Comparative Production Performance and Rumen Bacterial Diversity of Fattening Beef Cattle Supplemented with Different Levels Concentrated Feed

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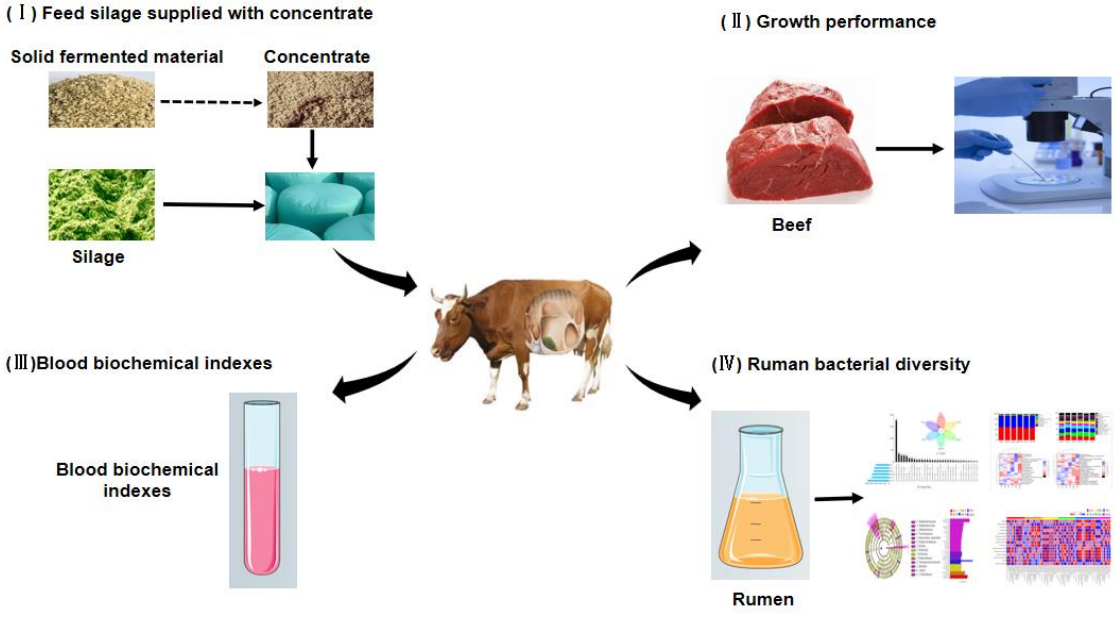
DOI: 10.15376/biores.19.2.2216-2243

GRAPHICAL ABSTRACT



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The production performance and rumen bacterial diversity were compared for different silage-based diets supplemented with common concentrate or bio-concentrate to develop an alternative of common concentrate for fatten cattle feeding. The daily gain of fattening cattle was increased by 0. 99 kg and 1.04 kg, respectively, when fed with single corn silage or mixed silage-based diet supplemented with bio-concentrate. There was no significant difference in water loss rate and cooked meat rate among groups ($P>0.05$), but the tenderness of beef in the bio-concentrate group was significantly higher than that in the common concentrate group ($P<0.05$). There were no adverse effects on beef quality and blood biochemical indexes in each group. Compared with the normal concentrate group, the OTU number and α-diversity index of rumen microorganisms of fattening cattle fed with mixed silage as the basic diet supplemented with bio-concentrate increased significantly. At generic level, the relative abundances of *Prevotella*, Porphyromonadaceae (unclassified), and *Succiniclasticum* were increased by adding bio-concentrate in the diets based on mixed silage and single sorghum silage. Relative abundances of *Bacteroidetes (*unclassified), Ruminococcaceae (unclassified), and *Firmicutes* (unclassified) decreased. In conclusion, the bio-concentrate might be a better choice than common concentrate for beef cattle breeding.

DOI: 10.15376/biores.19.2.2216-2243

Keywords: Silage feed; Finishing cattle; Concentrated feed; Growth performance; Meat quality and blood indexes; Rumen bacterial diversity; 16S rRNA sequence

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INTRODUCTION

In heavily cultivated countries such as China, the feeding system of ruminants is agricultural by-product dependent rather than grassland dependent. The efficiency of a ruminant feeding system in these countries, therefore, relies on how efficiently agricultural by-products are used. In the traditional extensive practice, beef cattle are fed crop stalks supplemented with a little concentrate. Silage such as woodgrass, hay, and crop straw is an important source of roughage for ruminants (Filik and Erturk 2023). Roughage contains a lot of crude fiber, which is an important source of energy for ruminants (Orskov 1998). Although ruminants do not produce cellulosic hydrolase or hemicellulosic hydrolase on

their own, the cellulose components in the feed are broken down and fermented by the microbial flora in the rumen to produce volatile fatty acids such as acetic acid, propionic acid, and butyric acid (Castillo-González *et al.* 2014). Volatile fatty acids are absorbed to provide energy to the ruminants. In addition, crude fiber can also stimulate animal chewing, gastrointestinal peristalsis, enrich the gastrointestinal tract, and regulate gastrointestinal microflora (Refat and Yu 2016).

Corn, sorghum, and wheat are the main sources of roughage. However, it is difficult to meet the nutrient needs of ruminants by feeding only these roughages with low levels of protein, calcium, and phosphorus (Santra and Karim 2009). In many feedlots, adding additives to the basal diet of ruminants is a practical way to increase production. To improve production performance, management and improvement of rumen fermentation have always been the goals of ruminant research (Dias *et al.* 2021; Jihene *et al.* 2022; Várhidi *et al.* 2022). The addition of concentrate provides more energy for the growth of ruminants (Hill *et al.* 2008). The mixture of the roughage and the concentrate greatly improves the utilization rate of the feed (Coverdale *et al.* 2004), improves the rumen environment (Khan *et al.* 2011), and reduces the abnormal behavior of ruminants during growth (Muhammad *et al.* 2016). In addition, optimization of dry matter intake reduces feed costs and improves feeding efficiency (Suarez-Mena *et al.* 2015), especially at the concentrate level. Studies have shown that high concentrate levels alter the feeding time of ruminants, shortening rumination times (Devries *et al.* 2007; Devries and Keyserlingk 2009).

The output of China's animal husbandry products ranks among the top in the world, but grain and feed production has always been a weak link, and the gap between supply and demand of grain as ordinary feed is getting bigger and bigger (Zhang *et al.* 2019; Kang *et al.* 2021). Therefore, finding a low-cost feed that does not adversely affect animals to replace the higher-cost traditional feed is one of the main research goals at present. Microbial fermentation is one of the ways (Yafetto *et al.* 2023). Nowadays, it is widely used. Based on the previous work, the research team improved the traditional general concentrate formula and added a solid fermented product. This type of feed is called bio-concentrate (Wang *et al.* 2017).

The study compared the growth performance, blood biochemical parameters, beef quality, and rumen bacterial diversity of different silage-based diets (corn silage, sorghum silage, sorghum, and corn silage) supplemented with the common concentrate or the biological concentrate. It was hypothesized that under the condition of a single silage diet, adding biological concentrate can improve the growth performance and beef quality of fattening cattle more effectively than adding common concentrate. The second purpose of the study was to compare the feeding effect of biological concentrate and common concentrate under the condition of mixed silage. It was hypothesized that diets supplemented with bio-concentrate would be palatable, high in energy, and provide maximum weighted gain and higher dressing percentage for fattening cattle. The goal is to reasonably develop a more nutritious, higher utilization rate and lower cost alternative than traditional ordinary feed, so as to provide a reference for beef cattle breeding.

EXPERIMENTAL

Experimental Animal Management

The study was conducted on a farm (the third farm of Dingle ecological industry group, Wuwei City) in Wuwei City, Gansu Province. There was a total of 18 castrated bulls

of about 20 months of age. All fattening cattle were purchased by the farm. Animals were quarantined for 3 weeks during which time they were vaccinated for levamisole (8 mg/kg weight), and de-warmed with Albendazole mainly against the adult stages of internal parasites. After the recovery of fattening cattle, the formal experiment began. Each of the cattle was weighed and placed in an individual pen and acclimated to the environment and experimental condition, which was followed by 145 days of feeding trial. All procedures and tests followed the Regulations on the Administration of Laboratory Animals promulgated and implemented by the State Science and Technology Commission of China and relevant national laws and regulations and animals were treated humanely.

