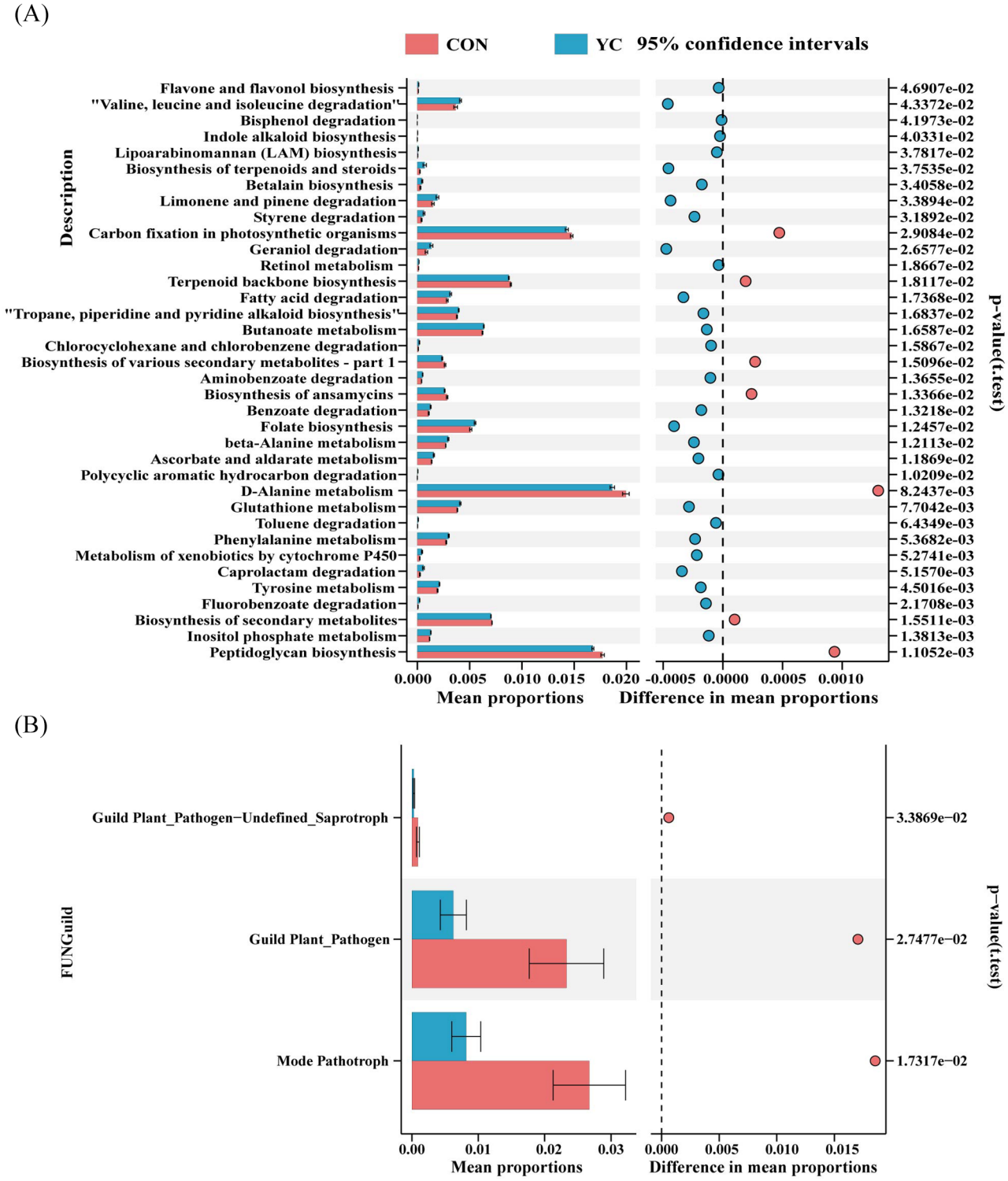

Figure 6. Microbial function prediction. (A) Differential enrichment pathway (level 3) analysis of PIRCUSt2 based on 16S sequencing data. (B) FUNGuild differential function prediction analysis based on ITS sequencing data.

(Gao et al. 2023), inducing liver cancer in rodents (Perera et al. 2017) and causing a carcinogenic effect on animals and humans (Kondratiuk et al. 2017).

