OXFORD

AMERICAN SOCIETY OF **ANIMAL SCIENCE**

Effects of ensiling sugarcane tops with bacteria-enzyme inoculants on growth performance, nutrient digestibility, and the associated rumen microbiome in beef cattle

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Abstract

Major challenges when ensiling sugarcane tops include fermentation that results in high quantities of alcohol and decrease in nutrient digestibility due to the accumulation of fiber components. Increased efforts to apply bacteria-enzyme inoculants in silage have the potential to improve nutrient digestibility. This study aimed to evaluate the effects of ensiling sugarcane tops with bacteria-enzyme inoculants or mixed bacterial inoculants on growth performance, nutrient digestibility, and rumen microbiome in beef cattle. Chopped sugarcane tops were ensiled in plastic bags for 60 d after application of 1) no inoculant (control check, **CK**); 2) bacteria-enzyme inoculants containing *Pediococcus acidilactici*, *Saccharomyces cerevisiae*, cellulase, and xylanase (T1, viable colony-forming units of each bacterial strain ≥10[8] CFU/g; enzyme activity of each enzyme ≥200 U/g); or 3) mixed bacterial inoculants containing *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae* (T2, viable colony-forming units of each bacterial strain ≥10[7] CFU/g). Silages were fed to eighteen Holstein bull calves ($n = 6$/treatment) weighing 163.83 ± 7.13 kg to determine intake in a 49-d experimental period. The results showed that beef cattle-fed T1 silage or T2 silage had a significantly higher ($P < 0.05$) average daily gain than those fed CK silage, but the difference in dry matter intake was not significant ($P > 0.05$). The apparent digestibility of crude protein (**CP**) and acid detergent fiber (**ADF**) were higher ($P < 0.05$) for beef cattle-fed T1 silage or T2 silage than for those fed CK silage. The rumen bacterial community of beef cattle-fed T1 silage or T2 silage had a tendency to increase ($P > 0.05$) abundance of *Firmicutes* and *Rikenellaceae_RC9_gut_group* than those fed CK silage. Rumen fungal communities of beef cattle-fed T1 or T2 silage had a tendency to increase ($P > 0.05$) abundance of *Mortierellomycota* and of *Mortierella* than those fed CK silage. Spearman's rank correlation coefficient showed that the apparent digestibility of ADF for beef cattle was positively correlated with *unclassified_p_Ascomycota* of the fungal genera ($P < 0.05$). *Neocalimastigomycota* of the fungal phyla was strongly positively correlated with the apparent digestibility of neutral detergent fiber (**NDF**) ($P < 0.05$). *Ruminococcus* was positively correlated with the apparent digestibility of CP ($P < 0.05$). It was concluded that both T1 and T2 improved the growth performance of beef cattle by improving the ruminal apparent digestibility of CP and ADF, and had no significant impact on major rumen microbial communities in beef cattle.

Lay Summary

Major challenges when ensiling sugarcane tops include fermentation that results in high quantities of alcohol and decrease in nutrient digestibility due to the accumulation of fiber components. Increased efforts to apply bacteria-enzyme inoculants in silage have the potential to improve nutrient digestibility. This study aimed to evaluate the effects of ensiling sugarcane tops with bacteria-enzyme inoculants or mixed bacterial inoculants on the growth performance, nutrient digestibility, and rumen microbiome in beef cattle. Chopped sugarcane tops were ensiled in plastic bags for 60 d after application of 1) no inoculant (control check, **CK**); 2) bacteria-enzyme inoculants (*Pediococcus acidilactici*, *Saccharomyces cerevisiae*, cellulase, and xylanase), termed treatment T1; or 3) mixed bacterial inoculants (*Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae*), termed treatment T2. Silages were fed to 18 Holstein bull calves ($n = 6$/treatment) weighing 163.83 ± 7.13 kg to determine intake in a 49-d experimental period. It was concluded that both T1 and T2 improved the growth performance of beef cattle by improving the ruminal apparent digestibility of crude protein and acid detergent fiber, and had no significant impact on major rumen microbial communities in beef cattle.

Key words: beef cattle, growth performance, inoculant, nutrient digestibility, ruminal microbial, sugarcane top silages

Abbreviations: ADF acid detergent fiber ADG, average daily gain; CK control check CP crude protein DM, dry matter; DMI, dry matter intake; EE ether extract FBW, final body weight; FM, fresh material; IBW, initial body weight; LAB, lactic acid bacteria; NDF neutral detergent fiber NE, net energy; OM organic matter OTUs, operational taxonomic units; PCoA, principal coordinate analysis; VFAs, volatile fatty acids; WSC, water soluble carbohydrate

Introduction

In 2019, the annual planting area and average yield of sugarcane in China reached 1.16 million ha and 76.9 t/ha, respectively (Qi et al., 2021). Sugarcane tops are a major residue of the sugarcane industry and account for 14% of the total harvest (Couto et al., 2017). Sugarcane tops are considered to be medium-quality forage. In one analysis, the moisture content of the fresh sugarcane top was 74.3%, and the air-dried matter of the sugarcane top contained 94.7% organic matter (**OM**), 6.8% crude protein (**CP**), and 1.8% ether

extract (**EE**). In terms of carbohydrates and fiber, sugarcane tops show, 76.1% neutral detergent fiber (**NDF**), 42.5% acid detergent fiber (**ADF**), and 7.9% water soluble carbohydrate (**WSC**) (Cai et al., 2020). The sugarcane harvest lasts from November to April of the following year. Due to high humidity and low temperatures during this period, drying for forage is not practical and prone to mildew if left in the field. Conversely, silage avoids the drawbacks of seasonal accumulation of sugarcane tops, and improves the palatability of sugarcane tops as animal feed. However, a major challenge when ensiling sugarcane tops is fermentation that results in high quantities of alcohol, resulting in high dry matter (**DM**) losses and poor-quality silage (Pedroso et al., 2005), and accumulating fiber components cause a decrease of the forage digestibility (Ferreira et al., 2007). The advantages of sugarcane top silage may therefore be limited by these factors.

Additives have been widely used to improve silage quality, improving its nutrient digestibility and enhancing the production efficiency of cattle (Guo et al., 2022; Singh et al., 2023). *Lactobacillus plantarum* is often used as a silage inoculant to decrease DM losses and the pH of silage and modulate ruminal fermentation by stabilizing the microbial ecosystem (Chen et al., 2022). Shah et al. (2017) reported that *L. plantarum* or *Pediococcus acidilactici* significantly reduced the pH and the acetic acid content of elephant grass silage, while the lactic acid was significantly increased. *Bacillus subtilis* can produce cellulases, which were shown to promote the growth of lactic acid bacteria (**LAB**) to increase lactic acid production rates by increasing the release of soluble sugars through hydrolysis of the plant cell walls, thereby promoting the nutritional quality of silage (Ning et al. 2017; Li et al. 2018). Lara et al. (2018a) also found that inoculation of corn silage with *L. plantarum* and *B. subtilis* increased both the metabolizable energy and digestible energy, and also resulted in the higher average daily gain (**ADG**) of lambs. *Aspergillus oryzae* increased the utilization of fiber in dairy cattle through an increase in microbial activity (Sun et al., 2017). The use of *Saccharomyces cerevisiae* increased aerobic stability of corn silage (Nair et al., 2019a) and apparent total tract digestibility with respect to OM, ADF, NDF, and DM in beef cattle (Nair et al., 2019b). More recently, increased efforts to apply enzyme additives in silage have become an attractive method of increasing the nutritive value of silage, thus increasing digestibility (Machado et al., 2020; Irawan et al., 2021). During ensilage, a number of fibrolytic enzymes such as xylanase and cellulose have been incorporated in inoculants (Li et al., 2018) that were expected to break down the cell wall carbohydrates. Furthermore, supplementation with additional sugar substrates was done to promote ensilage via LAB fermentation and thus improve the fermentation quality of the silage (Irawan et al., 2021) and its nutrient digestibility (Guo et al., 2022).

