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Research Article

Effects of Dietary Supplementation with *Clostridium butyricum* on Rumen Fermentation, Rumen Microbiota and Feces in Beef Cattle

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Abstract

This study investigated how *Clostridium butyricum* affected rumen fermentation and the microbial communities of rumen and feces in beef cattle. Twenty beef cattle were divided into two groups: the control group (CK) and the *C. butyricum* group (CB, fed 2.5 x 10^8 CFU/kg of dry matter intake per day). The results showed that *C. butyricum* increased rumen pH, ammonia-N concentration, and microbial crude protein (MCP) concentration (P<0.05). Ruminal propionate and butyrate concentration increased, while the ruminal acetate to propionate ratio decreased (P <0.05). For rumen microbiota, observed species, Chao 1, and ACE indices were higher (P<0.05) with supplemented *C. butyricum*. At the phyla level, the *C. butyricum* enhanced the proportion of Firmicutes and decreased Bacteroidota (P<0.01). *Christensenellaceae* R-7 group, *Methanobrevibacter, Oscillospiraceae* NK4A214 group, *Desulfovibrio, Streptococcus,* and *C. butyricum* were increased (P<0.05) at the genus and species levels in the CB group. The proportion of *Prevotella*, *Christensenellaceae* R-7 group, *Blautia*, and *Megasphaera elsdenii* increased, while *Escherichia coli* decreased (P<0.05) in feces. *E. coli and Salmonella* populations were significantly reduced (P<0.01). These results indicated that diets supplemented with *C. butyricum* could improve rumen fermentation by increasing the diversity and altering the microbial community structure of the rumen. Additionally, the supplemented *C. butyricum* changed the fecal microbiota and decreased the harmful bacteria population in beef cattle.

Keywords: *Clostridium butyricum*, Fecal *E. coli*, Fecal microbiota, Rumen fermentation, Rumen microbiota, Microbial population

Introduction

Ruminants have many microorganisms in their gastrointestinal tracts. They are crucial for animal health processes, such as digesting nutrients and mediating animal immune and physiological responses [1]. The rumen microorganisms enable ruminants to use energy stored in plant material through complex interactions [2]. Changing the rumen microbial composition can affect the energy-harvesting ability, health, and rumen function of ruminants [3]. A typical and stable microbial community is an essential guarantee of ruminant health, playing vital roles in promoting the development of gastrointestinal morphology and structure, maintaining normal immune function, resistance to exogenous pathogenic factors, and so on. The microbial composition in the gastrointestinal tracts is fundamental because it can affect production performance and animal health [4]. Understanding the relationships between microbial communities in the gastrointestinal tracts and the ruminant animal has been shown to provide essential animal benefits. It has been reported that dietary-supplemented probiotics can improve productive performance by altering gastrointestinal bacterial communities in ruminants [5].

Clostridium butyrium, a strictly anaerobic bacterium, is a gram-positive bacteria that can form endospores. Additionally, *C. butyrium* can tolerate complex environments in the gastrointestinal tracts of ruminants compared with *Lactobacillus* and *Bifidobacterium* [6]. Therefore, *C. butyricum* belongs to typical intestinal microorganisms and is used in feed additives. Furthermore, extensive

research studies have confirmed that *C. butyricum* could enhance intestinal health and function in weaned piglets [7] and chickens [8]. Additionally, diets supplemented with *C. butyricum* can improve the short-chain fatty acids (SCFAs) content, the microbial diversity of gastrointestinal tracts, and production performance in Pekin ducks [9]. Additionally, *C. butyricum* produces lipoteichoic acid, SCFAs, hydrogen, and bacteriocin, helping to enhance the anti-oxidant and anti-bacterial functions of the intestines of animals [10]. Probiotic effect of *C. butyricum* has been demonstrated in monogastric animals, but few studies have been revealed in ruminants. Ruminants have a complex digestive system, and diet digestion occurs initially in the rumen. Rumen microorganisms break down diet components such as carbohydrates, plant fiber, and proteins, producing short-chain fatty acids. Thus, we hypothesized that dietary supplements with *C. butyricum* would affect rumen microbiota and rumen fermentation function of beef cattle. In addition, rumen microorganisms have attracted considerable attention in ruminant nutrition [5], but limited attention has been focused on the hindgut microorganisms. Therefore, this research examined how *C. butyricum* affected rumen fermentation and the microbial communities of rumen and feces.

