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Effect of lactic acid bacteria preparations on calf fecal flora

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ABSTRACT - This experiment was conducted to investigate the effects of lactic acid bacteria preparations on microbial diversity and community structure of calves. On days 1 and 7 of the trial period, feces were collected into sterile tubes and labeled (Day 1: control group D1DZ, experimental group D1SY, and Day 7: control group D7DZ, experimental group D7SY). Twenty Angus calves (150±10 kg) were selected and randomly divided into two groups of 10 calves each. The control group fed a basal diet. In addition to feeding the basal diet, the experimental group was given 15 mL lactobacillus preparation orally at 09:00 and 16:00 h every day. Calves were allowed free feeding and drinking water. All other feeding environments and management conditions remained consistent with the experiment lasting for seven days. At the end of the experiment, the fecal microflora of the calves was analyzed using 16S rRNA sequencing techniques. The 16S rRNA analysis data were processed using the Excel 2007 software and analyzed by the IBM SPSS statistical software (Statistical Analysis System, version 22). The Alpha diversity index analysis showed that the Chao and the Ace indices were significantly different after feeding supplemented with lactic acid bacteria. The PCA analysis showed that the fecal flora structure differed significantly after supplementation with the lactic acid bacteria preparation. Further analysis showed that the lactic acid bacteria increased Firmicutes, Patescibacteria, Rikenellaceae_RC9_gut_group, Clostridium_sensu_stricto_1, and prevotellaceae_UCG-003 in the feces. Therefore, we speculate that lactic acid bacteria preparations play an important role in animal production and are beneficial to the diversity of the fecal microflora of the calves.

Keywords: 16S rRNA, mammal, microbiology, rumination, probiotic

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1. Introduction

Calf intestinal disease is primarily induced by comprehensive factors, including pathogens, nutrition, environment, and feeding management. Intestinal disorders in calves cause diarrhea, and continuous diarrhea in calves delays their growth leading to their death (Uetake, 2013), thus financially affecting the farm's economy. No matter the cause of diarrhea in calf intestinal disorders, antibiotics are the first-line choice for treatment. However, long-term antibiotic therapy interferes with the gut flora of calves, causing antibiotic resistance which has negative effects on animal, human, and environmental health (Plantinga et al., 2015).

The European Union has completely banned the addition of antibiotics in feed since January 1, 2006 (Castanon, 2007). In July 2019, the Ministry of Agriculture and Rural Affairs of the People's Republic of China issued Notice No. 194, stipulating the withdrawal of all kinds of growth-promoting pharmaceutical feed additives except traditional Chinese medicine from 2020 (Ministry of Agriculture and Rural Affairs, 2019). Therefore, the need to accelerate the research, development, and application of antibiotic substitutes to ensure livestock health and food safety is essential in protecting the health and safety of the natural environment.

After the policy of comprehensive prohibition was proposed, probiotic agents were studied as an alternative to antibiotics. Research has proposed the ability of beneficial microorganisms to aggregate and adhere to the intestinal epithelium, thus helping to colonize the gut and build a protective barrier against intestinal pathogen infection (Lebeer et al., 2008; Prabhurajeshwar and Chandrakanth, 2017). Lactobacillus is the most frequently used class of bacteria in probiotic preparations. Lactic acid bacteria help to avoid the potential risk of drug resistance in animals as well as the antibiotic residues found in animal products (Pupa et al., 2021). In addition, lactic acid bacteria are essential for maintaining the stability of the gastrointestinal tract, preventing intestinal infections, and supporting overall intestinal health (Gu et al., 2008; Jha et al., 2020).

Current studies have primarily focused on the effects of adding lactobacillus on the performance of calves. Frizzo et al. (2010) reported that adding lactic acid bacteria as a feed additive to cattle during the pre-weaning period improved the average daily gain (ADG) and feed efficiency. Oral administration of *L. flora*-rich probiotics in Holstein calves improved calf growth performance, nutrient digestibility, and relieved weaning stress (Zhang et al., 2016). Moreover, feeding calves *L. flora* GB LP-1 improved intestinal health (Casper et al., 2021), and the addition of fermented milk substitutes was also found to prevent diarrhea in calves (Kayasaki et al., 2021).

Lactic acid bacteria can improve the performance and immunity of calves. However, there are few reports regarding the effects of feeding lactic acid bacteria on the fecal microbial diversity in calves. In this study, 16S rRNA high-throughput sequencing technology was used to analyze the bacterial community structure within the feces of calves fed lactobacillus preparations, therefore, providing a reference for the use and application of lactobacillus preparations during the calf breeding process. We hypothesized that feeding lactic acid bacteria preparations to calves could alter the microbial diversity within their intestines.