In our previous research, we found that both bacteria-enzyme inoculants containing *P. acidilactici*, *S. cerevisiae*, cellulase and xylanase, and bacterial inoculants containing *L. plantarum*, *B. subtilis*, and *A. oryzae* could significantly decrease the pH as well as the butyric acid and NH_3–N content in sugarcane top silage, while significantly increasing the lactic acid, acetic acid, and CP content in sugarcane top silage. Moreover, both bacteria-enzyme inoculants and bacterial inoculants could significantly impact microbial communities of sugarcane top silage. The chemical composition and fermentation characteristics of sugarcane top silage are presented in Supplementary Table S1. The relative abundance of

the bacterial phylum and genus in the sugarcane top silage are shown in Supplementary Table S2. The relative abundance of the main fungi phylum and genus in the sugarcane top silage are shown in Supplementary Table S3. However, the effects of the bacteria-enzyme inoculants and bacterial inoculants on the nutrient digestibility of silage, growth performance, and rumen microbial community of Holstein bull calves have not been well evaluated. It was hypothesized here that the supplementation of Holstein bull diets with sugarcane top silage treated with bacteria-enzyme or bacterial inoculants may improve the growth performance of Holstein bull calves by improving nutrient digestibility and altering the rumen microbiome. The main objectives of this study were to assess the impacts on nutrient digestibility and growth performance in Holstein bull calves fed sugarcane top silage treated with the mixed inoculants, and to explore the microbial mechanism of improved growth performance using high-throughput sequencing.

Materials and Methods

Ethics statement

The experimental procedure was performed in accordance with the protocols approved by the Institutional Animal Care and Use Committee of Guangxi University (No. GXU2018-029).

Silage materials and silage production

The sugarcane tops were harvested from fields located at Fusui, Guangxi, China (N 22°38′, E 107°54′) in February 2019. The average chemical characteristics of the fresh sugarcane tops were as follows: 317.2 g DM/kg fresh material (**FM**), 67.6 g CP/kg DM, 672.2 g NDF/kg DM, 387.9 g ADF/kg DM, and 126.9 g WSC/kg DM. The preparation of sugarcane top silage was carried out at the Duchong Cattle Cooperative of Fusui, Guangxi, China in February 2019. Before ensiling, the harvested materials were cut into ~2 to 3 cm lengths with a forage cutter (Shandong Shengshi Machinery Manufacturing Co., Ltd, 9ZP-10). Sugarcane tops were ensiled after application of the following treatments: 1) distilled water (60 L/t, no inoculant, control check, **CK**); 2) bacteria-enzyme inoculants containing *P. acidilactici, S. cerevisiae*, cellulase, and xylanase (bacteria-enzyme inoculants were dissolved in distilled water and sprayed onto fresh sugarcane tops, 60 L/t, T1); and 3) mixed bacterial inoculants containing *L. plantarum*, *B. subtilis*, and *A. oryzae* (the expanded culture broth and distilled water were mixed at a volume ratio of 2:1, and then sprayed onto fresh sugarcane tops, 60 L/t, T2). Bacteria-enzyme inoculants (T1, viable colony-forming units of each bacterial strain ≥ 10^8 CFU/g; enzyme activity of each enzyme ≥ 200 U/g) consisted of the active fermentation agent obtained from the Yichun Strong Microbial Technology Co., Ltd (Jiangxi Province, China). Mixed bacterial inoculants (T2, viable colony-forming units of each bacterial strain ≥ 10^7 CFU/g) were isolated from natural sugarcane top silage by our laboratory (*L. plantarum* and *A. oryzae* have been entered into the strain bank of the China Center for Type Culture Collection, numbered M2019002 and M2018425, respectively, and *B. subtilis* has been identified by the Guangdong Detection Center of Microbiology). A straw silage baler (Shandong Xuheng Machinery Co., Ltd, ZYD-100) was used to pack the different processed sugarcane tops. Each bag contained about 50

kg of FM, and 4.5 metric tons of silage from each treatment were produced. Treated forages were stored for 60 d at an ambient temperature range of 15 to 29 °C and protected from sunlight. After sugarcane top silage was completed, four bags were randomly selected from each treatment to evaluate the effect of different mixed inoculants on the chemical composition, fermentation characteristics, and microbial communities of sugarcane top silage. The chemical composition and fermentation characteristics of sugarcane top silage are presented in Supplementary Table S1. The relative abundance of the bacterial phylum and genus in the sugarcane top silage are shown in Supplementary Table S2, and the relative abundance of the main fungi phylum and genus in the sugarcane top silage are shown in Supplementary Table S3.

Experimental animals and design

This feeding experiment was carried out at the Duchong Cattle Cooperative of Fusui, Guangxi, China in April 2019. Eighteen Holstein bull calves with similar body weights (163.83 ± 7.13 kg), were randomly fed one of the three silages (CK silage, T1 silage, or T2 silage). All Holstein bull calves were fed the same diet (Table 1) in each treatment, and the ratio of concentrate to roughage was 1:1 (air-dry basis). The total experimental period was 49 d, with an adaptation period over the first 7 d. During the experiment, food was provided twice daily at

Table 1. The ingredients and nutrient composition of experimental diets (DM basis%)

Items	Treatments[3]		
	CK	T1	T2
Ingredients			
Sugarcane top silage (FM[1])	50.00	50.00	50.00
Corn	26.00	26.00	26.00
Soybean meal	12.00	12.00	12.00
Wheat bran	9.00	9.00	9.00
Mountain flour	1.50	1.50	1.50
Salt	0.50	0.50	0.50
Premix[2]	1.00	1.00	1.00
Total	100.00	100.00	100.00
Nutrient composition			
DM	57.10	56.6	58.3
NE[4] (MJ/kg)	6.53	6.67	6.70
CP	11.52	11.96	11.75
NDF	51.80	52.79	51.59
ADF	22.95	23.84	21.94

[1]FM, fresh material.
[2]Premix per kilogram: vitamin A 130 to 150 KIU; vitamin D3 65 to 75 KIU; vitamin E 650 to 750 IU; iron 1.9 to 2.1 g; zinc 1.2 to 1.4 g; iodine 10 to 20 mg; selenium 10 to 15 mg; copper 0.35to 0.55 g; calcium 100 to 200 g; phosphorus 50 to 100 g; cobalt 8 to 15 mg; moisture ≤ 10%. The premix was supplied by Beijing Oriental Kingherd Biotechnology Co., Ltd.
[3]Treatments included no inoculant (CK), bacteria-enzyme inoculants containing *Pediococcus acidilactici*, *Saccharomyces cerevisiae*, cellulase, and xylanase (T1, viable colony-forming units of each bacterial strain ≥ 10[8] CFU/g; enzyme activity of each enzyme ≥ 200 U/g), and mixed bacterial inoculants containing *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae* (T2, viable colony-forming units of each bacterial strain ≥ 10[7] CFU/g).
[4]NE, Net energy.

0800 and 1600 hours. Holstein bull calves had free access to water at all times.

Chemical analyses

The DM of the fresh samples and silage was determined by oven drying at 65 °C for 72 h. The NDF and ADF contents were measured according to Van Soest's procedures (Van Soest et al., 1991). The CP content (CP = total $N \times 6.25$) was analyzed using a Kjeldahl apparatus (Germany, Gerhardt Vapodest 50s) according to the procedure described by the Association of Official Agricultural Chemists (1997). The WSC content was determined using the anthrone method (Mcdonald and Henderson, 1964).

Fermentation profile

After 60 d of silage, 15 g of each silage sample was thoroughly combined with 135 mL of Ringer's test solution, soaked for 30 min, evenly stirred for 2 min with a laboratory stirrer, and filtered through two layers of gauze. The pH was measured using a pH meter (pH 211; HANNA; Italy). The contents of acetic acid, propionic acid, and butyric acid were determined using gas chromatography (Shimadzu gc-2014). The NH_3–N content was determined by a phenol–hypochlorite reaction (Broderick and Kang, 1980). The lactic acid content was determined by colorimetry (Pryce, 1969).

Determination of growth performance and nutrient digestibility

The initial body weight (**IBW**) was measured after the pre-feeding period, and the final body weight (**FBW**) was measured at the end of the formal test experiment. The ADG was calculated based on the IBW, FBW, and feeding days. During the formal experiment, the 5-d feed intake of each group was recorded in the early, mid, and late stages of the experiment, and the DM intake (**DMI**) and feed-to-weight ratio of each group were calculated. The feed samples and fecal samples were collected 1 wk before the end of the experiment for five consecutive days. Feed samples were collected before feeding and dried at 65 °C. Fecal samples were collected in the morning and afternoon. The collected fecal samples were supplemented with 10% sulfuric acid for nitrogen fixation for 4 to 5 h, and then dried at 65 °C.

The apparent digestibility of nutrients in the diet was determined using the endogenous indicator method (acid insoluble ash). The formula is as follows:

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

where a is a certain nutrient content in feed (%); b is the nutrient content in feces (%); c is the content of the indicator in feed (%); and d is the content of the indicator in feces (%) (Keulen and Young, 1977).

Rumen sampling and analysis of microbial diversity

Ruminal fluid samples (200 mL) were collected from Holstein bull calves (n = 4/treatment) according to the reported method of Zou et al. (2019) on the 48th day. Ruminal fluid samples were stored at –20 °C and analyzed by Shanghai Meiji Biomedical Technology Co., Ltd. for identification and analysis of rumen microbial diversity.