Table 1. Test Diet

Groups	Coarse Fodder	Concentrate
SS-I	100% silage sorghum	Common concentrate
SS-II		Biological concentrate
SSC-I	50% silage sorghum+50% silage corn	Common concentrate
SSC-II		Biological concentrate
SC-I	100% silage corn	Common concentrate
SC-II		Biological concentrate

Table 2. Nutrient Composition of Silage Material

Nutrient Composition	Silage Sorghum	Silage Corn
Moisture	72.85	73.14
Protein	6.44	8.97
Ash	15.55	6.46
Ca	1.07	0.36
P	0.21	0.19
Fat	2.33	5.4
Fibers	30.94	26.31
Starch	21.59	33
pH	4.22	6.39

Experimental treatments were arranged by a 3×2 factorial array in a completely randomized block design. The test diet was set to three levels of coarse fodder and two levels of concentrate supplementation (Table 1). Eighteen cattle were grouped according to their initial body (350kg±25kg) weight and randomly assigned to one concentrate supplementation level, each consisting of three animals. The experiment feeds consisted of silage corn and silage sorghum (Table 2) as a basal diet and concentrate mix as a supplement. The ratio of concentrate to roughage was 70:30. All the feeds were mixed evenly and prepared into total mixed ration (TMR) for feeding and adjusted feed intake according to the actual situation. The nutritional metabolism and composition of the two different concentrates are shown in Tables 3 and 4. The preparation process of the key components, solid fermented material, in the bio-concentrate was as follows: First, with bean dregs, beer pomace, and apple pomace as the fermentation base, water was added to the fermentation tank, and the ratio of material to water was 60 to 70%; Then, fermentation bacteria such as activated *Aspergillus niger*, *Candida ruana*, and *Lactobacillus plantarum* were added. Last, the fermentation process was started for 2 to 5 days at 30 to 40 ℃. The test diet was offered twice a day in two equal portions at 8:00 and 16:00 hours. Clean water was available all the time.

Table 3. Nutritional and Metabolic Levels of Concentrate (Air-dried Basis)

Name of Nutrient	Company	Formula Nutrition		Name of Nutrient	Company	Formula Nutrition	
		Common concentrate	Biological concentrate			Common concentrate	Biological concentrate
Ca	%	0.591	0.66	Trp	%	0.176	0.213
Total phosphorus	%	0.576	0.586	Val	%	0.713	0.842
Available phosphorus	%	0.26	0.26	Bovine digestibility	MC/kg	3.309	3.272
Na	%	0.224	0.224	Bovine metabolizable energy	MC/kg	2.711	2.676
Cl	%	0.344	0.344	Comprehensive net energy of beef cattle	MC/kg	1.82	1.783
Protein	%	16.123	18.716	Net energy gain of beef cattle	MC/kg	1.526	1.501
Arg	%	1.148	1.357	Beef cattle energy unit	RND/kg	0.947	0.931
His	%	0.401	0.474	Neutral detergent fibers	%	15.62	15.437
Ile	%	0.552	0.689	Acid detergent fiber	%	5.138	5.572
Leu	%	1.277	1.471	Degraded protein (cattle)	%	6.464	8.077
Lys	%	0.667	0.862	Metabolism protein	%	10.994	12.488
Met	%	0.261	0.291	Bovine intestinal digestible protein	%	0.242	0.273
Cys	%	0.53	0.594	Bovine digestible protein	%	9.005	10.83
Phe	%	0.783	0.915	Digestible Nutrients of Cattle	%	64.276	63.953
Tyr	%	1.255	1.483	Total digestible nutrients	%	69.556	68.573
Thr	%	0.546	0.656	Total digestible protein	%	1.548	1.393

Table 4. Composition of Concentrate (Air Drying Foundation)

Raw Material Composition	Content (%)	
	Common concentrate	Biological concentrate
Corn	60	54
Soybean meal	10	7
Cottonseed meal	8	7
Bran	8	5
Wheat	12.85	9.85
$Ca(HCO_3)_3$	0.17	0.17
$CaCO_3$	0.33	0.33
Salt	0.5	0.5
Premix	0.15	0.15
Solid fermented material		16
Total	100	100

Determination of Slaughter Procedure and Growth Performance of Finishing Cattle

At the beginning of the feeding trial and the end of the fattening period, the fattening cattle were weighed on an empty stomach and recorded as initial and final body weights. The average daily gain (ADG) of individual fattening cattle was calculated by dividing the sum of the average daily gains over the trial period by the number of trial days. 145 days later, the slaughter was carried out at Dingle Jiahe Slaughterhouse, Wuwei City, Gansu Province, China, following the normal procedures of the National Inspection Slaughterhouse. Carcass weight was measured by weighing. The dressing percentage was calculated as carcass weight (kg)/final weight (kg)×100%.

Collection and Determination of Blood Samples of Fattening Cattle

On the last day of the fattening test, 3 h after feeding, 10 mL of blood was collected from the tail vein. After standing at room temperature for 60 min, serum was prepared by centrifugation at 2000 g for 10 min and frozen at -20 ℃ for testing. The blood sample was sent to Lanzhou University of Technology Hospital to determine the blood routine and serum biochemical indicators, which are shown in Table 7.

Analysis and Determination of Meat Quality of Finishing Cattle

To analyze physicochemical properties, samples of the longissimus muscle were collected from each of the finishing cattle. The collected longissimus muscle was minced, placed in a freeze dryer for 48 h, dampened at room temperature for 24 h, crushed, and stored in a self-sealing bag. It was used for the determination of meat moisture, crude protein (CP), crude fat (EE), and crude ash.

Meat pH was determined at multiple sites using a hand-held pH meter (Testo 205, Testo AG, Schwarzwald, Germany) with a sharp penetrating electrode, 3 times for each meat sample, and finally averaged to give meat pH. The color of the meat (lightness, L^*; redness, a^*; yellowness, b^*) at the section of the longissimus dorsi muscle was obtained using an automatic color difference meter (Minolta, Osaka, Japan). A portion of the longissimus dorsi muscle sample (6 cm × 4 cm × 4 cm) was weighed, placed in an aluminum steamer, and cooked in boiling water for 30 min, then cooled at 0 to 4 ℃ for 2 h. A paper towel was used to remove the surface water, and the cooking loss was calculated based on the weight difference of the sample before and after cooking. A sample of meat

after cooking loss was measured for the determination of shear force. Samples were taken parallel to the direction of the muscle fibers using a special sampler. The core was then sheared using a texture analyzer (TMS pro of Stirling Food Technology company, Virginia, USA) at a transverse velocity of 60 mm/min for a 1000 Newtons (N) tension/compression load cell. During this process, the maximum shear force was recorded. A portion of the longissimus dorsi muscle sample (6cm×4cm×4cm) was weighed and suspended at 0 to 4 ℃ for 24 h, and the drip loss was calculated as the weight difference before and after suspension.

Sampling and Determination of Rumen Fluid

On the last day of the fattening trial, 3 h after morning feeding, rumen fluid was collected using an oral rumen catheter inserted to a depth of approximately 200 cm. To avoid saliva contamination, the first 50 mL collected was discarded. Subsequently, 150 mL of rumen fluid was collected through an oral rumen catheter, filtered through four layers of sterile gauze, and stored in a 50 mL sterile centrifuge tube in a -80 ℃ ultra-low temperature refrigerator until microbial diversity analysis of bacterial DNA was performed. On the last day of the fattening trial, after 3 hours of morning feeding, rumen fluid was collected using an oral rumen catheter inserted to a depth of approximately 200 cm. To avoid saliva contamination, the first 50 mL collected was discarded. Subsequently, 150 mL of rumen fluid was collected through an oral rumen catheter, filtered through four layers of sterile gauze, and stored in a 50 mL sterile centrifuge tube in a -80 ℃ ultra-low temperature refrigerator until microbial diversity analysis of bacterial DNA was performed.

Rumen fluid samples were transferred to third-party testing institutions for high-throughput gene sequencing of bacterial flora. After total DNA of the sample is extracted, the double-end data is spliced, and quality control and chimera removal are carried out to obtain the final effective data. Finally, OTU division, diversity analysis, taxonomic annotation, and difference analysis of species were carried out.

Statistical Analysis

A randomized design was applied to determine the dietary effect. The animal was the experimental unit (n=45) in all analyses, as data were collected individually. Considering each of the cattle as an experimental unit, the average value of repeated measurements for each parameter was used to conduct a comparison analysis. The differences between least-square means were evaluated by Duncan's method, where $P<0.05$ were considered statistically significant, and P-values <0.10 were considered trends in the data.

The original data were statistically processed by EXCEL, and the data were analyzed by the One-Way ANOVA model in SPSS 22.0 software and compared by Duncan's multiple test. The P-values obtained were expressed in the form of means ± SE, with $P<0.01$ indicating a very significant difference, $0.01<P<0.05$ indicating a significant difference, and $0.05<P<0.1$, indicating a trend of difference. For sequencing data, multivariate statistical analyses were performed using the package 'vegan' from the R statistical program (R-3.5.0, Windows). To identify the featured microorganisms at the species level in different experimental groups, linear discriminant analysis (LDA) effect size (LefSe) was done using online tools (http://huttenhower.sph.harvard.edu/galaxy).

RESULTS AND DISCUSSION

Analysis of Growth Performance of Fattening Cattle

Table 5 lists the effects of diet on the growth performance of fattening cattle. The final weight of fattening cattle fed with 100% silage sorghum and mixed silage (50% silage sorghum+50% silage corn) as the basic diet and supplemented with biological concentrate was significantly higher than that of cattle fed with common concentrate ($P<0.05$), which was 15.34 kg and 28.67 kg higher, respectively. Correspondingly, the carcass weight of fattening cattle fed with 100% sorghum silage and mixed silage as a basic diet supplemented with biological concentrate was significantly higher than that of fattening cattle fed with a common concentrate diet ($P<0.05$). However, there was no significant difference in the growth performance of fattening cattle fed with 100% corn silage as the basic diet supplemented with biological concentrate and common concentrate. In addition, the growth performance of fattening cattle fed with silage sorghum was significantly better than that of fattening cattle fed with silage corn. The growth performance parameters clearly increased with varying levels of concentrate supplementation.