Material and Methods

Ethical Approval

The Institutional Animal Care and Use Committee of Northwest A&F University (NWAFAC1008) approved this animal study.

Animals, Experimental Design, and Feeding Management

This animal experiment was conducted at a beef cattle breeding base in Guangdong VTR Bio-Tech Co., Ltd. (Zhuhai, China). Twenty beef steers (500±34 kg) were divided into two groups according to body weight, with 10 beef cattle in an open-sided house. Beef steers in the control group (CK) were fed a basal diet, and the experimental group (CB) was fed a basal diet with 2.5 x 10^8 CFU/kg *C. butyricum* of dry matter intake, respectively. *C. butyricum* was deposited in the Guangdong Microbial Culture Collection Center (GDMCC) and provided by Guangdong VTR Bio-Tech Co., Ltd. The deposition number was GDMCC NO: 61311. The experimental period lasted 40 days. The basal diet was designed to meet the requirements for the growth of beef cattle based on the Feeding Standard of Beef Cattle (NY/Y 815-2004). Beef cattle were fed twice daily at 7:30 and 14:30 and allowed free access to water.

Collection of Samples

On the last days of the trial (day 40), a flexible oral stomach tube (the Laboratory of the Chinese University of Agriculture, Beijing, China) was used to collect rumen samples at 3, 6, and 9 h after feeding [11]. The first 50 mL of rumen samples were discarded to minimize contamination with saliva. Rumen samples from each beef cattle were homogenized and filtered using four layers of gauze to obtain rumen liquids [12]. The rumen liquid was immediately used to determine pH and stored at -20°C freezer for determining rumen fermentation parameters. Approximately 2 mL of rumen samples were placed in a sterile frozen tube, immediately frozen in liquid nitrogen, and stored at -80°C freezer for microbial community analysis. On the same day, rectal fecal samples were collected 4 h after feeding, placed in a sterile frozen tube, and stored at -80°C freezer for further analysis of the fecal community [13]. Approximately 20 g of fresh fecal samples were used to determine the microbial population.

Measurements of Rumen Fermentation Parameters

The pH value was obtained via a PHS-3C pH meter (INESA Scientific Instruments, Shanghai, China) after the collection of the rumen fluid. Then, the sample was centrifuged at 10.000 x g for 20 min, and an aliquot (3 mL) of supernatants was used to determine the contents of ammonia-N (NH_3-N), microbial crude protein (MCP), acetic acid, propionic acid, and butyric acid. First, the MCP content was measured using the spectrophotometric method [14]. Next, NH_3-N concentration was determined using the phenol/hypochlorite method [15]. Finally, the concentrations of acetic, propionic, and butyric acids were determined using gas chromatography (Agilent GC 8860, Agilent Company, US) [16]. Briefly, an aliquot (0.1 mL) of supernatants was added to 0.8 mL 25% (w/v) metaphosphoric acid. Then, the supernatant sample was injected into a silica column of GC after centrifuging at 12.000 × g for 20 min.

Microbial Community Analysis

Rumen and fecal samples were used to determine bacterial flora in the digestive tract and investigate microbial community changes after dietary supplementation with *C. butyricum*. First, total DNA was extracted from the rumen and fecal samples using the sodium dodecyl sulfate (SDS) method [17]. Subsequently, the integrity and concentration of the DNA were verified using 0.7% agarose gel electrophoresis. The distinct V3-V4 regions of 16S rRNA were sequenced on a sequencing platform (Novaseq6000, Novogene Technology Company, China). The raw sequence data were obtained after sequencing and stored as fastq format [11]. The sequence data were filtered to remove barcodes and primers. Then, the sequence data were spliced using Fast Length Adjustment of Short Reads (FLASH; Version 1.2.7, *http://ccb.jhu.edu/software/FLASH/)* according to Quantitative Insights Into Microbial Ecology (QIIME; Version 1.9.1, *http://*

qiime.org/scripts/split_libraries_fastq.html) process [18]. After quality filtering, the effective tags were assembled to obtain operational Taxonomic Units (OTUs) at a 97% similarity level by the clustering method of the UPARSE-OTU algorithm (Version 7.0.1001; *http://www.drive5.com/uparse/)* and relative abundance information. Based on the OTU results, alpha diversity analysis was obtained using QIIME [19]. Online repositories *(https://www.ncbi.nlm.nih.gov/PRJNA852290)* contained the datasets.