Functional classification of fungi revealed a significant decrease in plant_pathogen and pathotroph. It has been shown that *Dipodascus* includes pathogenic species (Kulesza et al. 2021) and *Gibberella* species are destructive plant pathogens (Karlsson et al. 2016), and the reduced abundance of both genera may explain the decrease in phytopathogenic and pathogenic trophic fungi. Thus, the study speculated that the decrease in the abundance of *Dipodascus* and *Gibberella* might be caused by the increase of flavone and flavonol biosynthesis pathway in YC. A reduction

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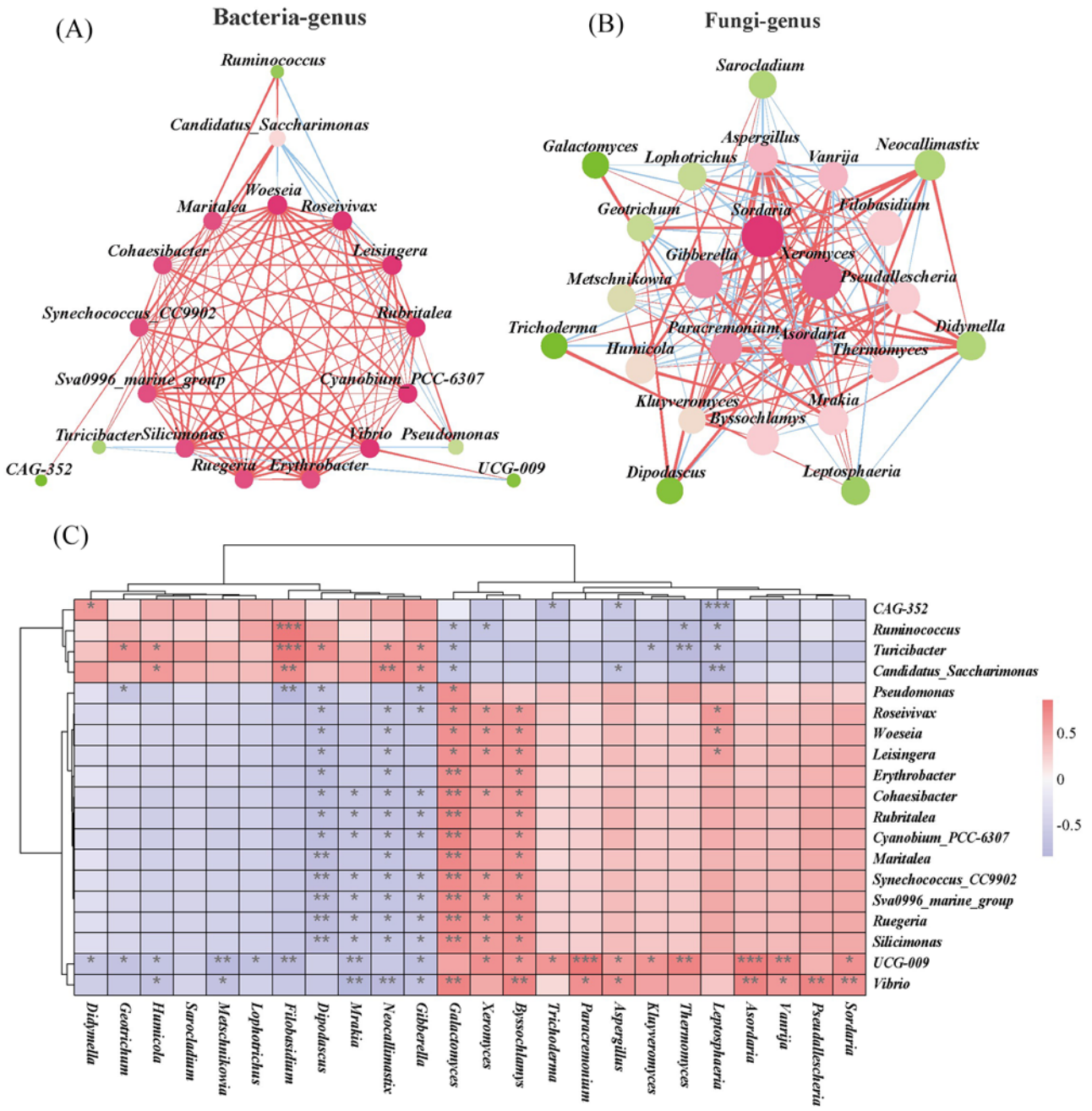