Analysis of Meat Quality and Chemical Composition of Beef

The beef quality parameters of fattening cattle are shown in Table 6. The pH values of beef from different fattening cattle were significantly different ($P<0.05$) in the range of 5.51 to 7.05. The pH of the SC-I group was slightly higher than that of the SC-II group. However, the pH of beef in the diet supplemented with biological concentrate was significantly higher than that in the diet supplemented with common concentrate ($P<0.05$). There was no significant difference in drip loss and cooked meat rate among the test groups ($P>0.05$), but the drip loss of beef of fattening cattle fed with a bio-concentrate diet was slightly higher than that of the common concentrate group. In addition, the meat color of each biological concentrate group was higher than that of the common concentrate group, and the difference in meat color among the experimental groups was not significant ($P>0.05$).

Analysis of the chemical composition parameters of beef in each experimental group, compared with the common concentrate group, the protein content and ash content of beef in the biological concentrate group were higher than those in the common concentrate group, but the fat content was lower than that in the common concentrate groups. The results showed that the beef chemical composition of fattening cattle was not affected by different concentrate levels ($P>0.05$).

Analysis of Blood Metabolites in Fattening Cattle

The serum biochemical indexes of fattening cattle were determined (Table 7). There was no significant difference among the parameters ($P>0.05$), and all the biochemical indexes changed within a reasonable range. The results showed that the fattening diet had no adverse effects on fattening cattle. The TG content of fattening cattle fed with single silage corn as the basic diet and biological concentrate was significantly lower than that of fattening cattle fed with an ordinary diet. TG level can reflect the level of fat metabolism, the lower the TG content, the higher the fat utilization rate, which indirectly indicates that adding bio-concentrate to the diet can improve the fat utilization rate of fattening cattle.

Table 5. Effects on Growth Performance of Beef Cattle

Item	Treatment Groups						SEM	P-value
	SS-I	SS-II	SSC-I	SSC-II	SC-I	SC-II		
	100% Silage sorghum		50% Silage sorghum+50% Silage corn		100% Silage corn			
	Common concentrate	Biological concentrate	Common concentrate	Biological concentrate	Common concentrate	Biological concentrate		
Initial weight (Kg)	546.67+5.03a	542.67+3.51a	491+4.36b	477+2.65c	468.67+3.21c	458.67+7.77d	4.55	0
Final weight (Kg)	640.33+48.81ab	685.67+40.08a	598.33+10.02b	627+11.27b	607+8.72b	594.17+22.83b	9.45	0.016
Daily weight gain (Kg)	0.65+0.33b	0.99+0.25ab	0.75+0.06ab	1.04+0.09a	0.96+0.04ab	0.94+0.19ab	0.05	0.161
Carcass weight (Kg)	336.67+25.11aab	358.33+21.03a	321.33+3.21b	324+5.57ab	319.33+10.69b	313.67+14.01ab	4.73	0.039
Slaughter rate (%)	52.58+0.11ab	52.27+1.24ab	53.71+0.97a	51.68+0.65b	52.6+1.02ab	52.79+1ab	0.23	0.225

Means with different superscript (a, b, c and d) in the same row are significantly different (P<0.05).

Table 6. Organoleptic Quality and Meat Chemical Composition of Beef Muscle from the Steers Fed Finishing Diets Based on Silage Sorghum or Silage Corn

Item	Treatments Groups						SEM	P-value
	SS-I	SS-II	SSC-I	SSC-II	SC-I	SC-II		
	100% Silage sorghum		50% Silage sorghum+50% silage corn		100% Silage corn			
	Common concentrate	Biological concentrate	Common concentrate	Biological concentrate	Common concentrate	Biological concentrate		
pH	5.51+0.4b	6.86+0.54a	5.77+0.66b	6.56+0.1a	7.05+0.36a	6.62+0.1a	0.16	0.004
Tenderness	3.78+0.24ab	3.45+0.42b	3.83+0.42ab	4.22+0.29a	4.08+0.03a	4.12+0.25a	0.16	0.004
Drip loss (%)	35.51+1.39ab	36.86+1.69a	29.17+3.78b	34.75+4.52ab	34.31+5.51ab	35.6+2.33ab	0.009	0.201
Cooking percentage (%)	41.05+1.32a	41.93+0.83a	40.04+1.29a	40.31+1.21a	42.02+0.81a	41.16+1.28a	0.0029	0.26
Colour								
*L**	28.01+1.6ab	30.58+1.78ab	26.73+1.27b	27.42+0.77ab	29.76+1.87ab	31.27+4.45a	0.608	0.16
*a**	11.06+0.91ab	14.65+1.83ab	10.11+2.15b	12.07+1.21ab	11.21+1.31ab	13.2+4.19a	0.571	0.222
*b**	5.22+0.94a	6.77+1.2a	4.7+1.17a	6.35+1.59a	5.3+0.55a	6.33+3.06a	0.37	0.601
Meat chemical composition								
Moisture (%)	67.4+0.18a	67.45+0.41a	67.42+0.11a	67.5+0.32a	67.6+0.19a	67.32+0.18a	0.054	0.816
Crude protein (%)	24.42+0.23a	24.8+0.26a	23.35+0.18b	24.18+0.98ab	24.15+0.64ab	24.47+0.23a	0.148	0.07
Fat (%)	3.53+0.34a	3.24+0.22ab	3.39+0.13ab	3.09+0.19ab	3.5+0.31a	2.95+0.02b	0.069	0.053
Crude ash (%)	1.43+0.01ab	1.49+0.02a	1.35+0.12b	1.44+0.02ab	1.39+0.04ab	1.43+0.02ab	0.015	0.116

Means with different superscript (a, b, c and d) in the same row are significantly different (P <0.05).

Table 7. Blood Metabolites of Beef from the Steers Fed Finishing Diets Based on Silage Sorghum or Silage Corn

Item	Treatments Groups						SEM	P-value
	SS-I	SS-II	SSC-I	SSC-II	SC-I	SC-II		
	100% Silage sorghum		50% Silage sorghum+50% Silage corn		100% Silage corn			
	Common concentrate	Biological concentrate	Common concentrate	Biological concentrate	Common concentrate	Biological concentrate		
Liver function								
TBIL (μmol/L)	1.63+0.4a	1.3+0.26a	1.2+0.42a	1.47+0.6a	1.8+0.66a	1.90+0.14a	0.233	0.571
D-BIL (μmol/L)	0.73+0.64a	1.2+0.61a	1.63+1.23a	1.33+0.76a	1.73+0.75a	1.30+0.46a	0.371	0.689
IBIL (μmol/L)	0.9+0.26a	0.35+0.21c	0.25+0.07c	0.25+0.21c	0.5+0.42bc	0.85+0.07ab	0.153	0.075
Renal function								
UA (μmol/L)	64.33+15.57a	45+6.24a	44.67+7.57a	67.00+42.43a	47.00+2.00a	68.00+48.34a	8.988	0.273
BUN (mmol/L)	2.2+1.11b	3.47+0.55ab	2.57+1.01ab	3.87+1.21ab	4.37+1.55ab	4.53+1.17ab	0.643	0.138
CREA (μmol/L)	62.33+9.71a	89+14.73a	76.33+22.94a	62+21.66a	61+22.52a	63.33+24.58a	5.608	0.48
BUN/CREA	0.03+0.01a	0.04+0.01a	0.03+0a	0.07+0.04a	0.08+0.04a	0.08+0.04a	0.008	0.17
Blood fat								
CHOl (mmol/L)	2.78+0.33b	4.35+0.54a	3.35+0.34ab	3.25+0.72ab	2.76+0.76b	3.29+1.01ab	0.19	0.112
TG (mmol/L)	0.87+0.62a	0.22+0.07a	0.66+0.7a	0.91+0.6a	1.22+0.82a	0.82+0.51a	0.36	0.505
HDL-C (mmol/L)	1.43+0.41a	1.79+0.08a	1.41+0.19a	1.65+0.13a	1.57+0.25a	1.50+0.15a	0.234	0.369
LDL-C (mmol/L)	0.53+0.05a	1.12+0.18a	0.86+0.13a	0.82+0.3a	0.61+0.27a	1.00+0.66a	0.598	0.443
Amino acid metabolism								
ALT (U/L)	15.67+6.43a	31+2.65a	30+11.53a	17.33+13.2a	20.33+10.97a	17.0+9.64a	3.71	0.266
AST (U/L)	55.33+9.5a	59.33+9.07a	51.67+9.45a	51.33+10.26a	50.33+8.33a	57.33+7.51a	5.487	0.778
AST/ALT	4+1.73a	1.91+0.21a	1.85+0.49a	6.06+6.32a	2.87+1.23a	4.01+1.70a	1	0.478
Electrolyte								
K	14.63+1.04a	15.11+1.6a	16.23+2.8a	17.74+1.7a	16.6+2.69a	17.11+1.67a	0.598	0.443
Na	134.53+1.98b	134.3+1.4b	134.4+0.17b	135.13+0.9ab	137.93+2.61a	135.13+1.27ab	1.141	0.118
Cl	103.57+1.67ab	103.1+1.99ab	102.83+1.17ab	104.5+0.17ab	101.57+2.86b	105.03+0.84a	0.961	0.228
pH	7.53+0.05a	7.37+0.04b	7.47+0.08ab	7.5+0.03a	7.44+0.06ab	7.43+0.08ab	0.026	0.062

Means with different superscript (a, b, c and d) in the same row are significantly different (P<0.05).