Microbial Population Analysis

The populations of *Escherichia coli*, *C. butyricum*, and *Salmonella* were measured using the spread-plate method. Briefly, samples (20 g) were homogenized in sterile water (180 mL) and shaken at room temperature for 20 min. The colonies were counted after inoculating serial dilutions on agar plates and spreading them evenly [19]. *E. coli* was incubated on MacConkey agar at 37°C for 24 h. *C. butyricum* was incubated using a reinforced medium for *Clostridia* agar at 37°C under anaerobic conditions for 18 h. *Salmonella* was incubated using Selenite Cystine Broth agar for 24 h.

Statistical Analysis

A one-way analysis of variance (ANOVA) was used to analyze all data based on a completely randomized using SPSS Statistics (version 22.0; IBM Corp., Armonk, N. Y., USA) general linear model procedure. Replications were considered experimental units. A P-value <0.05 was defined as significant, and a P-value <0.01 as extremely significant.

Results

Rumen Fermentation Parameters

The results of ruminal pH, ammonia-N, MCP, acetate, propionate, and butyrate concentrations are shown in *Table 1*. There was a tendency to an increase in rumen pH (P=0.078) by adding *C. butyricum*. The contents of ammonia-N and MCP in the CB group were higher (P<0.05) than those of the CK group. Propionate and butyrate concentrations increased (P<0.05) by adding *C. butyricum*. At the same time, the acetate-to-propionate ratio decreased (P<0.05). The acetate concentrations did not differ in the two groups.

Rumen Microbiota

These OTUs of the rumen sample that are shared and unique among the two groups are shown in *Fig. 1*. An evaluation of the distribution of OTUs was conducted using the Venn and Flower diagrams. In total, 2521 OTUs were clustered. Of the 1.952 common OTUs, 202 and 367 were unique to the CK and CB groups. The population of OTUs was more enhanced than in the CK group (2.319 vs. 2.154). Alpha diversity can reflect the species richness and diversity of the microbial community. The alpha diversity indices of the rumen samples in the two groups are given in *Table 2*. The observed species, Chao 1, and ACE indices were increased (P<0.05) with added *C. butyricum*. There were no effects on the Shannon, Simpson, and PD-whole-tree indices in the two experimental groups with added *C. butyricum*. Our results indicated that dietary added *C. butyricum* positively affected rumen microbial structure.

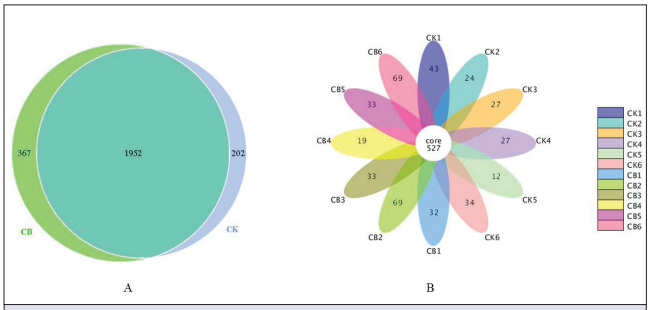

Fig 1. Venn analysis of operational taxonnmic units (OTUs) of rumen sample. (A), Each circle represented a group. The common OTUs were showed in the overlapping part, while the numbers in the non-overlapping part represent unique OTUs in each group. (B), Each petal represented a sample, while different colors represented different samples. The numbers of common OTUs were showed in the overlapping part. CK, the control group; CB, the *C. butyricum* group

Table 1. *Ruminal fermentation parameters of beef cattles with C.butyricum supplementation*