High-throughput sequencing and bioinformatics analysis

The bacterial 16S rDNA amplicon sequencing used the 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) universal primers, which amplify the V3 and V4 region of the 16S rRNA gene (Srinivasan et al., 2012). The fungal ITS amplicon sequencing used the ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) universal primers. These primers amplify the ITS1 region of the fungal ITS gene (Liu et al., 2015). The microbial DNA was analyzed by Shanghai Meiji Biomedical Technology Co., Ltd, using an Illumina MiSeq 2500 platform. The sequence data reported in this study (bacteria and fungi) have been deposited in the NCBI database (Accession No. PRJNA884864 and Accession No. PRJNA885232).

FLASH software (Magoč and Salzberg, 2011) was used to remove low-quality reads, and to read and splice double-ended sequences to generate original tags. Usearch software (Edgar, 2013) was used for sequence filtering and operational taxonomic unit (**OTU**) clustering (similarity threshold was 97%). An RDP classifier (http://rdp.cme.msu.edu/) using a Bayes algorithm (Qiong et al., 2007) was used to compare the Silva 16S rRNA gene database (https://www.arb-silva.de) and Fungi Unite8.0 gene database (https://unite.ut.ee) to annotate the OTU species taxonomy and analyze the representative sequences of the OTUs with a 97% similarity level. Alpha diversity metrics (Chao1, Shannon, Simpson, Ace, and Sobs) were calculated using the mothur software platform.

Statistical analyses

In this study, sugarcane top silage treated with no inoculant (CK), T1, or T2 were fed to eighteen Holstein bull calves ($n = 6$/treatment). The experimental design was a completely randomized design. One-way analysis of variance and Duncan's multiple comparison test were used to evaluate the silage fermentation parameters, microbial communities of sugarcane top silage, the growth performance of beef cattle, and ruminal microbial alpha diversity using SPSS 22.0 (IBM Corp., Armonk, NY, USA) for Windows. Results were considered significant at $P < 0.05$. The microbial beta diversity was determined through principal coordinates analysis (**PCoA**). Ruminal microbial community composition at the genus and species levels was analyzed using the Kruskal–Wallis H test with a false discovery rate correction and Tukey–Kramer as a post hoc test to elucidate differences across treatment groups. Spearman's correlation coefficient (r) was used to determine the potential relationship between rumen bacteria and fungus with different growth performances and nutrient digestibility. The correlations marked with an asterisk ("*") were significant at $P < 0.05$, and "**" represents $P < 0.01$.

Results

Animal growth performance

The growth performance of beef cattle is shown in Table 2. Beef cattle had similar IBW values at 163.6 to 164.2 kg and FBW values of 182.8 to 189.2 kg among the treatments ($P > 0.05$). The ADG ($P < 0.05$) and DMI ($P > 0.05$) of beef cattle-fed T1 silage (0.53 and 3.97 kg/d, respectively) or T2 silage (0.60 and 4.21 kg/d, respectively) were higher than those fed CK silage (0.46 and 3.58 kg/d, respectively), and the feed-to-weight ratio of beef cattle-fed T1 silage (7.42) or T2 silage (7.01) showed a decreasing trend compared to those fed CK silage (7.95).

Nutrient digestibility

Nutrient digestibility in beef cattle is shown in Table 3. The apparent digestibility of the CP ($P < 0.05$) and ADF ($P < 0.05$) in the beef cattle-fed T1 silage (71.26% and 48.27%, respectively) and T2 silage (72.46% and 48.60%, respectively) were higher compared to the beef cattle-fed CK silage (69.79% and 46.67%, respectively).

Ruminal bacterial community profile

Bacteria sequencing information and the alpha diversity of the bacterial community in ruminal liquid are shown in Table 4. A total of 143,507 valid sequences were obtained by high-throughput sequencing. Coverage values exceeded 0.99 in all the samples, indicating that the sampling depth had adequately captured most of the bacterial communities.

Table 2. Effects of different additives applied to silage sugarcane tops on the growth performance in beef cattle-fed over a period of 49 d

Items	Treatments[1]				
	CK	**T1**	**T2**	**SEM**	***P*-value**
IBW[2] (kg)	163.70	163.60	164.20	7.13	1.00
FBW[3] (kg)	182.80	185.80	189.20	7.38	0.74
ADG[4] (kg/d)	0.46[b]	0.53[a]	0.60[a]	0.01	0.04
DMI[5] (kg/d)	3.58	3.97	4.21	0.08	0.48
Feed-to-weight ratio	7.95	7.42	7.01	0.17	0.06

[1]Treatments included no inoculant (CK), bacteria-enzyme inoculants containing *Pediococcus acidilactici*, *Saccharomyces cerevisiae*, cellulase, and xylanase (T1, viable colony-forming units of each bacterial strain ≥ 10⁸ CFU/g; enzyme activity of each enzyme ≥ 200 U/g), and mixed bacterial inoculants containing *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae* (T2, viable colony-forming units of each bacterial strain ≥ 10⁷ CFU/g).
[a,b]Values with different superscripts within each row are significantly different ($P < 0.05$).
[2]IBW, Initial body weight.
[3]FBW, Final body weight.
[4]ADG, average daily gain.
[5]DMI, dry matter intake.

Table 3. Effects of different additives applied to silage sugarcane tops on the nutrient apparent digestibility in beef cattle-fed over a period of 49 d

Items	Treatments[1]				
	CK	**T1**	**T2**	**SEM**	***P*-value**
CP (%)	69.79[b]	71.26[a]	72.46[a]	0.37	0.03
OM (%)	71.92	72.29	72.67	0.24	0.49
NDF (%)	58.84	59.47	59.88	0.28	0.74
ADF (%)	46.67[b]	48.27[a]	48.60[a]	0.35	0.03

[1]Treatments included no inoculant (CK), bacteria-enzyme inoculants containing *Pediococcus acidilactici*, *Saccharomyces cerevisiae*, cellulase, and xylanase (T1, viable colony-forming units of each bacterial strain ≥ 10⁸ CFU/g; enzyme activity of each enzyme ≥ 200 U/g), and mixed bacterial inoculants containing *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae* (T2, viable colony-forming units of each bacterial strain ≥ 10⁷ CFU/g).
[a,b]Values with different superscripts within each row are significantly different ($P < 0.05$).

Table 4. Statistical diversity values of fungal and bacterial communities in ruminal fluid of beef cattle-fed silage sugarcane tops treated with different additives in a 49-d experimental period

Treatments[1]	Qualified reads	OTUs	Sobs	Shannon	Simpson	Ace	Chao	Coverage (%)
Bacterial								
CK	47,938	865	864.50	4.31	0.07	1,050.81	1,056.99	99.26
T1	52,145	1013	1,013.25	5.11	0.02	1,171.33	1,180.67	99.24
T2	43,424	982	981.75	5.04	0.02	1,169.69	1,182.22	99.05
SEM	2,701.00	44.06	44.06	0.20	0.01	37.99	37.44	0.06
P-value	0.46	0.38	0.38	0.22	0.21	0.37	0.32	0.34
Fungal								
CK	52,746	250 [a]	249.75 [a]	2.84	0.18	292.70[ab]	300.14[ab]	99.95
T1	56,562	235 [a]	235.00 [a]	3.4	0.14	240.83[a]	243.17[a]	99.98
T2	62,348	341[b]	341.25[b]	3.49	0.11	344.01[b]	344.93[b]	99.99
SEM	4,794.71	20.75	20.75	0.16	0.02	17.11	16.86	0.01
P-value	0.74	0.04	0.04	0.17	0.46	0.03	0.04	0.13