Sequencing Results and Diversity Index Analysis Based on Rumen Fluid of Fattening Cattle

In 18 samples, 507 039 quality sequences were obtained, 454 886 high-quality sequences were obtained after double-end splicing, quality control, and chimeric filtering, and each sample produced 25 271 sequences on average. After removing the chimeric sequence, 97% of the sequence similarity was used as the cut-off value to allocate the total sequence to 364 124 OTUs. At the OTU level, the differences and similarities between different samples were statistically analyzed and displayed by the Venn diagram and Upset Plot (Fig. 1). In the Upset plot, the bars on the left show the total number of elements contained in each raw dataset. For the part of the lower intersection point, the point refers to the name of the corresponding data set on the left side through the horizontal corresponding relationship; the connection between points is realized vertically to indicate that there is the intersection between the corresponding data sets; and the vertical corresponding relationship corresponds to the upper histogram to indicate the number of intersection elements in the case of intersection. The total number of OTUs produced by the 6 diet groups was 1778, and the number of OTUs produced by the 6 diet groups alone was 37, 103, 38, 65, 43, and 45.

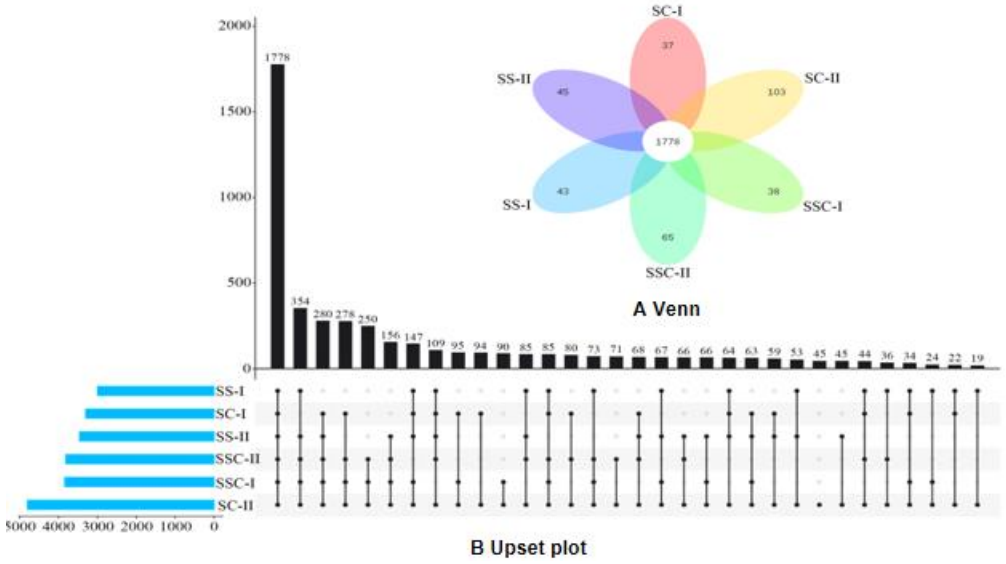

Fig. 1. Upset Plot and Venn diagram based on the average reads of bacteria community in the rumen of beef cattle

Alpha-diversity describes the species diversity within a single sample, including the indices of Observed, Chaol, Shannon, and Simpson (Fig. 2). Among them, the chao1 index and Observed species index mainly reflect the number of OTU (species) in the samples, while Shannon index and Simpson index also reflect the number of species in the samples and the average or uniformity of species abundance of different species in the samples. Chaol index and Observed index show that the addition of bio-concentrate significantly changed the effective number of species ($P<0.05$) compared with the control group.

Similarly, the Shannon index and Simpson index showed that the addition of different concentrates significantly changed the evenness of bacterial flora ($P<0.05$). In addition, it was also found that the Chaol index and Observed index of SSC-I were significantly different from those of SC-I ($P<0.05$), but not significantly different from

those of SS-I (P>0.05). Shannon index and Simpson index showed that there was no significant difference between SSC-I and SC-I (P>0.05), but there was a significant difference between SSC-I and SS-I (P<0.05). It is speculated that feeding mixed silage and feeding single sorghum silage had no significant effect on the rumen microorganisms of fattening cattle.

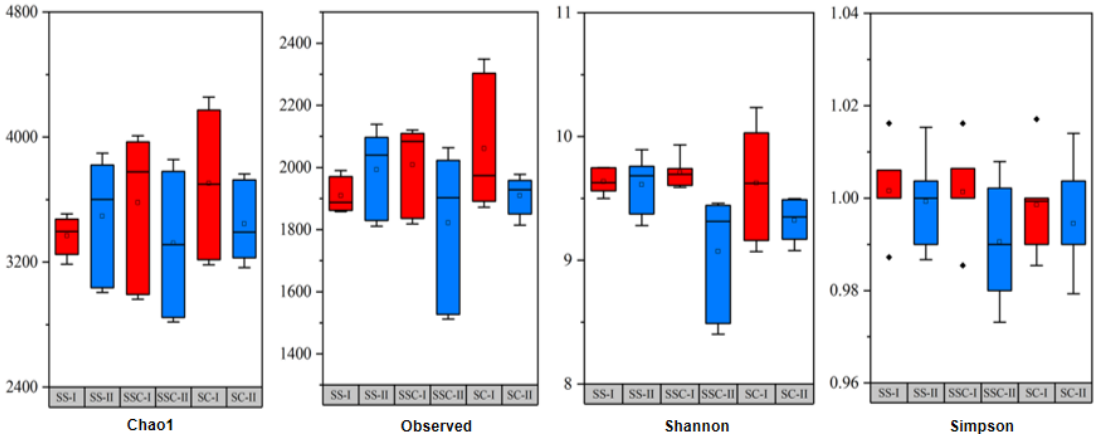

Fig. 2. Sequence statistics and alpha diversity of bacterial in Rumen fluid

Community Composition

The changes in bacterial species and abundance are shown in Fig. 3, respectively. A total of 19 bacterial phyla were identified (Fig. 3A). *Firmicutes* and *Bacteroidetes* were relatively abundant in rumen (total abundance 92.0% to 93.9%). However, the relative abundance of these dominant phyla did not differ significantly between rumen populations. The relative abundance of *Firmicutes* and *Bacteroidetes* was higher than that of common concentrate. At the genus level, 179 genera belonging to 19 phyla were detected (Fig. 3B). Sixteen genera with relative abundance of more than 1% are considered to be the most important bacteria affecting the rumen environment and digestive system. *Prevotella* and *Succiniclasticum* are relatively abundant genera. Most unknown genera could be classified as *Bacteroidetes*, Ruminococcaceae, *Firmicutes*, Lachnospiraceae, Porphyromonadaceae, *Clostridiales*, Prevotellaceae, and *Bacteria*. *Prevotella* and *Succiniclasticum* are the dominant bacterial genera of known rumen fluid bacterial flora. The results showed that there were significant differences in the species and abundance of rumen microorganisms in fattening cattle fed with different concentrates.

After clustering the abundance distributions of the phylum and genus horizontal taxa in the bacterial flora, a heatmap is obtained, which reflects the differences and similarities between the samples (Fig. 3C and 3D). The results showed that SC-I and SC-II groups had higher similarity. There were significant differences among the groups fed with single-silage corn or mixed silage as a basic diet. Feeding biological concentrates changed the relative abundance of taxonomic units of beef cattle rumen samples. The results showed that the biological concentrate improved the composition of beef cattle rumen flora, thereby changing the microbial activity.