Item	Treatment		SEM	P-value
	CK	CB		
pH	6.58	6.65	0.021	0.078
Ammonia-N (mg/100 mL)	8.01^{b}	8.18^{a}	0.043	0.034
MCP (mg/mL)	6.11^{b}	6.22^{a}	0.190	0.001
Acetate (mmol/L)	54.36	56.89	1.432	0.401
Propionate (mmol/L)	15.14^{b}	19.89^{a}	1.117	0.025
Butyrate (mmol/L)	9.68^{b}	13.32^{a}	0.955	0.050
Acetate/propionate	3.73^{a}	2.88^{b}	0.208	0.033

CK, the control group; CB, the C. butyricum group; SEM, standard error of the mean; MCP, microbial crude protein

Table 2. *OTUs number of alpha diversity indices of microbial community of rumen*

Items	Observed-Species	Shannon	Simpson	Chao1	ACE	Goods-Coverage	PD-Whole-Tree
CK	1287[b]	7.915	0.986	1396.8[b]	1404.1[b]	0.995	94.919
CB	1403[a]	7.892	0.982	1512.2[a]	1516.4[a]	0.995	99.476
SEM	29.543	0.0997	0.002	28.029	27.373	0.0002	2.283
P	0.044	0.916	0.373	0.031	0.032	0.664	0.342

CK, the control group; CB, the C. butyricum group. SEM, standard error of the mean

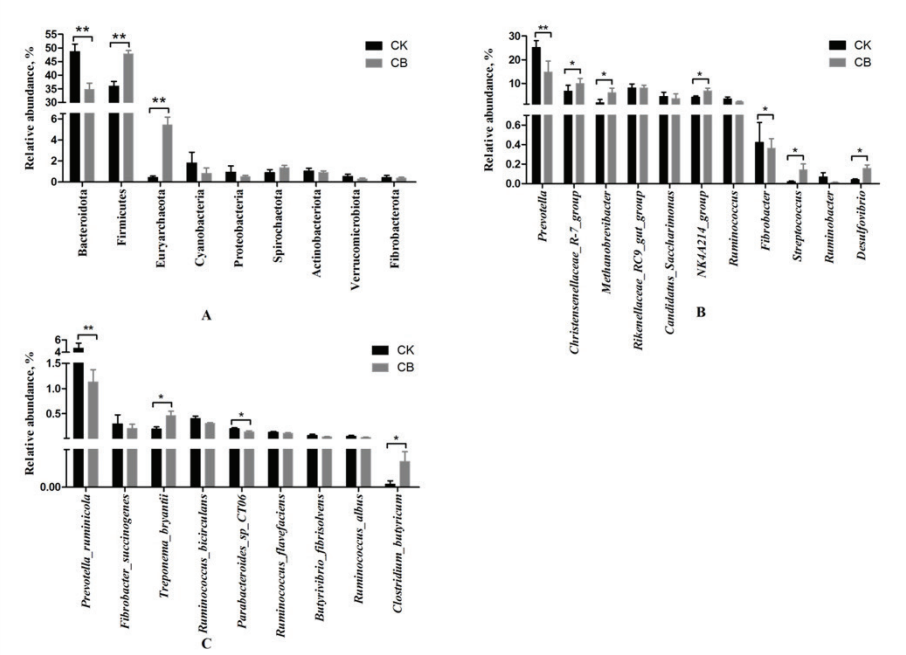

Fig 2. The relative abundances of rumen microbiota composition at the phylum level (A), genus (B), and the species level (C). CK, the control group; CB, the *C. butyricum* group