[1]Treatments included no inoculant (CK), bacteria-enzyme inoculants containing *Pediococcus acidilactici*, *Saccharomyces cerevisiae*, cellulase, and xylanase (T1, viable colony-forming units of each bacterial strain ≥ 10^8 CFU/g; enzyme activity of each enzyme ≥ 200 U/g), and mixed bacterial inoculants containing *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae* (T2, viable colony-forming units of each bacterial strain ≥ 10^7 CFU/g).
[a,b]Values with different superscripts within each column are significantly different ($P < 0.05$).
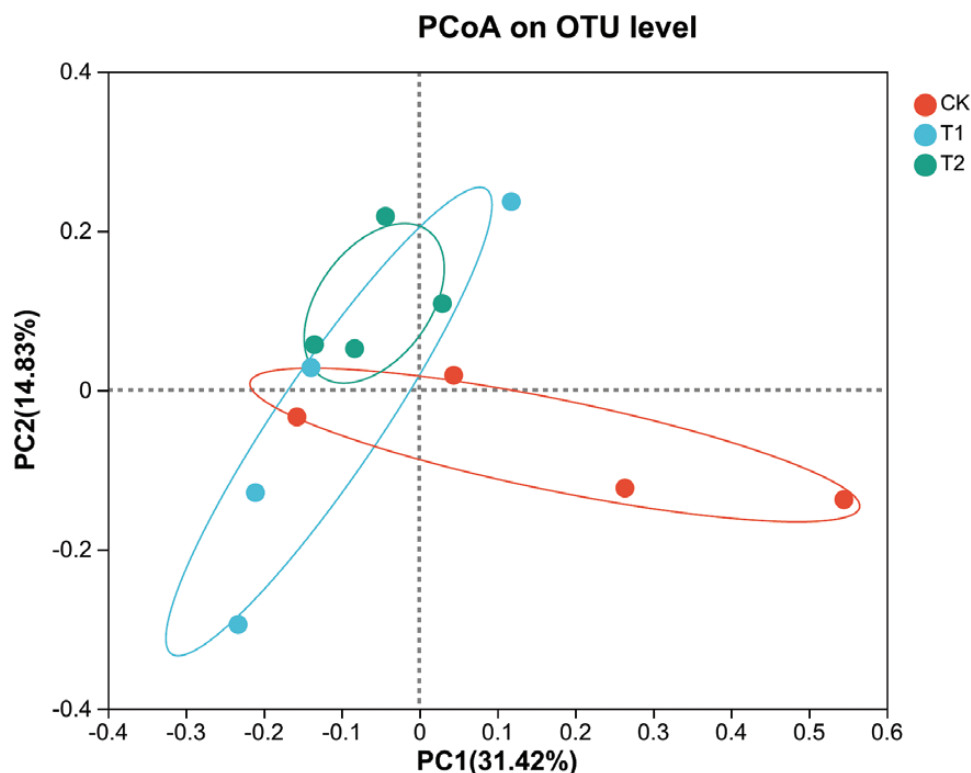

Figure 1. PCoA of the bacterial community in beef ruminal fluid. Treatments included no inoculant (CK), bacteria-enzyme inoculants containing *Pediococcus acidilactici*, *Saccharomyces cerevisiae*, cellulase, and xylanase (T1, viable colony-forming units of each bacterial strain ≥ 10^8 CFU/g; enzyme activity of each enzyme ≥ 200 U/g), and mixed bacterial inoculants containing *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae* (T2, viable colony-forming units of each bacterial strain ≥ 10^7 CFU/g).

The alpha diversity indexes of the rumen bacterial community of beef cattle showed no significant difference between groups ($P > 0.05$). Comparisons of bacterial communities by PCoA based on the Bray–Curtis distance matrix showed no significant separation between the samples obtained from beef cattle-fed CK silage, T1 silage, or T2 silage (Figure 1).

Changes in the bacterial community at the phylum level in the rumen liquid of beef cattle are shown in Figure 2. The bacterial phyla community in the rumen liquid of beef cattle was mainly represented by two phyla, *Bacteroidetes* and *Firmicutes*, and *Bacteroidetes* was the dominant bacterial phylum in all the samples.
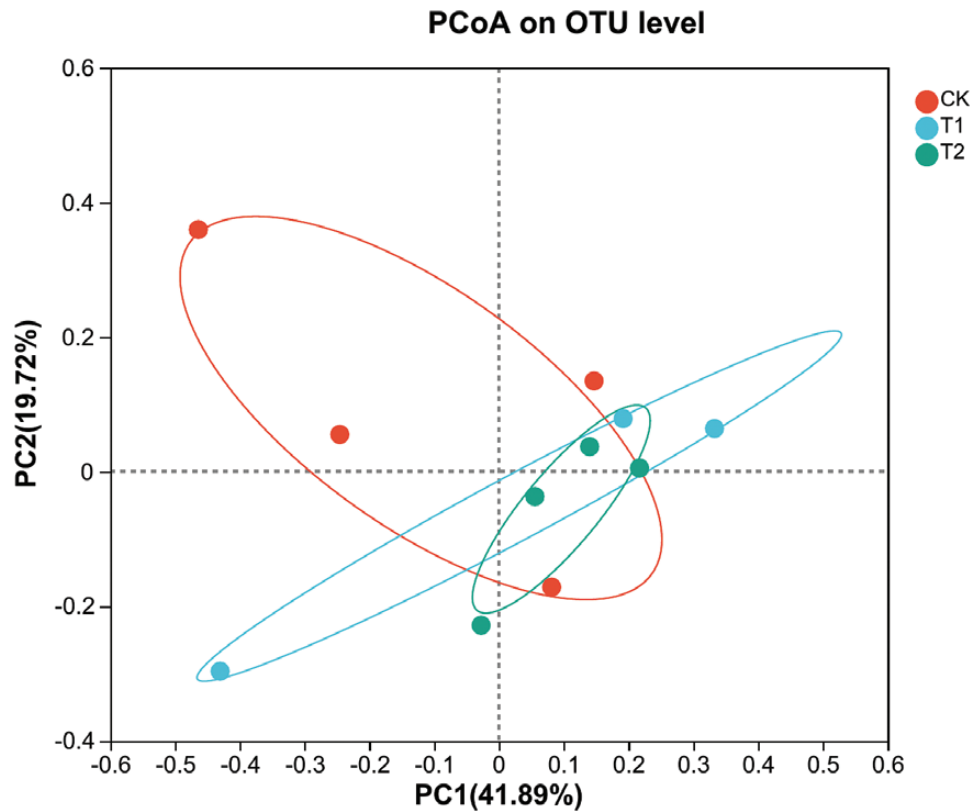
PCoA on OTU level

Figure 2. Relative abundance of bacterial phyla in beef ruminal fluid (average relative abundance > 0.5%). Treatments included no inoculant (CK), bacteria-enzyme inoculants containing *Pediococcus acidilactici*, *Saccharomyces cerevisiae*, cellulase, and xylanase (T1, viable colony-forming units of each bacterial strain ≥ 10^8 CFU/g; enzyme activity of each enzyme ≥ 200 U/g), and mixed bacterial inoculants containing *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae* (T2, viable colony-forming units of each bacterial strain ≥ 10^7 CFU/g).

Changes in the bacterial community at the genus level in the rumen liquid of beef cattle are shown in Figure 3. *Prevotella*, *Rikenellaceae_RC9_gut_group*, *norank_f_F082*, *NK4A214_group*, *Prevotellaceae_UCG-003*, *norank_f_Bacteroidales_RF16_group*, *Succiniclasticum*, *Prevotellaceae_UCG-001*, *Lachnospiraceae_NK3A20_group*, *Ruminococcus*, and *Christensenellaceae_R-7_group* were the most abundant genera with an average abundance ≥ 1%. *Prevotella* was the dominant bacterial genera in all the samples, followed by *Rikenellaceae_RC9_gut_group*.

As shown in Supplementary Figures S1 and S2, there were no significant differences observed in the relative abundance of the major rumen bacterial compositions at the phylum or genus levels among the three groups ($P > 0.05$).

Ruminal fungal community profile

Fungi sequencing information and the alpha diversity of the fungal community in the rumen liquid are shown in Table 4. A total of 171,656 valid sequences were obtained by high-throughput sequencing. Coverage values exceeded 0.999 in all the samples, indicating that the sampling depth had adequately captured most of the bacterial communities. The alpha diversity indexes of the rumen fungi community, including OTUs, Sobs, Ace, and Chao, differed among the three treatments in the following order: T2 > CK > T1. This indicated that T2 increased the abundance and diversity of fungi in the beef cattle rumen, however, T1 reduced the abundance and diversity of fungi in the beef cattle rumen.

Comparisons of fungal communities by PCoA based on the Bray–Curtis distance matrix showed no significant separation between the samples obtained from beef cattle-fed CK silage, T1 silage, or T2 silage (Figure 4).

Changes in the fungal community at the phylum level in the rumen liquid of beef cattle are shown in Figure 5. *Ascomycota*, *Mortierellomycota*, *Neocallimastigomycota*, *Basidiomycota* and *unclassified_k_Fungi* were the most abundant phyla with an average abundance ≥ 1%. *Ascomycota* was the dominant fungal phyla in all the samples.

Changes in the fungal community at the genus level in the rumen liquid of beef cattle are shown in Figure 6. *Mortierella*, *Pichia*, *Issatchenkia*, *unclassified_p_Ascomycota*, *Aspergillus*, *unclassified_k_fungi*, *Fusarium*, *Penicillium*, *Talaromyces*, *and Schizothecium* were the most abundant genera with an average abundance ≥ 1%. *Mortierella* was the dominant fungal genus in all the samples.