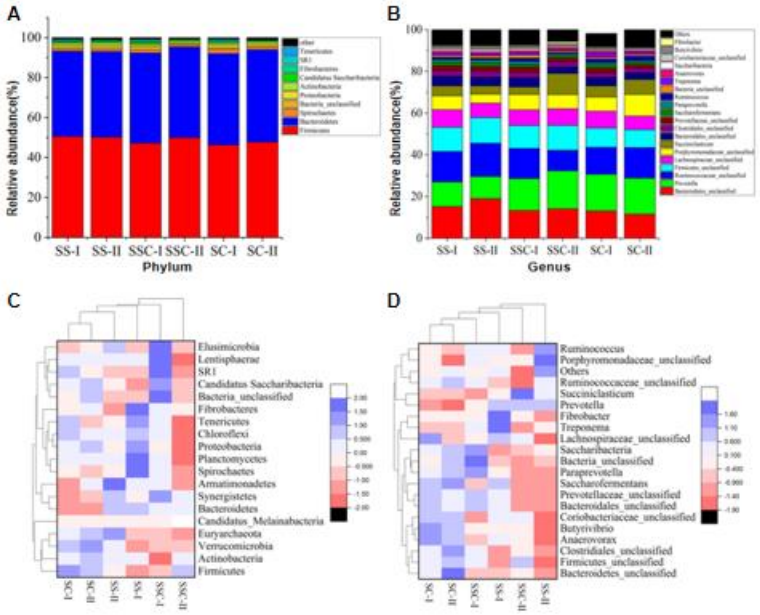

Fig. 3. The microbial community structure, heat maps, and clustering of the rumen bacterial communities

Analysis of Significant Differences

The cladogram shows taxa that play an important role in the microbial community (highlighted by small circles and shading) (Fig. 4A). At the family level, the relative abundance of Paenibacillaceae in the SC-II group was significantly higher than that in the common concentrate addition group, while Methanobacteriaceae, Porphyromonadaceae, *Bacillace*, Thermoactinomycetaceae, and Veillonellaceae were significantly higher in the SS-II group. At the family level, the relative abundance of *Bacteroidales* (unclassified) in the SC-I group was significantly higher than that in the common concentrate group. The relative abundance of Nocardiopsaceae in the SS-I group was significantly higher, while the relative abundance of *Bacillaceae1* and *Billaceae2* in SSC-I group was significantly higher.

Distribution histograms of LDA scores indicated significant differences in species richness in different rumen environments (Fig. 4B). The length of the bar indicates the magnitude of the species' impact. According to the results of LDA, the rumen microbes of SS-II group were mainly enriched in *f*-Porphyromonadaceae, *g-Porphyromonadaceae* (unclassified), and *s*-Porphyromonadaceae (unclassified). The rumen microbes of SS-I group were mainly enriched in *f*-Nocardiopsaceae, *g*-Desulfovibrionaceae (unclassified), *g-Thermobifida*, and *s-Thermobifida* (unclassified). The rumen microbes of SSC-II group were mainly enriched in *d-Bacteria.* The rumen microbes of SSC-I group were mainly enriched in *g-Bacillaceae1* (unclassified), *f-Bacillaceae1*, *s-Oceanobacillus* (unclassified), *s-Bacillaceae1* (unclassified), *g-Oceanobacillus*, and *f-Bacillaceae2*. The rumen microbes of SC-II group were mainly enriched in *s*-Paenibacillaceae (unclassified), *g*-Paenibacillaceae (unclassified), and *f*-Paenibacillaceae. The rumen microbes of SC-I group were mainly enriched in *f-Bacteroidales* (unclassified), *g-Bacteroidales* (unclassified), *and s-Bacteroidales* (unclassified).

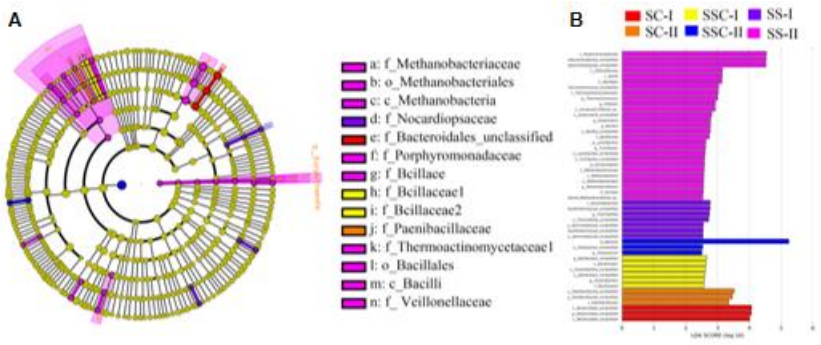

Fig. 4. Microbial community structure

Correlation Analysis of Rumen Microorganisms and Growth Performance and Blood Biochemical Indexes of Finishing Cattle

The correlation results of the top 14 bacteria in the total abundance of rumen microbial genera in fattening cattle are shown in Fig. 5. According to that cladogram and LDA scoring chart, Paenibacillaceae, Methanobacteriaceae, Porphyromonadaceae, *Bacillace*, Thermoactinomycetaceae, Veillonellaceae, *Bacteroidales* (unclassified), and *Nocardiopsaceae* were the main enriched species. Under the condition of taking the single silage corn as the basal diet, among the bacterial communities with a relatively high abundance at the genus level in the SC-I group, the genus *Bacteroidetes* (unclassified) mainly was positively associated with Ruminococcaceae (unclassified) and *Bacteroidales* (unclassified), while it was negatively associated with *Prevotella*, Porphyromonadaceae (unclassified), Lachnospiraceae (unclassified), and *Treponema*. In the SC-II group, the genus *Bacteroidetes* (unclassified) mainly was positively associated with *Prevotella* and *Porphyromonadaceae* (unclassified), while it was negatively associated with Lachnospiraceae (unclassified) and *Saccharofermentans*. Under the condition of taking the mixed silage as the basic daily ration, among the bacterial communities with a relatively high abundance at the genus level in the SSC-I group, the genus *Bacteroidetes* (unclassified) mainly was positively associated with *Paraprevotella* and *Treponema*, while it was negatively associated with *Bacteria* (unclassified). In the SSC-II group, the genus *Bacteroidetes* (unclassified) mainly was positively associated with Prevotellaceae (unclassified) and *Paraprevotella*, while it was negatively associated with *Firmicutes* (unclassified), *Porphyromonadaceae* (unclassified), *Succiniclasticum*, and *Ruminococcus*. Under the condition of taking the single sorghum silage as the basic diet, among the bacterial communities with a relatively high abundance at the genus level in the SS-I group, the genus *Bacteroidetes* (unclassified) mainly was positively associated with *Bacteria* (unclassified), while it was negatively associated with *Succiniclasticum*. In the SS-II group, the genus *Bacteroidetes* (unclassified) mainly was positively associated with *Bacteroidales* (unclassified), *Paraprevotella*, and *Bacteria* (unclassified), while it was negatively associated with *Succiniclasticum* and *Saccharofermentans.*

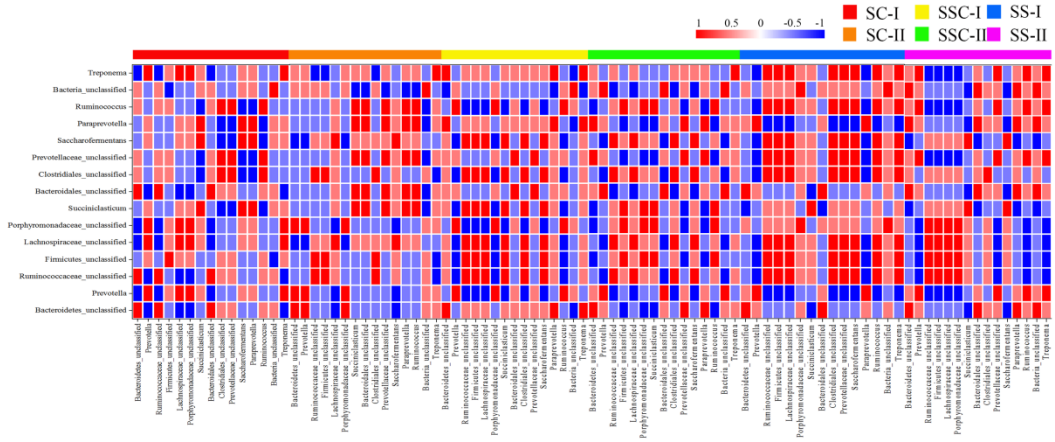

Fig. 5. Correlation of rumen microbial genus levels in fattening cattle

The correlation analysis between rumen microorganisms and meat quality indicators of fattening cattle is shown in Fig. 6. Under the condition of taking the single corn silage as the basal diet, in the SC-I group the pH mainly was positively associated with *Succiniclasticum*, *Saccharofermentans*, and *Paraprevotella*, while it was negatively associated with *Clostridiales* (unclassified), Prevotellaceae (unclassified), and *Ruminococcus.* The Tenderness and Drip loss mainly was positively associated with *Succiniclasticum*, *Bacteroidales* (unclassified), Prevotellaceae (unclassified), *Paraprevotella*, and *Ruminococcus*. Meat color mainly was positively associated with *Clostridiales* (unclassified), Prevotellaceae (unclassified), and *Ruminococcus*. In the SC-II group, the Tenderness and Drip loss mainly was positively associated with *Succiniclasticum*, *Bacteroidales* (unclassified), Prevotellaceae (unclassified), *Paraprevotella*, and *Ruminococcus*.