The proportion of ruminal microbiota is shown in *Fig. 2*. At the phyla level, a diet supplemented with *C. butyricum* had an enhanced proportion of Firmicutes (36.0 vs. 47.9; P<0.001), Euryarchaeota (0.43 vs. 5.44; P<0.001), and decreased the proportion of Bacteroidota (48.7 vs. 34.9; P=0.003). In addition, dietary supplemented with *C. butyricum* enhanced the proportion of *Christensenellaceae* R-7 group (4.08 vs. 9.94; P=0.026), *Methanobrevibacter* (0.48 vs. 3.88; P=0.019), *Oscillospiraceae* NK4A214 group (4.18 vs. 6.92; P=0.043), *Desulfovibrio* (0.04 vs. 0.16; P=0.010), *Streptococcus* (0.02 vs. 0.15; P=0.042), and reduced the proportion of *Prevotella* (27.2 vs. 11.2; P<0.001), and *Fibrobacter* (0.21 vs. 0.42; P=0.038) at the genus level. At the species level, dietary supplemented with *C. butyricum* enhanced the proportion of *Treponema bryanti* (0.20 vs. 0.46; P=0.022), *C. butyricum* (0.000 vs. 0.003; P=0.022), and decreased *Prevotella ruminicola* (4.67 vs. 1.13; P=0.002), *Parabacteroides* sp._CT06 (0.20 vs. 0.14; P=0.039). On the other hand, *Ruminococcus bicirculans* tended to decrease with *C. butyricum* supplementation (0.40 vs. 0.31; P=0.093).

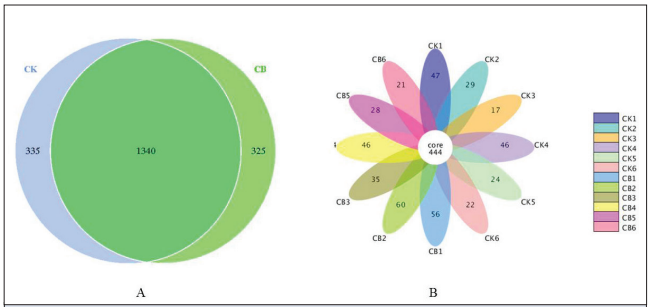

Fig 3. Venn analysis of operational taxonomic units (OTUs) of fecal sample. (A), Each circle represented a group. The common OTUs were showed in the overlapping part, while the numbers in the non-overlapping part represent unique OTUs in each group. (B), Each petal represented a sample, while different colors represented different samples. The numbers of common OTUs were showed in the overlapping part. CK, the control group; CB, the *C. butyricum* group

Fecal Microbiota

The shared and unique OTUs of the fecal samples among the two groups are illustrated in *Fig. 3*. An evaluation of the distribution of OTUs was carried out using the Venn and Flower diagrams. In total, 2000 OTUs were clustered.

Table 3. OTUs number of alpha diversity indices of microbial community in feces							
Items	**Observed-Species**	**Shannon**	**Simpson**	**Chao1**	**ACE**	**Goods-Coverage**	**PD-Whole-Tree**
CK	1011[b]	7.683[b]	0.989	1093.06	1091	0.997	71.103
CB	1083[a]	7.897[a]	0.989	1137.85	1145	0.997	79.374
SEM	16.249	0.053	0.0005	15.429	15.95	0.0001	2.310
P	0.016	0.035	0.640	0.155	0.088	1.000	0.070
CK, the control group; CB, the C. butyricum group. SEM, standard error of the mean							