2. Material and Methods

The animal experiments were conducted in Tongliao (43°37'39" N, 122°14'54" E), located in Nei Monggol, China. Animal research was approved by the Medical Ethics Committee of Inner Mongolia University for Nationalities (License number: DK3178). All calves were raised under standard conditions and had *ad libitum* access to feed and water. Lactic acid bacteria preparations were provided by Hui 'an Sentient Beings Biotechnology Development (Beijing) Co., Ltd. These preparations were mainly *Lactobacillus plantarum*, and the total number of viable bacteria was 1×10^{10} cfu/g.

Twenty non-siblings, healthy, four-month-old male Angus calves with similar body weight (150 g±10 kg) were randomly divided into two groups with 10 calves per group. The control group fed the basal diet. In addition to feeding the basal diet, the experimental group was given 15 mL lactobacillus preparation orally at 09:00 and 16:00 h daily. Other feeding environments and management conditions remained constant, and the experimental period lasted seven days. Diets were formulated according to NRC (2000) (Table 1).

On days 1 and 7 of the trial period, fresh feces from three calves were randomly collected from each group and placed into sterile tubes. After that, they were labeled (D1: first day of the trial, D7: day 7 of the trial; DZ: control group, and SY: test team), sealed, and stored in a –80 °C refrigerator for subsequent fecal flora analysis.

Table 1 - Composition and nutrient level of calf particles

Ingredient	Content (%)	Dietary nutrition level	Value
Oat grass	52	Metabolizable energy (kcal kg^{-1})	3.73
Corn	16	Crude protein (%)	17.42
Soybean meal	6	Neutral detergent fiber (%)	38.12
Expanded soybean	15	Acid detergent fiber (%)	23.12
DDGS	8	Ca (%)	0.76
Stone powder	1.2	P (%)	0.42
$CaHPO_4$	0.2		
Salt	0.6		
Premix[1]	1		
Total	100		

[1] Premix provided the following per kg of diet: vitamin A, 2300 IU; vitamin D3, 1130 IU; vitamin E, 20 IU; Cu, 12 mg; Fe, 47 mg; Mn, 21 mg; Zn, 26 mg; I, 0.3 mg; Se, 0.25 mg.

Microbial DNA was extracted from the fecal samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's protocols. The final DNA concentration and purification were determined via a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was verified using a 1% agarose gel electrophoresis. The V3-V4 hypervariable regions of the 16S rRNA gene of bacteria were amplified with primers 338F (5'- ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') via a PCR thermocycler system (GeneAmp 9700, ABI, USA). The PCR reactions were conducted using the following thermocycler settings: 3 min of denaturation at 95 °C, 27 cycles at 95 °C for 30s, 30s annealing at 55 °C, 45s elongation at 72 °C, and a final extension at 72 °C for 10 min. Polymerase chain reactions were performed in triplicate using a 20-μL mixture volume containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. The resulting PCR products were extracted from a 2% agarose gel, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol.

Purified amplicons were pooled in equimolar ratios and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols used by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

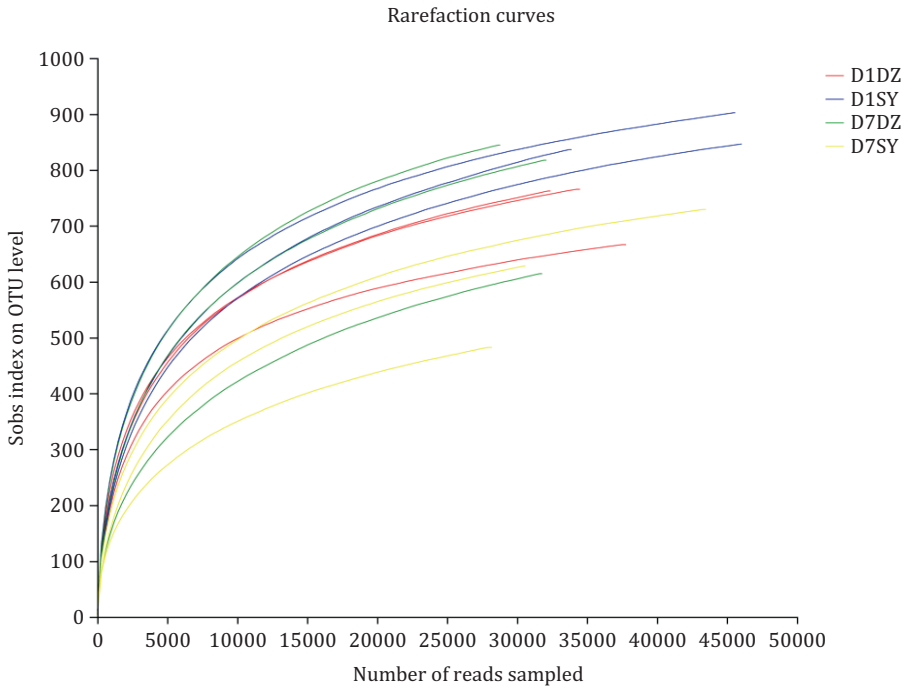
Raw fastq files were quality-filtered by Trimmomatic and merged using FLASH with the following criteria: the reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window; sequences whose overlap was longer than 10 bp were merged according to their overlap with a mismatch of no more than 2 bp; sequences of each sample were separated according to barcodes (exactly matching), and primers (allowing two nucleotide mismatching). The reads containing ambiguous bases were removed.