As shown in Supplementary Figures S3 and S4, there were no significant differences observed in the relative abundance of the major rumen fungal compositions at the phylum or genus level among the three groups ($P > 0.05$).

Correlations between growth performance, nutrient digestibility, and microbial community composition

The correlations between growth performance, nutrient digestibility, and microbial community composition are shown in Figure 7. According to Spearman's rank correlation coefficient, the ADG was strongly negatively correlated with
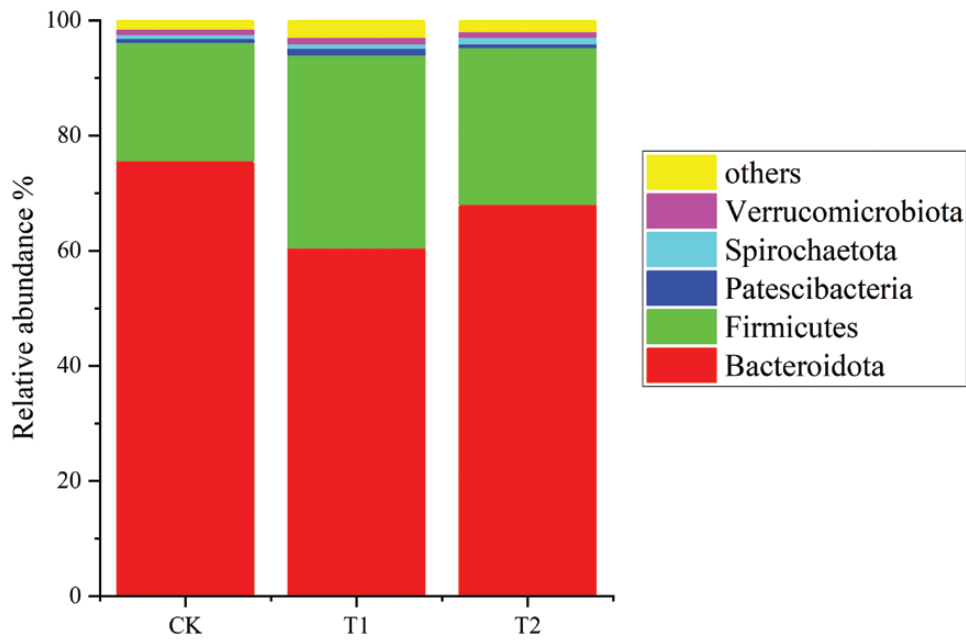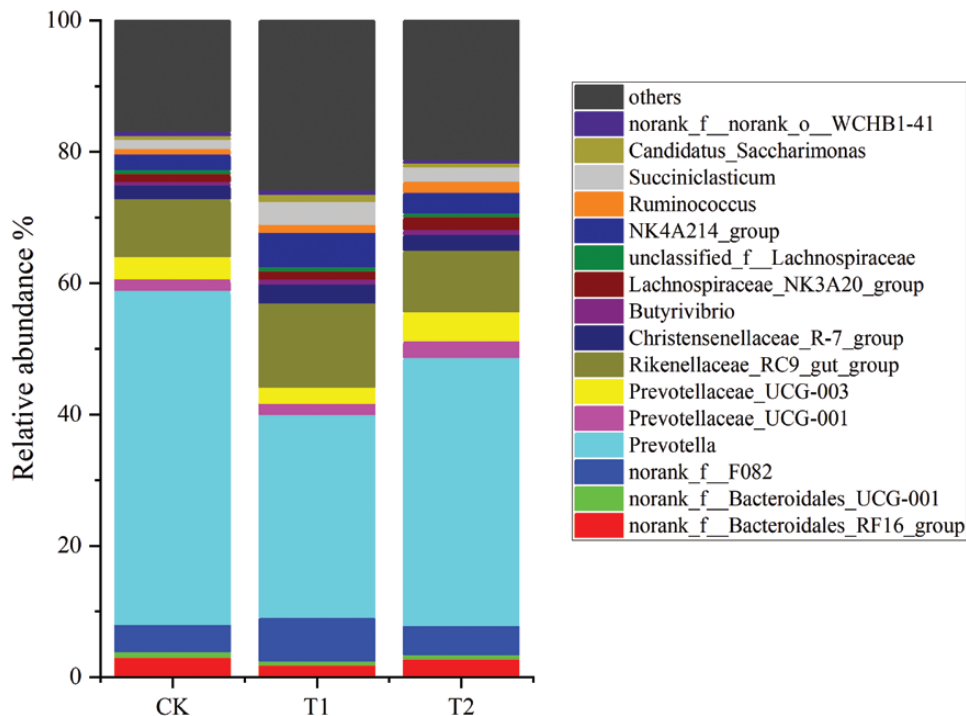
Figure 3. Relative abundance of bacterial genera in beef ruminal fluid (average relative abundance > 0.5%). Treatments included no inoculant (CK), bacteria-enzyme inoculants containing *Pediococcus acidilactici*, *Saccharomyces cerevisiae*, cellulase, and xylanase (T1, viable colony-forming units of each bacterial strain ≥ 10^8 CFU/g; enzyme activity of each enzyme ≥ 200 U/g), and mixed bacterial inoculants containing *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae* (T2, viable colony-forming units of each bacterial strain ≥ 10^7 CFU/g).

Figure 4. PCoA of the fungal community in beef ruminal fluid. Treatments included no inoculant (CK), bacteria-enzyme inoculants containing *Pediococcus acidilactici*, *Saccharomyces cerevisiae*, cellulase, and xylanase (T1, viable colony-forming units of each bacterial strain ≥ 10^8 CFU/g; enzyme activity of each enzyme ≥ 200 U/g), and mixed bacterial inoculants containing *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae* (T2, viable colony-forming units of each bacterial strain ≥ 10^7 CFU/g).

Ascomycota of the fungal phyla and *Pichia* of the fungal genera ($R = -0.61$, $P = 0.046$; $R = -0.62$, $P = 0.042$, respectively). The apparent digestibility of NDF was strongly positively correlated with *Neocallimastigomycota* of the fungal phyla ($R = 0.79$, $P = 0.0037$). The apparent digestibility of ADF

was strongly positively correlated with *unclassified_p_Ascomycota* of the fungal genera ($R = 0.65$, $p = 0.032$). Among the bacterial genera, *Ruminococcus* was strongly positively correlated with the apparent digestibility of CP ($R = 0.62$, $P = 0.043$).
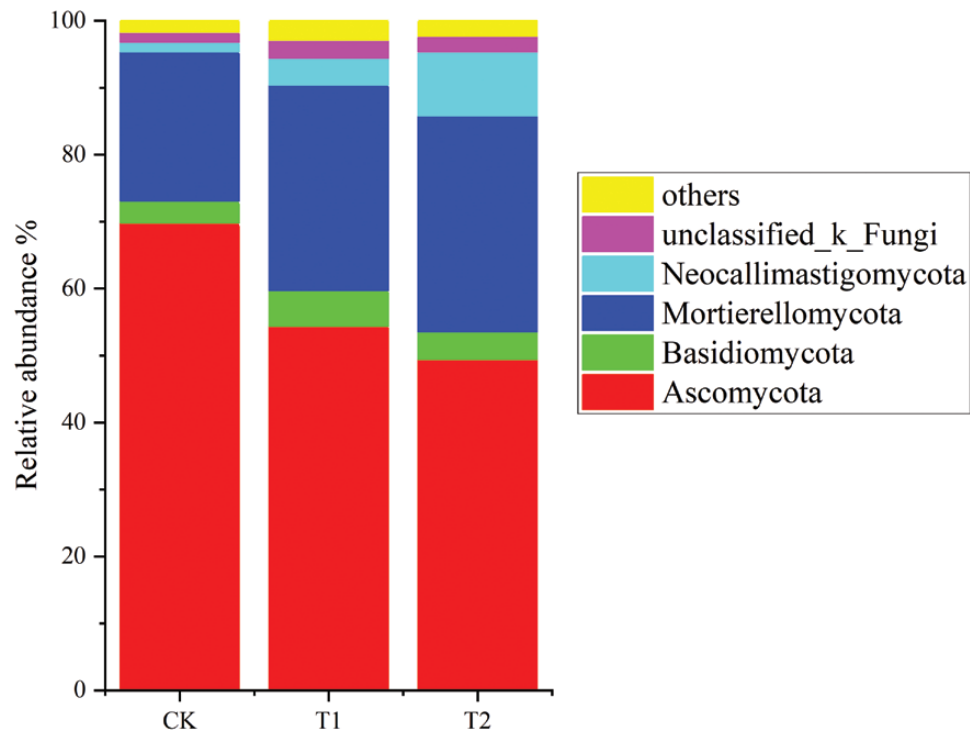
Figure 5. Relative abundance of fungal phyla in beef ruminal fluid (average relative abundance > 0.5%). Treatments included no inoculant (CK), bacteria-enzyme inoculants containing *Pediococcus acidilactici*, *Saccharomyces cerevisiae*, cellulase, and xylanase (T1, viable colony-forming units of each bacterial strain ≥ 10^8 CFU/g; enzyme activity of each enzyme ≥ 200 U/g), and mixed bacterial inoculants containing *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae* (T2, viable colony-forming units of each bacterial strain ≥ 10^7 CFU/g).