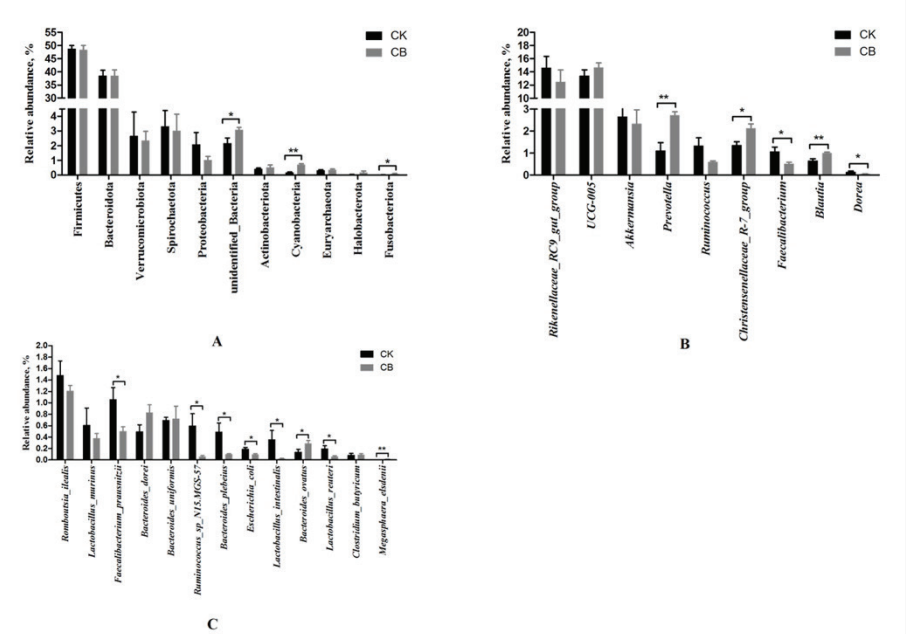

Fig 4. The relative abundances of fecal microbiota composition at the phylum level (A), genus (B), and the species level (C). CK, the control group; CB, the *C. butyricum* group

Table 4. Effects of C. butyricum on the microbial population of rumen and fecal sample				
Items	**Rumen *C. butyricum***	**Feces**		
		C. butyricum	***E. coli***	***Salmonella***
CK	1.22[b]	3.18	4.84[a]	2.96[a]
CB	5.54[a]	2.98	3.76[b]	1.74[b]
SEM	0.653	0.102	0.185	0.210
P	<0.001	0.332	<0.001	<0.001
Bacterial number is expressed as Log_{10} colony forming units per fecal contents. CK, the control group; CB, the C. butyricum group. SEM, standard error of the mean				

Of the 1340 common OTUs, 335 and 325 were unique to the CK and CB groups. The alpha diversity indices of the fecal samples in the two groups are given in *Table 3*. The observed species and Shannon indices were higher (P<0.05) than the CK group. In addition, ACE and PD-whole-tree indices increased with added *C. butyricum*. However, the two groups did not differ in Simpson, Chao 1, or goods-coverage indices.

The microbial relative proportions of the fecal sample are shown in *Fig. 4*. At the phyla level, dietary supplemented with *C. butyricum* led to an enhanced relative proportion of Cyanobacteria (0.14 vs. 0.69; P<0.001), Unidentified Bacteria (2.16 vs. 3.07; P=0.046), and Fusobacteriota (0.006 vs. 0.079; P=0.023). At the genus level, dietary supplemented with *C. butyricum* enhanced the proportion of *Prevotella* (1.11 vs. 2.72; P=0.002), *Christensenellaceae* R-7 group (1.36 vs. 2.12; P=0.014), *Blautia* (0.65 vs. 0.99; P=0.007) and reduced the proportion of *Faecalibacterium* (1.07 vs. 0.50; P=0.031), *Dorea* (0.14 vs. 0.05; P=0.050). *Ruminococcus* decreased in the CB group compared to the

CK group (1.33 vs. 0.59; P=0.070). At the species level, dietary added *C. butyricum* enhanced the proportion of *M. elsdenii* (0.002 vs. 0.013; P=0.003) and decreased the proportion of *Faecalibacterium prausnitzii* (1.06 vs. 0.50; P=0.033), *Ruminococcus* sp._N15.MGS-57 (0.60 vs. 0.06; P=0.029), *Bacteroides plebeius* (0.49 vs. 0.10; P=0.034), *Lactobacillus reuteri* (0.20 vs. 0.06; P=0.031), *Lactobacillus intestinalis* (0.42 vs. 0.02; P=0.033) and *E. coli* (0.19 vs. 0.10; P=0.005).

Microbial Rumen Population and Fecal Samples

The microbial rumen population and fecal samples are shown in *Table 4*. The *C. butyricum* rumen population in the CB group increased (P<0.001), while the *E. coli* and *Salmonella* feces populations decreased significantly (P<0.001). There were no observed effects on the *C. butyricum* population in feces with dietary *C. butyricum* supplementation.