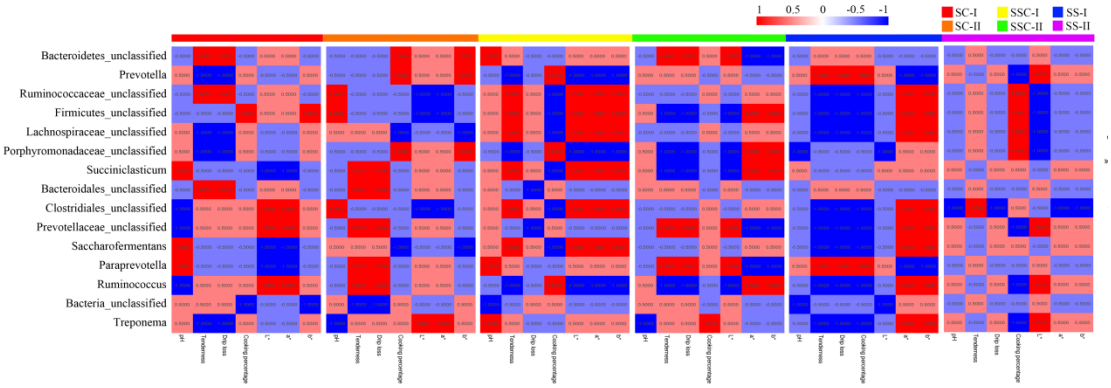

Fig. 6. Correlation analysis between rumen microorganism and meat quality indexes in finishing cattle

Meat color mainly was negatively associated with Ruminococcaceae (unclassified) and *Firmicutes* (unclassified). Under the condition of taking the mixed silage as the basic daily ration, in the SSC- II group the Meat color mainly was positively associated with Ruminococcaceae (unclassified), *Firmicutes* (unclassified), Lachnospiraceae (unclassified), *Succiniclasticum*, *Clostridiales* (unclassified), and *Saccharofermentans*. In the SSC-I group, the Meat color mainly was positively associated with *Firmicutes* (unclassified), *Porphyromonadaceae* (unclassified), *Succiniclasticum*, and *Ruminococcus*. Under the condition of taking the single sorghum silage as the basic diet, in the SS-I group

the Tenderness, Drip loss and cooking percentage mainly was negatively associated with *Clostridiales* (unclassified), Prevotellaceae (unclassified), *Saccharofermentans*, *Ruminococcus*, *Treponema*, Ruminococcaceae (unclassified), *Firmicutes* (unclassified), and *Lachnospiraceae* (unclassified). In the SS-II group, the cooking percentage mainly was positively associated with Ruminococcaceae (unclassified), *Firmicutes* (unclassified), and Lachnospiraceae (unclassified).

To determine whether the effect of different concentrates on rumen microbial flora of fattening cattle is related to serum biochemical indicators of fattening cattle, the correlation between abundant and significantly different bacteria and serum biochemical indicators in different groups of fatting cattle was analyzed, as shown in Fig. 7.

Under the condition of taking the single silage corn as the basal diet, in the SC-I group, the IBIL, BUN/CREA, ALT, and BUN mainly were positively associated with *Firmicutes* (unclassified), while they were negatively associated with *Bacteria* (unclassified). The UA mainly was positively associated with *Bacteroidetes* (unclassified), Ruminococcaceae (unclassified), and *Bacteroidales* (unclassified), while it was negatively associated with *Bacteria* (unclassified). CHOl mainly was positively associated with *Clostridiales* (unclassified), Prevotellaceae (unclassified), and *Ruminococcus*. In the SC-II group, the IBIL mainly was positively associated with *Bacteroidetes* (unclassified), *Prevotella*, and Porphyromonadaceae (unclassified); the BUN/CREA and CHOl mainly were positively associated with *Ruminococcaceae* (unclassified), *Firmicutes* (unclassified), and *Clostridiales* (unclassified); the ALT and BUN mainly were positively associated with Lachnospiraceae (unclassified) and *Saccharofermentans*, while they were negatively associated with *Bacteroidetes* (unclassified), *Prevotella*, and Porphyromonadaceae (unclassified); the UA mainly was positively associated with *Bacteria* (unclassified), while it was negatively associated with *Succiniclasticum*, *Bacteroidales* (unclassified), Prevotellaceae (unclassified), *Paraprevotella*, and *Ruminococcus*. Under the condition of taking the mixed silage as the basic feed, in the SSC-I group, the IBIL and UA mainly were positively associated with Ruminococcaceae (unclassified), *Firmicutes* (unclassified), Lachnospiraceae (unclassified), *Succiniclasticum*, *Clostridiales (unclassified)*, and *Saccharofermentans*, while they were negatively associated with *Prevotella*, Porphyromonadaceae (unclassified), and *Ruminococcus*; the BUN/CREA, BUN, and CHOl mainly were negatively associated with *Bacteroidales* (unclassified) and Prevotellaceae (unclassified). In the SSC-II group, the IBIL, BUN/CREA, BUN, and UA mainly were positively associated with *Prevotella*, *Bacteroidales* (unclassified), and *Bacteria* (unclassified), while they were negatively associated with Ruminococcaceae (unclassified), Lachnospiraceae (unclassified), *Clostridiales* (unclassified), and *Saccharofermentans*; the ALT and CHOl mainly were positively associated with Ruminococcaceae (unclassified), Lachnospiraceae (unclassified), *Clostridiales* (unclassified), and *Saccharofermentans*.

Under the condition of taking the single sorghum silage as the basic diet, in the SS-I group, the IBIL mainly was positively associated with Ruminococcaceae (unclassified), *Firmicutes* (unclassified), Lachnospiraceae (unclassified), *Clostridiales* (unclassified), Prevotellaceae (unclassified), *Saccharofermentans*, *Ruminococcus*, and *Treponema*; the UA and CHOl mainly was positively associated with *Bacteroidetes* (unclassified) and *Bacteroidales* (unclassified). In the SS-II group, the IBIL and BUN/CREA mainly were positively associated with *Bacteroidetes* (unclassified), *Bacteroidales* (unclassified), *Paraprevotella*, and *Bacteria* (unclassified); the ALT and CHOl mainly were positively associated with *Succiniclasticum* and *Saccharofermentans*, while they were negatively

associated with *Bacteroidetes* (unclassified), *Bacteroidales* (unclassified), *Paraprevotella*, and *Bacteria* (unclassified).

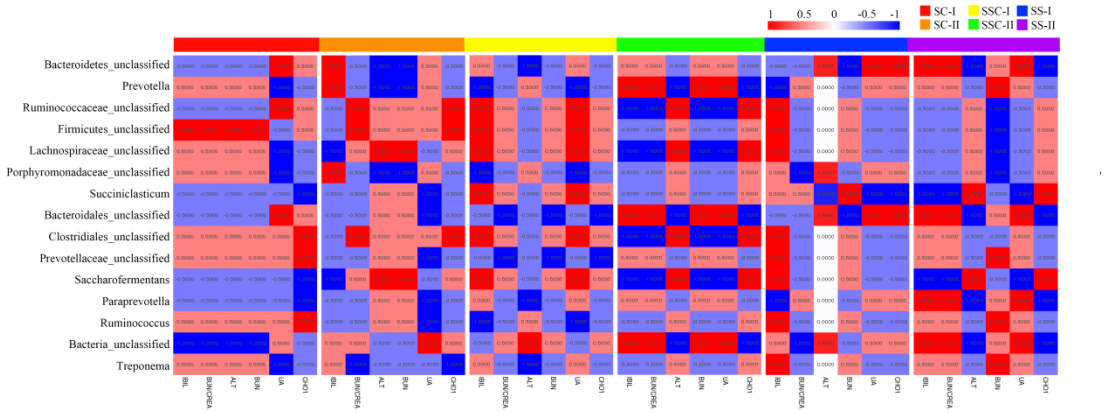

Fig. 7. Correlation analysis between rumen microorganism and blood index of fattening cattle

DISCUSSION

To meet the growing demand for beef in both domestic and export markets, many feedlots are introducing concentrates and streamlining the production process, which reduces the time required for fattening cattle to reach slaughter standards (Missio *et al.* 2009). Adding different proportions of concentrate in the fattening diet of fattening cattle will have different effects on the fattening process of beef cattle. In order to improve feed efficiency, concentrated feed has been widely used in the feeding process of fattening cattle (Gionbelli *et al.* 2012). The solid fermentation obtained by microbial treatment and fermentation not only increases the content of crude protein, fat, minerals, vitamins, and a variety of high-energy nutrients that can be used by animals, but it also contains active beneficial microorganisms, which can improve the gastrointestinal microbial environment of feeding animals and improve the growth performance of animals (Nadeem *et al.* 2016). In addition, due to its advantages of easy operation, low pollution, and wide source of raw materials, it can greatly save costs. Nowadays, bio-concentrates with solid fermentation are widely used. Kim *et al.* (2020) evaluated the growth performance of steers in the fattening stage fed rice distiller's grains and found that the experimental group not only gained total weight and average daily gain, but also had significantly higher feed intake and feed efficiency than the control group, indicating that the fermented rice distiller's grains could be used as an alternative feed source for ruminants. Similarly, Liu *et al.* (2023) found that fermented Chinese herbal residues could replace 10% of corn husks for beef cattle farming. Different dietary formulas not only affect the production performance of animals, but also may endanger the health level of animals. Therefore, three dietary regimens were evaluated in this work to explore the optimal dietary formulation for fattening cattle. In the first scheme, corn silage was used as the basic diet, which was mixed with common concentrate and bio-concentrate respectively. Silage maize is widely used as the basic diet of animal husbandry because of its high starch yield, low cost (Wilkinson and Rinne 2018), flexibility as feed or grain (Tharangani *et al.* 2021), fast harvesting, and easy silage (Muck *et al.* 2018). In the second scheme, sorghum silage and corn silage were used as the basic diet. Different levels of concentrate are added respectively. What is more, sorghum silage was used as the basic diet independently in the third scheme, adding common and biological concentrate respectively. In recent years, sorghum has been widely

used in animal husbandry, and is considered as the best alternative crop for corn silage. Because it has better water use efficiency than maize, it can produce more biomass under drought and saline-alkali conditions (McLoughlin *et al.* 2020). Therefore, this study analyzed the effects of different concentrate levels and different basal diets on production performance, blood biochemical parameters, and rumen microorganisms of fattening cattle.