Discussion

Effects of different additives applied to silage sugarcane tops on the growth performance of beef cattle

It has been reported that silage inoculated with LAB improved the growth performance of animals (Zhang et al., 2019; Nair et al., 2020). In this study, the ADG of beef cattle-fed sugarcane top silage with added T2 or T1 was significantly higher than that of beef cattle-fed CK, indicating that the two mixed inoculants improved the growth performance of beef cattle, which may be the result of improved nutrient preservation during the fermentation process and conservation of a higher proportion of digestible nutrients, thus stimulating the activity of rumen microorganisms. Similarly, silage inoculants had a great impact on the performance of cattle-fed sugarcane silage, with the ADG increasing by 80% (Rabelo et al., 2016). Homofermentative microbial inoculant and a mixture of microbial inoculant and chemical additives increased the ADG of beef cattle-fed temperate grasses silage (Menezes et al., 2022). Guo et al. (2022) also reported that sheep fed whole-plant corn silage treated with lignocellulose-degrading bacteria exhibited higher ADG than those fed untreated whole-plant corn silage. However, Addah et al. (2016) reported that microbial additives and enzyme-mixed additives failed to improve the growth performance of beef cattle, which was inconsistent with our research. These contrasting results could be due to differences among additive varieties and application rates, differences among animals, as well as variations in the magnitude of the intake responses and their inherent passage rates among the studies (Basso et al., 2014). In addition, in this study, we found that neither T1 or T2 affected the DMI of beef cattle, which indicated that the inoculants used in this study did not affect the silage palatability or feed intake. Currently, there are inconsistent reports on the effects of microbial or enzymatic silage additives on DMI. Some studies have shown that animals showed improved DMI when fed inoculated corn silages (Zhang et al., 2019; Guo et al., 2022), but other studies have shown that inoculated corn silage (Rabelo et al., 2018; Nair et al., 2020) and barley silage (Addah et al., 2014) had no significant effect on DMI. The improvement of DMI was usually attributed to increased NDF digestibility or more favorable fermentation conditions, but the improvement of DMI is not always recorded in studies (Bayatkouhsar et al., 2011). The feed-to-weight ratio is an important production index of beef cattle. The smaller the feed-to-weight ratio, the higher the production level. In this study, the feed-to-weight ratios of beef cattle-fed sugarcane top silage with added T2 or T1 were lower than those fed CK, but there were no significant differences detected among the three treatments. The effects of the feed-to-weight ratio of beef cattle have been shown elsewhere to depend on diet composition, the strain type of inoculants added, and dosage (Sartori et al., 2017). However, Rabelo et al. (2016) and Zhang et al. (2019) both observed that inoculation of maize silage did not produce any effect on the feed conversion ratio (feed/gain) of animals.

Effects of different additives applied to sugarcane top silage on the nutrient apparent digestibility of beef cattle

Although inoculants have mostly been used to improve silage fermentation, some reviews have reported the positive effects
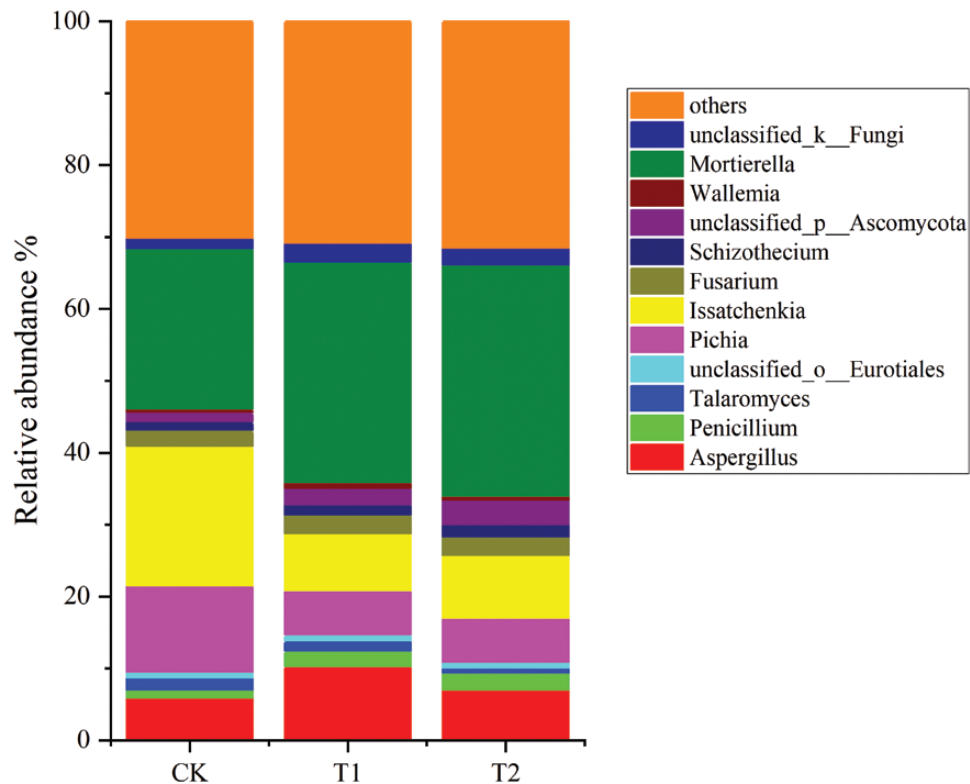
Figure 6. Relative abundance of fungal genera in beef ruminal fluid (average relative abundance > 0.5%). Treatments included no inoculant (CK), bacteria-enzyme inoculants containing *Pediococcus acidilactici*, *Saccharomyces cerevisiae*, cellulase, and xylanase (T1, viable colony-forming units of each bacterial strain ≥ 10^8 CFU/g; enzyme activity of each enzyme ≥ 200 U/g), and mixed bacterial inoculants containing *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus oryzae* (T2, viable colony-forming units of each bacterial strain ≥ 10^7 CFU/g).

of inoculation on the nutrient utilization of ruminants (Li et al., 2022; Mueller et al., 2022). In this study, it was found that apparent digestibility of CP was significantly increased in beef cattle-fed sugarcane top silage with added T2 or T1. Similarly, Lara et al. (2018b) and Nkosi et al. (2010) showed that the addition of bacterial or enzymatic inoculants to silage increased the apparent ruminal digestibility of CP. Li et al. (2022) also reported that ferulic acid esterase-producing *L. plantarum* in alfalfa silage resulted in substantial increases in apparent digestibility of CP in lactating dairy goats compared to the control group. This finding might be attributed to the effect of inoculants, which promoted LAB fermentation. LAB can produce sufficient lactic acid to result in a rapid drop in pH, inhibiting the growth of harmful bacteria and decreasing protein degradation in silage (Ying et al. 2017), thereby resulting in the higher apparent ruminal digestibility of CP. Moreover, the reason for the increase in the ruminal apparent digestibility of CP observed in the present study might be the possible probiotic effect exerted by T1 and T2 by producing beneficial substances or by inhibiting detrimental microbes, which may promote specific rumen microbial populations related to protein digestion. Monteiro et al. (2021) reported that high-producing dairy cows supplemented with alfalfa silage inoculated with a LAB inoculant showed significantly increased ruminal apparent digestibility of NDF. However, the present study found that the application of T1 and T2 to sugarcane top silage had no effect on the apparent digestibility of NDF in beef cattle. A similar result was also found by Rowghani et al. (2008). During the ensiling process, cellulolytic enzymes are likely to react positively to plants with low WSC (Khota et al., 2016), but sugarcane tops are major