Discussion

Ruminal fermentation parameter includes a range of indicators and can reflect the function and health status of the rumen. Ruminal pH is mainly related to the dietary digestion rate, especially the degradation rate of concentrate grains. Therefore, it is a critical fermentation parameter for the rumen environment and function [20]. It has been reported that added probiotics resulted in a high ruminal pH in cows [15]. Cai et al. reported that dietary supplemented with *C. butyricum* increased ruminal pH in goats [16]. Our results showed that ruminal pH increased with added *C. butyricum*, potentially associated with the rising proportion of bacteria that utilize lactic acid [15]. The result indicated that added *C. butyricum* could stabilize the ruminal pH as other probiotics.

Furthermore, the appropriate ammonia-N concentration can provide a nitrogen source for microorganisms and promote the synthesis of MCP [21]. The principal fermentation products of rumen are acetate, propionate, and butyrate, which account for as much as 70% of the overall metabolizable energy provision in ruminants [22]. A previous study reported that added *C. butyricum* enhanced ruminal NH_3-N concentration but did not affect the MCP concentration in goats [16]. In our research, adding *C. butyricum* improved the concentration of NH_3-N, propionate, and butyrate but decreased the acetate-to-propionate ratio in beef cattle. Our results indicated that adding *C. butyricum* enhanced ruminal protein and energy supply and positively affected rumen fermentation. Ruminal fermentation parameters can reflect the situation of the dietary digestion in the rumen and are closely related to ruminal microorganisms [2]. Therefore, we analyzed the ruminal microbial flora to find out why *C. butyricum* affected rumen fermentation.

Rumen microbiota is a vital factor in immune function and the efficiency of nutrient digestion. There is a large number of microorganisms in the gastrointestinal tracts of ruminants. The degradation of nutrients by ruminal microorganisms produces acetate, propionate, butyrate, and MCP, which supply energy and protein for ruminants. Therefore, determining the function of rumen microbiota is essential for understanding their role in animal metabolism [4]. In the research, adding *C. butyricum* enhanced the number of OTUs and the observed species, Chao 1, and ACE indices. The results indicated that *C. butyricum* positively affected microbial diversity, consistent with previous research [23].

Bacteroidota and Firmicutes were the most abundant bacteria in the rumen [24]. Many microorganisms belonging to Firmicutes can degrade fiber from dietary compounds and produce SCFAs [24]. The Firmicutes to Bacteroidota (F/B) ratio is a valuable indicator of the ability to absorb and store energy [25]. Our results indicated that added *C. butyricum* enhanced the proportion of Firmicutes but reduced the proportion of Bacteroidota. The F/B ratio was enhanced, which indicated that added *C. butyricum* improved the energy absorption capacity of the rumen microbiota. At the genus level, *Christensenellaceae* R-7 group and *Oscillospiraceae* NK4A214 group were the top two species belonging to Firmicutes. Our results showed that supplemented with *C. butyricum* could enhance the proportion of *Christensenellaceae* R-7 group and *Oscillospiraceae* NK4A214 group, thereby enhancing the relative proportion of *Firmicutes*. Changing the rumen microbial composition can affect the energy-harvesting ability and rumen function of ruminants [14]. The results indicated that added *C. butyricum* changed the ruminal microbial flora structure, affecting the rumen fermentation in beef cattle.

The effects on the fermentation parameters were closely related to the rumen flora structure [14]. Specifically, *Butyrivibrio fibrisolvens*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes* belong to cellulolytic bacteria and primarily produce acetate by degrading plant fiber [12]. Our results indicated that supplementation with *C. butyricum* did not affect the proportion of dominant cellulolytic bacteria including *F. succinogenes, B. fibrisolvens*, *R. albus*, and *R. flavefaciens*. Therefore, the acetate concentration showed no difference between the two groups. *Streptococcus* and *Ruminobacter amylophilus* are amylolytic bacteria that produce propionate [25]. *R. bromii* can degrade resistant starch and xylan, while *Ruminococcus* degrades complex deoxy sugars, such as fucose and rhamnose [11]. Our study indicated that the proportion of *Streptococcus* increased. This result was probably why the propionate content in the CB group was higher. *Provotella*, the most abundant

bacterial genus [26], can produce SCFAs by metabolizing dietary fiber [12]. Our study showed that the proportion of *Provotella* decreased with added *C. butyricum*. The low ruminal pH can promote the growth of *Prevotella* [26]. Therefore, the reduced proportion of *Provotella* in the CB group was probably due to the higher rumen pH. The increased rumen pH might be linked to *Desulfovibrio*, a lactate-utilizing bacteria. The proportion of *Desulfovibrio* increased with added *C. butyricum*. The *C. butyricum* also stimulated the development of lactate-fermenting bacteria via outcompeting lactate-producing bacteria for using sugar [25], thereby inhibiting lactate accumulation and increasing ruminal pH.