A large number of studies at home and abroad have shown that there is a significant positive correlation between the level of concentrate supplementation in the diet and the daily gain of fattening cattle. Dung *et al.* (2013) improved the performance of fattening cattle by increasing the level of concentrate and found that the daily gain of fattening cattle increased significantly. Da Silva *et al.* (2015) found that a 10 g/kg increase in concentrate increased total and average daily weight gain by 1.16 kg and 9.90 g, respectively. Koralagama *et al.* (2008) achieved higher daily weight gain by adding concentrate to a single corn diet. Wang *et al.* (2020) found that increasing the proportion of concentrate in the ration could increase the final weight and daily weight gain of Hainan yellow cattle in the late fattening period. In the present study, it was found that there was no significant difference ($P>0.05$) in daily gain between adding bio-concentrate and adding common concentrate to single sorghum silage diet. The average daily gain of fattening cattle was 0.99 kg and 1.04 kg when fed with a single corn silage or mixed silage as the basic diet supplemented with biological concentrate, respectively. However, the average daily gain of fattening cattle was 0.65 kg and 0.75 kg, respectively, when fed with single corn silage or mixed silage supplemented with common concentrate, which was significantly lower than that of biological concentrate group ($P<0.05$). The possible reason for our analysis is that the effect of weight gain and daily weight gain of cattle depends on the level of the energy intake under the condition of certain cattle breeds and dietary concentrate-to-forage ratio. The nutrients, especially protein, in bio-concentrate are richer than those of common concentrate, helping to absorb energy. The higher the energy intake, the higher the average daily weight gain (Mahgoub and Lodge 1994). On the other hand, it was found that the average daily gain (ADG) of fattening cattle fed mixed sorghum and corn silage was significantly higher than that of fattening cattle fed single silage ($P < 0.05$), indicating that mixed silage may enhance energy absorption efficiency. This was attributed to the relatively low digestibility of the single silage corn, low crude protein content, low effective mineral, and vitamin content. Singh and Olsen (2011) showed in a previous study that feeding a single grain residue resulted in significant weight loss in animals. Therefore, according to the present study, bio-concentrate provides a relatively high proportion of protein content, which helps to improve the nutrient utilization rate of fattening cattle. O as to improve the fattening effect; and compared with single silage, the mixed silage of sorghum and corn has a more remarkable fattening effect.

The speed and intensity of muscle glycogen glycolysis after slaughter were significantly correlated with the pH value. The lower the pH value, the stronger the bacteriostatic effect, which was conducive to prolonging the storage time. Glycogen in muscle is converted to lactic acid after glycolysis, resulting in a decrease in pH, which slowly increases with the time of acid excretion (Kadim *et al.* 2009). In this study, the pH value of beef from different fattening cattle ranged from 5.51 to 7.05, and the pH value of beef in the bio-concentrate group was significantly higher than that in the common concentrate group ($P<0.05$). Similar results were obtained by Rabelo *et al.* (2016). In a study on the effect of concentrate-to-feed ratio on meat quality in Nellore bulls. The effect of dietary levels on meat pH may be due to changes in muscle glycogen stores at slaughter,

which are inversely proportional to final pH (Souza *et al.* 2016). In addition, water loss and cooking loss are important indicators of beef quality, which measure the ability of muscle to retain water under different conditions. Recent studies have shown that different levels of concentrate have a significant impact on the sensory characteristics of meat products. This was reported in a study of Simmental cattle fed concentrate and barley straw as a total mixed ration. A recent study on the effect of meat quality in Korean cull cows found that beef quality was related to the concentrate/crude ratio of the forage (Ku *et al.* 2021). In the present study, there was no significant difference ($P>0.05$) in water loss rate and cooked meat rate among the groups. But the beef tenderness of the bio-concentrate group was significantly higher than that of the common concentrate group ($P<0.05$). Differences in tenderness may be due to differences in muscle fiber size (Mandell *et al.* 1998).

Meat color is another major factor in measuring meat quality. The breed of fattening cattle, age, storage time of meat, and so on will affect meat color (Suman and Joseph 2013). In this study, there was no significant difference in meat color between the groups ($P>0.05$). Previous studies have shown that different levels of concentrate have no significant effect on meat color (Alberti *et al.* 2014). The change in meat routine components can directly and objectively reflect the quality of meat. According to Barros *et al.* (2015), there was no significant effect of concentrate levels on meat quality and composition. Webb *et al.* (2012) also speculated that the crude protein content of longissimus dorsi muscle in steers generally varies very little when dietary factors are considered. Alqaisi *et al.* (2021) also found that the carcass meat quality of Holstein steers was not affected significantly by the dietary concentrate level. In the present study, there was no significant difference ($P>0.05$) in conventional meat quality components among the groups. It is worth noting that the protein content and ash content of beef in the bio-concentrate group were higher. But that fat content was slightly lower than that of the common concentrate group. The high moisture content and low protein content of meat in each group may be related to the difference in tissue development caused by different physiological maturity stages (Lawrie and Ledward 2006). It is speculated that there was no significant effect on meat quality and meat chemical composition of fattening cattle by adding different levels of concentrate.

To analyze the effects of different concentrates and diets on the health status of fattening cattle, the blood physicochemical indexes of fattening cattle were determined. Blood biochemical indicators are usually used to evaluate the health level and nutritional status of animals, in which ALT, AST, Glu, TG, CHOl, and TP concentrations are the main representative parameters of nutritional metabolism and organ function. This may be influenced by dietary factors, environmental factors, growth stage, and other factors (Zhang *et al.* 2008). There were no significant differences between the blood parameters in the present work. Notably, fattening cattle fed diets supplemented with biological concentrate exhibited lower cholesterol concentrations, and fattening cattle fed mixed silage had higher cholesterol concentrations than those fed single silage. The results showed that the activity of energy metabolism of fattening cattle was lower when they were fed with a single silage or common concentrate. Therefore, more metabolites need to be excreted. The level of triglyceride (TG) in serum can reflect the level of lipid metabolism (Farnier *et al.* 2021). The TG content of fattening cattle fed with single silage and biological concentrate was lower, which indirectly indicated that the fat utilization rate was higher. The index of urea nitrogen (BUN) and the content of creatinine can reflect the balance of protein in feed and the metabolic level of nitrogenous substances *in vivo* (Kim *et al.* 2023). If the bun index in the serum of the animal is obviously higher than that of the same kind of animal, it can be inferred that the protein metabolism in the animal is disordered. Or the

protein in the diet is too high, which causes damage to their kidney function. Serum creatinine reflects renal function status. Among them, the glomerular filtration rate can detect the level of protein metabolism in the body (Ma *et al.* 2023). In the present study, fattening cattle fed a diet supplemented with biological concentrate exhibited higher BUN, possibly due to the higher protein content of the biological concentrate diet. These results were consistent with growth performance. The relatively high proportion of protein content provided by bio-concentrate was helpful in demonstrating the nutrient utilization of fattening cattle, thereby improving the fattening effect. Compared with single silage, the mixed silage of sorghum and maize had a more significant fattening effect. The fluctuation of blood biomarkers is closely related to the performance of animals. In general, understanding diet-induced fluctuations in blood parameters is challenging due to complex homeostatic regulation.

The rumen is rich in beneficial flora, which can produce digestive enzymes that benefit ruminants, helping them digest cellulose, hemicellulose, and other hard-to-use nutrients (Sheikh *et al.* 2022). Numerous studies have reported that bacterial type can significantly affect the diversity and richness of bacterial communities (McCann *et al.* 2014, Henderson *et al.* 2015, Zhang *et al.* 2017). The α diversity index showed that the microbial diversity and richness of the bio-concentrate group were higher than those of the normal concentrate group, indicating that different feed types had a direct impact on the rumen microbial composition of fattening cattle. Based on previous studies on ruminants (Hua *et al.* 2017; Khafipour *et al.* 2016; Pinnell *et al.* 2022), it was found that the rumen core microbiota of fattening cattle was mainly composed of *Bacteroidetes* and *Firmicutes*. *Firmicutes* are mainly involved in the decomposition of fibrous materials such as crude fiber, while *Bacteroidetes* are mainly involved in the degradation of non-fibrous materials such as amylopectin (Jose *et al.* 2017). In addition, significant fluctuations in *Prevotella* and *Succinolyticus* were commonly observed in this and previous studies, and *Bacteroidetes*, Ruminococcaceae, *Firmicutes*, Lachnospiraceae, Porphyromonadaceae, *Clostridiales*, Prevotellaceae, *etc.* fluctuate slightly (Plaizier *et al.* 2017).