Figure 7. Differential microbial interaction networks. (A) Differential core bacterial genera Spearman correlation analysis ($|R| > 0.6$, $P < 0.05$), line red represents positive correlation; blue represents negative correlation; the darker the red color of the node, the closer it is to the core of the microbial network; node size represents closeness centrality. (B) Spearman correlation analysis of different fungal core fungi ($|R| > 0.6$, $P < 0.05$). (C) Heatmap of bacterial and fungal correlations, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

in the abundance of these two fungal genera is likely to have a beneficial effect on the health of dairy goats. In this study, rumen fermentation parameters were also examined, and the result showed that feeding YC had a significant effect on ruminal environmental indices, pH, NH_3-N levels increased, total VFA, acetate, propionate and butyrate levels decreased, acetate and propionate ratio did not change significantly, and fermentation pattern was not altered. The extant study corroborates earlier research, which demonstrated that YC increased pH and reduced acetate levels in dairy cows (Halfen et al. 2021), and that yeast supplementation also reduced rumen butyrate levels in dairy heifers (Lascano et al. 2009). However, under the condition of heat stress in bulls, after supplementation of YC, pH, NH_3-N, butyrate, or acetate/propionate did not differ significantly, while the rumen acetate, propionate, and total VFA content increased significantly (Zhang et al. 2022). However, dietary with YC had no significant effect on rumen fermentation parameters of Baluchi lambs (Malekkhahi et al. 2015). The different effects of feeding YC in different studies may be due to different species and physiological conditions. In addition, RDA analysis showed that among the differential microbes, differential fungi contributed more to the rumen environment, including *Gibberella* and *Dipodascus*.

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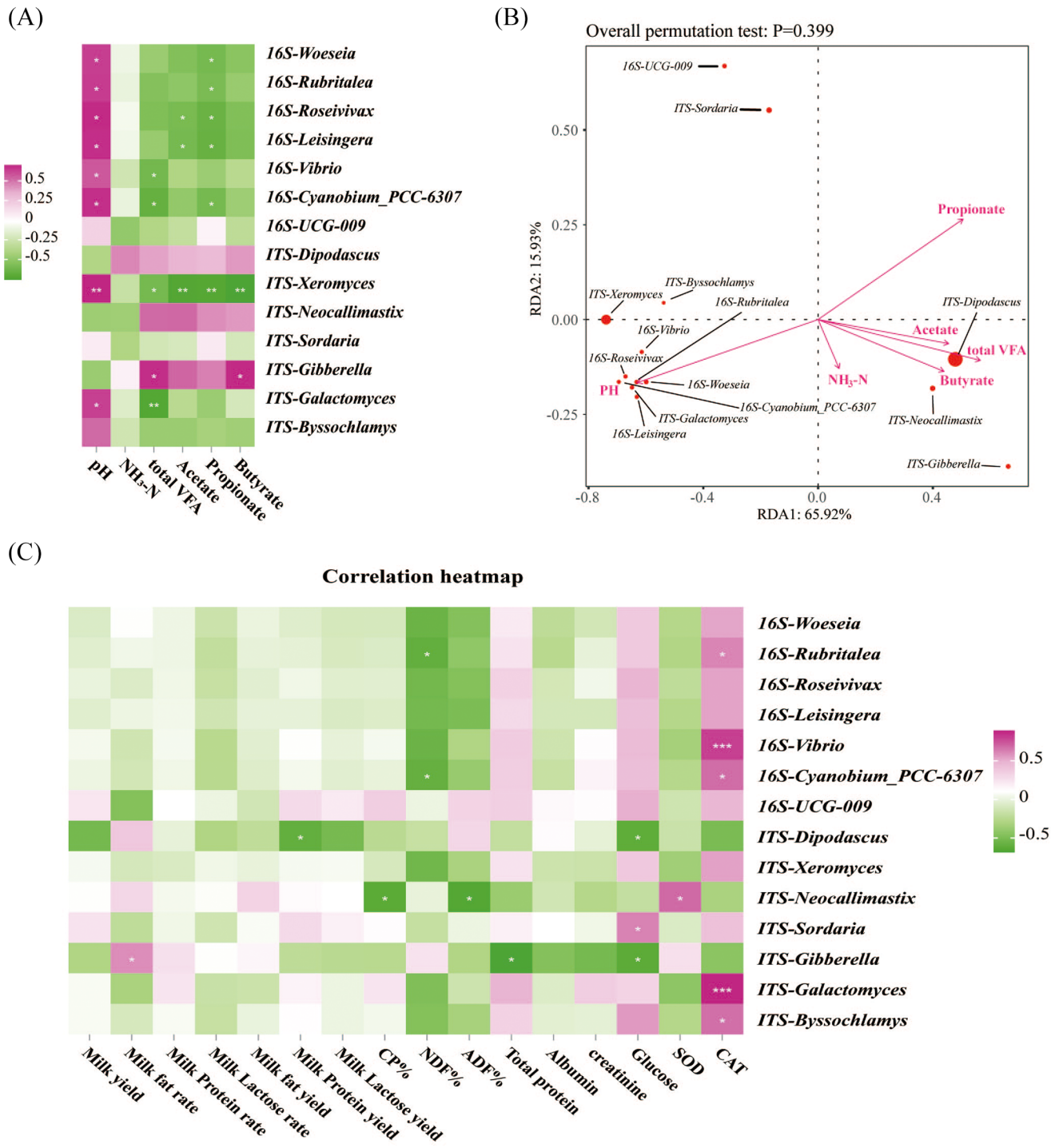