by-products of the sugarcane industry and have a high WSC content. This might be the main reason why T1 did not play a role in the degradation of NDF in sugarcane top silage, thus affecting the improvement of the ruminal apparent digestibility of NDF. In addition, the application of T2 to sugarcane top silage had no effect on the apparent digestibility of NDF, which may be due to the fact that the silage environment is not suitable for *A. oryzae* to achieve high activity, so it affects the function of T2. Hu et al. (2022) showed that the best temperature for enzyme production by *A. oryzae* was 28 to 35 °C. During this study, the silage environment in the laboratory was lower than 25 °C, which may be the main reason for the limited effect of *A. oryzae*. In this study, it was also found that the application of T1 and T2 to sugarcane top silage significantly increased the ruminal apparent digestibility of ADF. Similarly, it was reported that the use of LAB inoculants in silage led to the higher ruminal apparent digestibility of ADF (Nair et al., 2019b; Monteiro et al., 2021). The improvement of the ruminal apparent digestibility of ADF in this study may be due to the increased degradation of plant cellulose and the stimulation of rumen microbial activity caused by T1 and T2. Various reports indicated that the apparent digestibility of OM was significantly increased in ruminants fed inoculated silages (Ozduven and Onal, 2010; Nkosi et al., 2011). However, in some studies, inoculant-treated silage did not affect the ruminal OM apparent digestibility (Nkosi et al., 2010; Basso et al., 2014). Lara et al. (2018b) reported that the increased apparent ruminal digestibility of OM and DM was possibly a result of increased ADF and NDF digestibility. In the present study, both T1 and T2 had no effect on the apparent digestibility of OM in beef cattle, which may be because
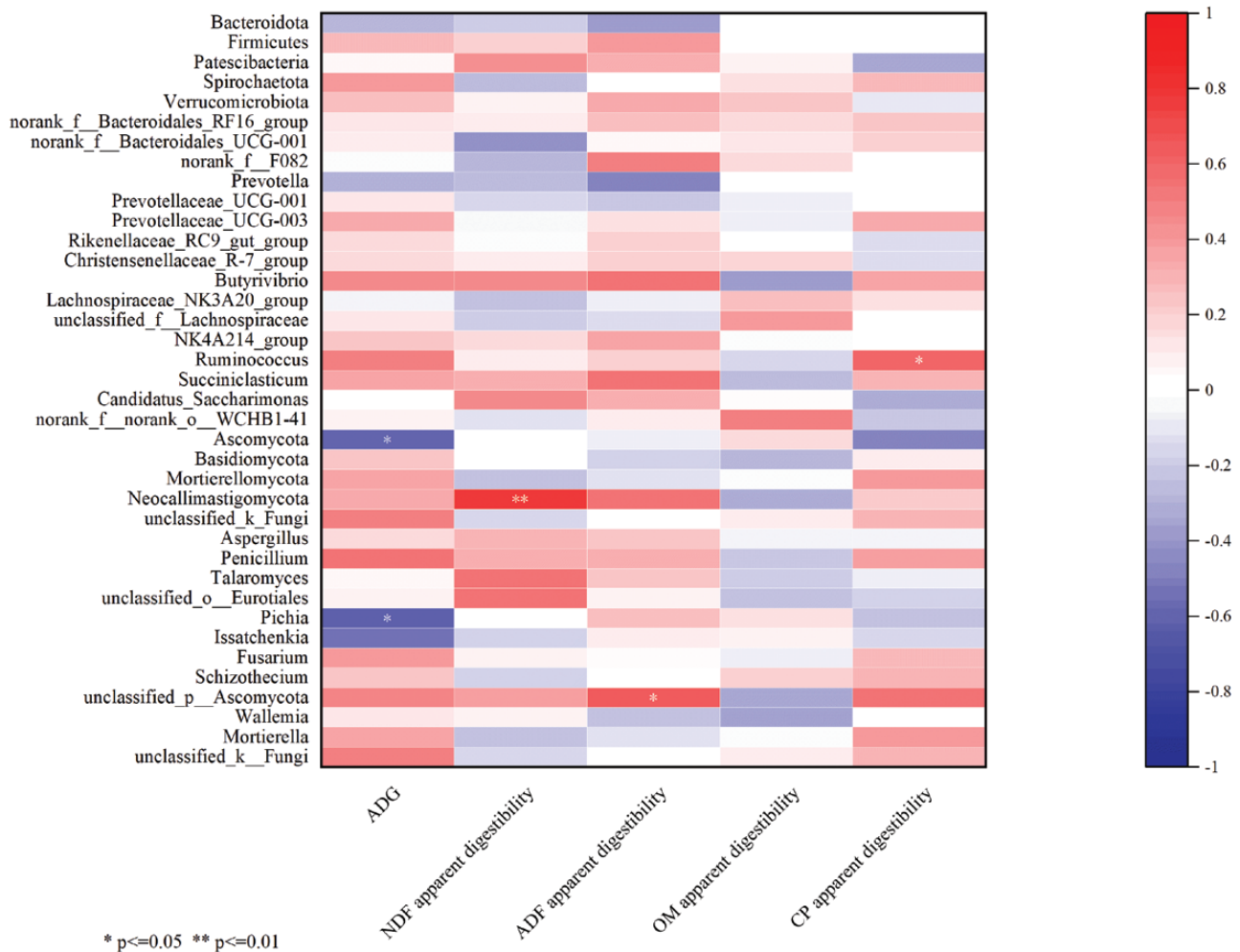
Figure 7. Relationships between growth performance, apparent nutrient digestibility, and rumen microbiome in beef cattle. The correlations marked with an asterisk ("*") are significant at *P* < 0.05, and "**" represents *P* < 0.01. Abbreviations: ADG, average daily gain; DMI, dry matter intake.

both T1 and T2 had no effect on the apparent digestibility of NDF in beef cattle. Direct-fed microbials, including *S. cerevisiae*, *L. plantarum*, *B. subtilis*, and *A. oryzae*, have been shown to improve the health status and production performance of cattle (Buntyn et al., 2016). In this study, the apparent digestibility of CP and ADF in beef cattle-fed sugarcane top silage with added T2 or T1 were higher compared to beef cattle-fed CK silage, which suggested that the silage inoculant likely acted as a DFM, modulating ruminal fermentation and enhancing the ruminal environment so as to promote CP and fiber digestibility.

Effects of different additives applied to silage sugarcane tops on rumen bacterial communities of beef cattle

In this study, the relative abundance of the dominant phyla and the majority of the genera with a relative abundance ≥ 0.5% were not affected by T1 or T2. PCoA of the bacterial community results also verified no significant differences in ruminal bacterial community structures among the three groups. Fomenky et al. (2018) found that supplementing the diet of pre-weaned calves with *S. cerevisiae Boulardii* CNCM I-1079 increased the microbial diversity index, but no significant difference in ruminal bacterial abundance at the genus level was found. Zhang et al. (2022) reported that the ruminal bacte-

rial community composition was not significantly changed by calcium propionate supplementation in early-lactation dairy cows, which may be due to the same total mixed ratio. In general, diet is considered the most important determining factor of ruminal microbial composition (Liu et al., 2021). In our study, the three groups had similar levels of protein and energy, and the beef cattle were raised under the same ambient conditions, which might account for the similarities in rumen bacterial microbiota. In contrast, Guo et al. (2022) reported that whole-plant corn silage treated with lignocellulose-degrading bacteria fed to sheep significantly increased the relative abundance of the most abundant bacterial genera. The difference may be related to the experimental animals, additive varieties, application rates, and diets.

At present, researchers have noted that *Bacteroides* and *Firmicutes* are the most abundant bacterial phyla in rumen liquid (Guo et al., 2022). *Bacteroidetes* benefit their hosts by assisting them in polysaccharide decomposition (Spence et al., 2006), while *Firmicutes* are mainly involved in decomposition of fibrous substances (Pitta et al., 2014). In this study, the bacterial phyla community in the rumen liquid of beef cattle mainly was represented by two phyla, *Bacteroidetes* and *Firmicutes*, and *Bacteroidetes* was the dominant bacterial phyla in all the samples. This was in line with previous studies (Guo et al., 2022). However, the rumen bacterial community

of beef cattle-fed sugarcane top silage with added T2 or T1 had a tendency to reduce the abundance of *Bacteroidetes* and increase the abundance of *Firmicutes* compared to those fed CK silage. *Firmicutes* degrade fibrous substances, which indicates that both T1 and T2 may be beneficial for improving the ability of beef cattle to degrade crude fiber in rumen. In addition, both T1 and T2 may have a certain inhibitory effect on *Bacteroidetes* in rumen liquid.