Methanobrevibacter, an essential member of methanogenic archaea, could produce methane by using H_2 as a substrate to reduce CO_2 [27]. In the study, the proportion of *Methanobrevibacter* was increased, potentially associated with the rising proportion of fiber-degrading bacteria, including carbohydrate-fermenting and H_2-producing bacteria [27]. In addition, *C. butyricum* positively affected production performance and ruminal nutrition digestibility [16]. Therefore, the increased proportion of *Methanobrevibacter* did not cause adverse effects on beef cattle.

Rumen microorganisms have attracted considerable attention, but limited attention has been paid to the hindgut microorganisms of ruminants. Diverse gut microorganisms are prominent in host metabolism, nutrient digestion, growth performance, and overall animal health [17]. Previous studies have reported that diets supplemented with *C. butyricum* could affect the intestinal microbiota by increasing bacterial abundance and diversity [28]. The observed species, Chao 1, and ACE indices increased with *C. butyricum* supplementation. These results showed that added *C. butyricum* enhanced the diversity of fecal microbial communities. The diversity of the intestinal microbiota serves as the foundation for nutrient digestion, intestinal functions, and promoting intestinal immune system development in animals [29]. Zeng et al.[8] found that Firmicutes, Bacteroidota, and Proteobacteria are the dominant phyla in the feces of ruminants. Added *C. butyricum* enhanced the Firmicutes proportion but reduced the Proteobacteria proportion [23]. However, our results showed that the addition of *C. butyricum* improved the abundance of Cyanobacteria and Fusobacteriota without affecting the proportion of Firmicutes, Bacteroidota, and Proteobacteria in feces. This lack of impact on impact on the latter phyla may be attributed to the presence of rumen microbial communities in ruminants.

This research revealed that by adding *C. butyricum* to the diets of the analyzed ruminants, the proportions of *Prevotella* and *M. elsdenii* increased, while the relative proportion of *E. coli* decreased. *Provotella* can decompose hemicellulose and is essential for utilizing non-fibrous polysaccharides [6]. Such an addition improves the production of acetates, propionates, and butyrates with the degradation of starch, xylan, and proteins [30]. The proportion of *Provotella* is predominant in animal feces [31]. *M. elsdenii* is an essential bacteria that converts lactate to acetate, propionate, butyrate, and valerate [32]. A previous study reported that *C. butyricum* could enhance the concentrations of SCFAs in feces [32]. The SCFAs could promote beneficial bacteria proliferation and inhibit the harmful bacteria *E. coli*, possibly due to the reduced pH [33]. Adding *C. butyricum* to diets reduced the proportion of *E. coli* and regulated the intestinal microbial structure by enhancing the amino acid metabolism and recombining proteins related to microbiota [34].

Broilers fed with *C. butyricum* reduced the *E. coli* and enhanced *Bifidobacterium* and *Lactobacillus* populations [34]. Zhang et al.[32] observed that *C. butyricum* could benefit the gut ecosystem by increasing the *Lactobacillus* population and reducing the counts of *C. perfringens*. The microbial composition in the gastrointestinal tracts of ruminants is fundamental because it can affect production performance and animal health. The results indicated that added *C. butyricum* reduced the harmful bacteria *E. coli* and *Salmonella* population compared with the CK group. This result may be attributed to the ability of *C. butyricum* to produce various beneficial materials and compete with pathogens for nutrition and attachment sites, thereby inhibiting the growth of harmful bacteria [35]. In this experiment, beef cattle fed *C. butyricum* could decrease the harmful bacteria counts and were beneficial to the gastrointestinal tract and animal health.

Declarations

Availability of Data and Material: The corresponding author can provide the datasets of this research upon reasonable request.

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