In the present study, the addition of bio-concentrate to the basal diet improved the diversity, richness, and composition of rumen bacteria, which may help to improve the fattening performance of beef cattle. Based on 16s rDNA gene sequencing, it was found that the number of OTU and α-diversity index were significantly increased in the bio-concentrate group compared with the normal concentrate group, especially in the mixed silage-based diet. Previous studies have reported that biological concentrates increased the abundance of *Prevotella*, *Fibrobacter*, and *Ruminococcus* and improved subacute rumen acidosis (SARA) in dairy cows (Plaizier *et al.* 2017). Khafipour *et al.* (2016) concluded that the increased ratio of *Firmicutes* to *Bacteroidetes* resulting from a high concentration diet is an undesirable outcome that interferes with the degradation and digestion of dietary fiber. What is more, bio-concentrates have also been found in other animals to cause changes in the gastrointestinal microbe (Súarez *et al.* 2019). The present study showed that the addition of bio-concentrate to mixed silage and sorghum silage-based diets resulted in an increase in *Firmicutes* and a decrease in *Bacteroidetes*, indicating the ability of bio-concentrate to optimize rumen bacterial microbiota. At The level of genus, the relative abundance of *Prevotella*, Porphyromonadaceae (unclassified), and *Succiniclasticum* were increased by adding bio-concentrate to the diets based on mixed silage and single sorghum silage. But it reduced the relative abundance of *Bacteroidetes* (unclassified), *Ruminococcaceae* (unclassified), and *Firmicutes* (unclassified). However, supplementation of silage corn-based diets with bio-concentrate increased the relative

abundance of *Bacteroidetes* (unclassified), Ruminococcaceae (unclassified), and *Firmicutes* (unclassified).

Based on previous studies, *Bacteroidetes* have been detected in yaks (Pang *et al.* 2022), beef cattle (Popova *et al.* 2017), and dairy cows (Schären *et al.* 2018). *Bacteroides* is mainly involved in the degradation of NFC in the rumen microbial flora, but the bacteria of *Bacteroides* lack real cellulase, so they cannot directly degrade plant cellulose, and only in the case of symbiosis with cellulose-degrading bacteria can they effectively utilize xylan and fructose (Purushe *et al.* 2010); Most cellulose-decomposing bacteria in the rumen belong to *Firmicutes*. There is a symbiotic relationship between *Firmicutes* and *Bacteroidetes*. They jointly promote the host to absorb or store energy. Therefore, *Bacteroidetes* and *Firmicutes* in the digestive tract are very important for the fermentation of polysaccharides (Hook *et al.* 2011). These results may be related to the function of rumen bacteria. We also found some small phyla of bacteria in the rumen microbiota. *Proteobacteria* is a large group of bacteria consisting of a variety of taxonomically diverse bacteria that can degrade a variety of feed ingredients (Zhao *et al.* 2021). *Spirochaetae* also make up only a small fraction of rumen bacteria. They are shared by all groups, which may indicate that they play an important role in the rumen ecosystem. Spirochetes have been reported to play a role in the degradation of cellulose, pectin, and phosphoric acid, the utilization of fermentable carbohydrates, and the production of organic volatile fatty acids as energy sources in the rumen ecosystem (McLoughlin *et al.* 2020).

At present, the correlation analysis between animal production or animal product quality and rumen microflora is mainly carried out on the impact of rumen microbial changes on animal production, but there are few reports on the causal relationship between animal production or animal product quality and rumen microbial community. The change in diet is the external cause, and the change in rumen microorganism level may be the internal important factor to determine the effect of diet and other factors. There is a symbiotic relationship between ruminants and rumen microbiota, and microbial enzymes degrade complex polysaccharides that cannot be digested by most animal-derived digestive enzymes (Jose *et al.* 2017). A link has been found between certain bacterial communities and feed use efficiency in ruminants (Rius *et al.* 2012). Improving feed efficiency can reduce the negative impact of animals on the environment and improve economic benefits (Herrero *et al.* 2013). Therefore, the regulation of the rumen microbial community can improve animal production. The present work analyzed the correlation between genus-level microorganisms and fattening beef quality as well as blood biochemical indicators. It was found that the correlation between microbe and beef quality and blood biochemical parameters changed significantly under different dietary conditions.

There was a significant positive correlation between beef tenderness and *Prevotella* under the conditions of a single silage sorghum diet and ordinary concentrate feeding. However, there was a negative correlation between beef tenderness and *Prevotella* under the conditions of supplemented biological concentrate feeding. There was a positive correlation between the pH value of beef and *Prevotella* when the beef was fed with corn silage as the basic diet supplemented with common concentrate. In addition, there was a significant negative correlation between *Prevotella* and IBIL under the condition of feeding corn and sorghum silage as the basic diet supplemented with common concentrate. However, there was a significant positive correlation between *Prevotella* and IBIL under the condition of feeding with bio-concentrate. *Prevonella*, which is conducive to the efficient biosynthesis of ruminant nutrients, can significantly improve the negative impact of ruminant rumen metabolism on the environment and is a key species of carbohydrate

and hydrogen metabolism (Betancur-Murillo *et al.* 2023). *Prevotella* does not produce cellulolytic enzymes, and only symbiosis with cellulose-degrading bacteria can effectively utilize xylem and other polysaccharides in plant cellulose (Gharechahi *et al.* 2015). The high abundance of the genus can be explained in two ways: first, the bacterial genus may have a broad metabolic niche due to genetic relatedness or high genetic variability, which allows the genus to occupy different microenvironments within the rumen. Secondly, *Prevotella* plays an important role in the degradation and utilization of non-cellulosic polysaccharides, proteins, starches, and xylenes in plants. The increase in the abundance of species in this genus may be attributed to changes in dietary nutrition, such as increased dietary protein, cellulose, and starch. The difference in fattening beef quality and blood biochemical indicators might be due to the change of rumen microorganisms caused by dietary changes. The rumen is a large "fermenter" with an anaerobic, weakly acidic, and temperature-stable environment. This particular environment is ideal for the reproduction and growth of rumen microorganisms. The rumen fermentation and digestion process, mediated by different symbiotic microorganisms, can convert the fiber components of food into potent nutrients (Öztürk and Gur 2021).

In summary, no harm was found to be caused by the bio-concentrate to fattening cattle in this study, because this study tested the blood biochemical indexes of different experimental groups. The addition of bio-concentrates, especially the SSC-II group, can regulate rumen microbial diversity, improve rumen fermentation, and indirectly promote the fattening effect of fattening cattle. As a result, fattening cattle are able to make effective use of the nutrients in their diets, resulting in a significant increase in meat weight. The bio-concentrate fermented by microorganisms is used to utilize the waste residue of the agricultural and sideline products processing industry, and the operation is simple, which can reduce the cost of feed and improve economic benefits. Bio-concentrate has good development potential and is expected to replace the traditional ordinary concentrate. Furthermore, mixed silage is preferred over single silage, probably because of the improved energy conversion efficiency. But the exact reasons for this remain to be studied. Based on this research, the authors will continue to explore related topics in-depth, such as formula ratio optimization, dosage control, process development, etc., to provide reference for beef cattle breeding.

CONCLUSIONS

1. The three experimental diets in the study had no adverse effects on growth performance, slaughter performance, blood physical and chemical indicators, rumen diversity, and other aspects of fattening cattle.
2. Beef cattle fed with corn silage and sweet sorghum silage in fattening period can obtain higher growth performance.
3. The beef cattle fed with the bio-concentrate instead of the common concentrate had no adverse effects on the quality indicators of beef cattle, and adding the bio-concentrate in the mixed silage feed might be a better dietary choice for beef cattle.

ACKNOWLEDGMENTS

This research was supported by the Department of Science and Technology of Tibet (XZDZKJ-2021-01-09; XZ202101ZD003N; XZ202101ZD003N), and the scientific research project of Gansu Agriculture and Animal Husbandry Department (No. 20170204).

Authors' Contributions

Xiaoli Wang and Shaowei Li conceived the idea of the study; Changze Han, XinQiang Zhu and Kai Chen conducted the experiments and drafted the paper; Feifan Leng and Yonggang Wang analyzed the data, interpreted the results; All authors discussed the results and revised the manuscript.

Data Availability

We certify that all data in the article are true and reliable. This manuscript is original and has not been submitted elsewhere for publication.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Statement

All procedures and tests are following the Regulations on the Administration of Laboratory Animals promulgated and implemented by the State Science and Technology Commission of China and relevant national laws and regulations. All animals received humane care as outlined in the Guide for the Care and Use of Experimental Animals of the National Institutes of Health. All animal experiments were performed such that suffering was minimized.

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Article submitted: November 14, 2023; Peer review completed: December 16, 2023; Revised version received and accepted: January 16, 2024; Published: February 19, 2024.
DOI: 10.15376/biores.19.2.2216-2243