Prevotella was the dominant bacterial genera in all the samples, followed by *Rikenellaceae_RC9_gut_group*. *Prevotella* was the most abundant bacterial genera in the rumen liquid, which was consistent with the previous research results on sheep (Guo et al., 2022) and dairy cows (Rinne et al., 2022). This may indicate that *Prevotella* is the dominant bacterial genera of rumen microorganisms of most ruminants. *Prevotella* promotes the decomposition of protein and carbohydrate and cooperates with cellulose-decomposing bacteria to improve the host's ability to decompose cellulose, increasing the utilization rate of nutrients in feed (Matsui et al., 2000). However, the rumen bacterial community of beef cattle-fed sugarcane top silage with added T2 or T1 had a tendency to have reduced abundances of *Prevotella* compared to those fed CK silage. This showed that both T1 and T2 might inhibit the growth of *Prevotella* in the rumen. The second dominant bacterial genus in the rumen liquid was *Rikenellaceae_RC9_gut_group*, which was consistent with the results of Zhang and Wang (2018). In another study, the *Rikenellaceae_RC9_gut_group* dominated in all the samples of rumen fluid from grassland red cattle (Guo et al., 2019). Conte et al. (2022) reported that *Rikenellaceae_RC9_gut_group* was related to rumen lipid metabolism. The dimethylacetals are derivatives of plasmalogen lipids, which were associated with the increased abundance of *Rikenellaceae_RC9_gut_group* in animals characterized by the best growth performances (Daghio et al., 2021). This study also found that both T1 and T2 had a tendency to increase the abundance of *Rikenellaceae_RC9_gut_group*, indicating that both T1 and T2 may be beneficial for promoting rumen lipid metabolism, thereby improving the ADG of beef cattle.

Effects of different additives applied to silage sugarcane tops on the rumen fungal community of beef cattle

Anaerobic fungi can produce various highly active enzymes, such as xylanases, cellulases, and esterases, and degrade the cell wall of ingested plants using various physical and chemical methods (Griffith et al., 2009). In our study, we found that T2 increased the abundance and diversity of fungi in the beef cattle rumen, thereby implying that T2 supplementation promoted the proliferation and metabolism of rumen fungi. The addition of microorganisms to the rumen can change the microbial community and the microbial community changes in response to changes in feed and feed levels in the rumen (Miguel et al., 2019). The dietary changes have important impacts on rumen bacterial communities (Bi et al., 2018). In this study, there were no significant differences observed in the relative abundance of the major rumen fungal compositions at the phylum and genus level in any of the samples, which may be due to similar diets, additive varieties, and application rates. Similarly, Cherdthong et al. (2021) reported that Thai-indigenous beef cattle-fed ensiled rice straw with *Lactobacillus casei* TH14, molasses, and cellulase enzymes did not have an altered ($P > 0.05$) rumen fungal population.

Supplementation effects of *Clostridium Saccharobutylicum* on the rumen microbiome in Holstein-Friesian cows indicated no differences in the abundance of general anaerobic fungi among treatments (Miguel et al., 2019).

Anaerobic fungi are known to be key players in the degradation of lignocellulosic plant fiber in the rumen (Lee et al., 2000). We revealed that *Ascomycota* was the dominant fungal phyla in all the samples in this study (49.50% to 66.43%). Similarly, Han et al. (2019) reported that *Ascomycota* was the most abundant fungal phyla in the rumen liquid of goats (53.37% to 77.79%). However, this result disagreed with Xue et al. (2018), who analyzed the diversity of fungi in the rumen liquid of dairy cows by metagenomic sequencing, showing that *Ascomycota*, *Basidiomycota*, and *Chytridiomycota* were dominant phyla in all samples. This discrepancy may be caused by different factors, such as diet and animals. Functionally, the *Ascomycota* phylum is known to play a key role in degrading lignin (Hu et al., 2021). It was also found that both T1 and T2 tended to reduce the abundance of *Ascomycetes*, and the abundance of other fungal phyla showed an increasing trend. This indicated that both T1 and T2 might have an inhibitory effect on the *Ascomycota* and promoted growth of *Mycophyta*. Figure 5 showed that *Neocalimastigomycota* also accounted for a relatively high proportion at the phylum level (>1%). Because it has strict anaerobic characteristics, causes a wide range of cell bindings, affects cellulose decomposition, hemicellulose decomposition, glycolysis, and protein hydrolysis, and promotes digestion and utilization of cellulose in the rumen, it has attracted more attention as a rumen juice microorganism (Liggenstoffer et al., 2010; Xue et al., 2018). Compared with CK, T1 and T2 had a tendency to increase the abundance of *Neocallimastigomycota*, indicating that both T1 and T2 may help to improve the digestion and utilization of cellulose in the rumen of beef cattle.

Sunil et al. (2013) reported that *Orpinomyces* (48%), uncultured clones (29%), *Cyllamyces* (9%), *Piromyces* (8%), and *Anaeromyces* (6%) were the predominant anaerobic fungal genera in the rumen. In addition, Mao et al. (2014) observed that the predominant anaerobic fungi detected in the rumen were from the genera *Neocallimastix*, *Orpinomyces*, and *Piromyces*, which collectively accounted for 57% of the anaerobic fungal reads. However, we found that *Mortierella* was the dominant fungal genera in all samples. *Mortierella*, which belongs to the order *Mucorales* within the class *Zygomycetes* (phylum Zygomycota), has attracted notable attention within this research area due to its potential as a lipid producer and the fact that a significant portion of fungal lipids contain essential fatty acids (Dyal and Narine, 2005). This study found that both T1 and T2 had a tendency to increase the abundance of *Mortierela*, indicating that both T1 and T2 may be beneficial for promoting rumen lipid metabolism in the rumen.

Correlation analysis

To better understand the relationships between the production traits (ADG and apparent digestibility of CP, NDF, ADF, and OM) and rumen bacterial and fungal populations after beef cattle were fed T1 silage and T2 silage, a Spearman's rank correlation coefficient heatmap was generated for ruminal microbial strains at the phylum and genus level (Figure 7).

The Spearman's rank correlation coefficient analysis showed that the ADG was strongly negatively correlated with *Ascomycota* of the fungal phyla and *Pichia* of the fungal

genera. However, this study found that both T1 and T2 had a tendency to reduce the abundance of *Ascomycetes* and *Pichia*. The above results thus explain why the application of T1 and T2 to sugarcane top silage significantly increased the ADG of beef cattle. *Neocalimastigomycota* can promote the digestion and utilization of cellulose in the rumen (Xue et al., 2018). In this study, both T1 and T2 increased the abundance of *Neocalimastigomycota*, and *Neocalimastigomycota* of the fungal phyla, which was strongly positively correlated with the apparent digestibility of NDF. However, the present study found that the application of T1 and T2 to sugarcane top silage had no effect on the apparent digestibility of NDF in beef cattle. This is due to the fact that the influence of rumen microorganisms is much less than the influence of raw material properties. The apparent digestibility of ADF was strongly positively correlated with *unclassified_p_Ascomycota* of the fungal genera. *Unclassified_p_Ascomycota* was positively correlated with most volatile substances (Fu et al., 2021). Fibers are fermented by rumen microorganisms in the rumen and transformed into VFAs (Wang et al., 2020); therefore, *Unclassified_p_Ascomycota* may play a very important role in the degradation of fibers in the rumen. The above results thus explain why the application of T1 and T2 to sugarcane top silage significantly increased the apparent digestibility of ADF in beef cattle. The *Ruminococcaceae* bacteria attain nutrients mainly through decomposing fibers and their fermentation products mainly consist of xylose and glucose, and *Ruminococcus* is related to the utilization efficiency of nitrogen in the gastrointestinal tract of goats (Wang et al., 2019). In the present study, both T1 and T2 had a tendency to increase the abundance of *Ruminococcus*, and *Ruminococcus* of the bacterial genera was positively and strongly correlated with the apparent digestibility of CP. This may explain why the apparent digestibility of CP was significantly increased in beef cattle-fed sugarcane top silage with added T2 or T1. The results also showed that the apparent digestibility of OM was not strongly correlated with any rumen microorganisms, which may have been one of the reasons why the apparent digestibility of OM could not be significantly improved.

Conclusions

Improved beef cattle performance occurred when the inoculants (T1 and T2) evaluated in this study were used, as indicated by a significantly increased ADG and ruminal apparent digestibility of CP and ADF compared with CK. In addition, both T1 and T2 had no significant impact on the major rumen bacterial and fungal community composition. Spearman's rank correlation coefficient analysis revealed that the apparent digestibility of ADF for beef cattle was strongly positively correlated with *unclassified_p_Ascomycota* of the fungal genera. *Neocalimastigomycota* of the fungal phyla was strongly positively correlated with the apparent digestibility of NDF. Ruminococcus of the bacterial genera was strongly positively correlated with the apparent digestibility of CP. The two mixed inoculants improved the growth performance of beef cattle. This may have been the result of improved protein preservation during the fermentation process and the regulation of the growth of cellulose-degrading bacteria and fungi in the rumen of beef cattle, which improved the ruminal apparent digestibility of CP and ADF.

Conflict of interest statement

The authors declare that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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