Operational taxonomic units (OTU) were clustered with a 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) with a novel "greedy" algorithm that performs chimera filtering and OTU clustering simultaneously. The taxonomy of each 16S rRNA gene sequence was analyzed via an RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using a confidence threshold of 70%. Venn analysis was performed in the R project Venn Diagram package (version 3.3.1). The alpha indexes were calculated in mothur (version v.1.30.2), processed using Excel 2007 software, and analyzed via the IBM SPSS Statistics software (Statistical Analysis System, version 22). Mothur was used to calculate the Alpha diversity index under different random samplings, and the R project tool was used to produce the Rarefaction curve. Principal component analysis (PCA) was performed in R project (version 3.3.1). Significant differences between groups were analyzed using the R (version 3.3.1) stats and Python SciPy packages.

3. Results

Fresh fecal samples from three calves were randomly collected from each group on days 1 and 7 of the trial period. A total of 12 samples were used for sequencing analysis. After sequencing, raw sequences of the 12 fecal samples were filtered using 423,304 optimized sequences. The growth rate of the sparse curves slowed (Figure 1), and the increased sequencing amount only produces a small number of new OTU, indicating that the amount of sequencing data currently obtained is sufficient to cover the vast majority of species within the sample to satisfy sample diversity. The resulting effective sequences were clustered into OTU based on 97% similar levels, yielding a total of 1451 OTU. These sequences fall into 13 phyla, 22 classes, 58 orders, 109 families, 246 genera, and 441 species.

The alpha diversity indices (Table 2) include Shannon, Simpson, Ace, and Chao, whereas the Shannon and Simpson indices primarily reflect community diversity. The larger the Shannon index, the smaller the Simpson index indicating a higher community diversity, while the Ace and Chao indices reflect



D1DZ - control group on the first day; D1SY - experimental group on the first day; D7DZ - control group on day 7; D7SY - experimental group on day 7; OTU - operational taxonomic unit.
The abscissa represents the amount of randomly selected sequencing data, and the ordinate represents the number of observed species (e.g., Sobs).

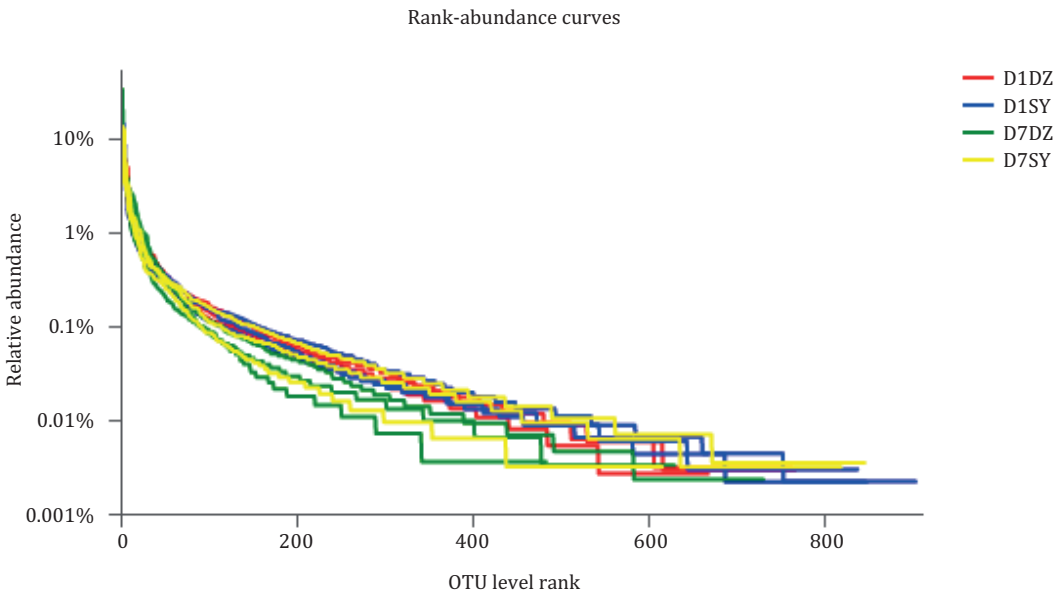
Figure 1 - Rarefaction curve of the sample.