Figure 8. (A) Pearson correlation analysis of rumen core differential microorganisms and differential fermentation parameters, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (B) Redundancy analysis (RDA) of microbial community changes with rumen environmental variables. (C) Correlating rumen microbiological with lactation performance and serum indicators, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Serum levels of TP, ALB and CR reflect the metabolism of dietary proteins and the immune status of the body (He et al. 2018). The results of the present study found that feeding YC increased the serum levels of TP, ALB, CR and glucose in dairy goats, which suggests that YC improves metabolism levels of dairy goats. Propionate is an important substrate for glucose (Kawas et al. 2007). Thus, the elevated serum glucose in the present study was partly due to increased fatty acid utilization in the rumen of ruminants, which may also contribute to the reduction in rumen VFA. YC increased serum SOD and CAT levels in dairy goats, possibly due to the antioxidant content from YC (Krizková 2001). This is consistent with previous studies, indicating that YC has resulted in increased antioxidant activity in organisms (Bu et al. 2019; Xv et al. 2021). Correlation analysis showed that SOD was

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positively correlated with *Neocallimastix*, which is an anaerobic fungus in rumen (Comlekcioglu et al. 2010). CAT was positively correlated with *Galactomyces* and *Vibrio. Galactomyces* can be isolated from rumen fluid and it has potential for use as a feed additive in ruminant production (Suntara et al. 2021). *Vibrio* is a rumen microorganism associated with rumen fermentation (Ramos et al. 2022).

YC increased DMI, milk yield, and milk lactose yield in dairy goats, which is consistent with the results of YC studies in cows (Bruno et al. 2009) and Nili-Ravi buffaloes (Ali et al. 2023). Correlation analysis results found that milk yield was negatively correlated with *Gibberella* and *Dipodascus* ($P > 0.05$). Milk lactose yield was negatively correlated with *Gibberella* and *Dipodascus* ($P > 0.05$), and milk fat rate was positively correlated with *Gibberella* proportions. As the relationship between these two fungi and lactation performance has not been reported, we speculated that feeding YC to dairy goats resulted in changes in the core differential microorganisms, *Gibberella* and *Dipodascus*, in the rumen of dairy goats, which further affected the bacterial–fungal interaction network and ultimately led to a decrease in VFA levels and an increase in serum glucose levels, finally increased lactose production, milk yield, and milk protein yield. Correlation analysis indicated that there was a stronger association between fungal diversity and dairy goat lactation index. Correlation analyses remain a nascent field of study, and the relationship between rumen microbes and lactation performance is yet to be fully elucidated.

Conclusions

The addition of YC to the diet improved lactation performance by altering the rumen bacterial and fungal composition of lactating dairy goats, affecting rumen fermentation parameters, increasing serum TP, CR, and Glu levels, and increasing serum levels of antioxidant indices such as SOD and CAT. Importantly, we further revealed that the improved lactation performance induced by YC was associated with decreased abundances of *Dipodascus* and *Gibberella* in the rumen.

Author contributions. Huaiping Shi: project administration, data curation, supervision. Liyan Ge and Shuying Bai: conceptualization, validation, visualization, writing – original draft, software. Huijun Shen and Kela Sha: validation, methodology, writing – original draft. Yuexin Shao: methodology, formal analysis. The authors have all read and approved the manuscript.

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Conflict of interest. The authors declare no competing financial interest.

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