Table 2 - Alpha diversity analysis

Item	D1DZ	D1SY	D7DZ	D7SY	P-value
Shannon	4.60±0.23	4.53±0.39	4.04±0.49	4.37±0.48	0.406
Simpson	0.03±0.01	0.04±0.02	0.07±0.06	0.04±0.02	0.601
Ace	845.70±73.94ab	988.86±19.54a	736.86±112.28c	915.18±99.97a	0.033
Chao	861.70±55.74ab	1004.21±31.81a	765.06±88.79c	917.68±102.53a	0.026
Coverage	0.996	0.996	0.996	0.994	0.184

D1DZ - control group on the first day; D1SY - experimental group on the first day; D7DZ - control group on day 7; D7SY - experimental group on day 7.
Data are presented as mean ± standard error.
Different letters in the same row are statistically different (a,b - P<0.05).

community richness, and higher values indicate larger community abundance. As seen from the grade-abundance curve of the fecal samples (Figure 2), the coverage index within this test is greater than 0.99, revealing that the sequencing results can represent the actual situation of the fecal microorganisms in each sample. Significant differences were found between the Ace (P = 0.033) and Chao (P = 0.026) index groups across the four groups (Table 2), indicating differences in community abundance. There was no significant difference between Shannon and Simpson indices among the four groups (P = 0.406 and P = 0.601, respectively).



D1DZ - control group on the first day; D1SY - experimental group on the first day; D7DZ - control group on day 7; D7SY - experimental group on day 7; OTU - operational taxonomic unit.
The abscissa represents the number ranking of species (or OTU) at taxonomic level, and the ordinate represents the relative percentage of species at that taxonomic level.

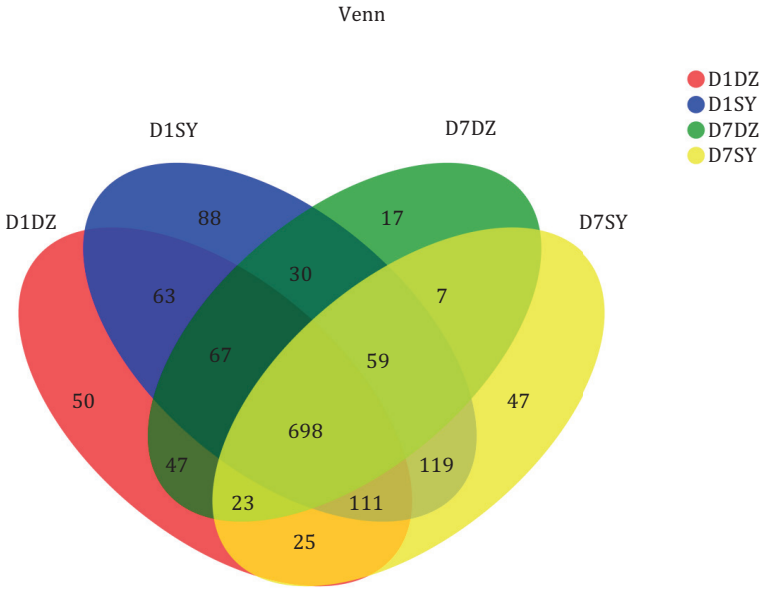
Figure 2 - Rank-abundance curves.

A total of 1451 OTU (Figure 3) were found in four groups out of the 698 OTU, accounting for 48.10% of the total OTU. After seven days, 33 and 41 unique OTU were reduced in the control and test groups, respectively.

Differences in Beta diversity via PCA were used to investigate the fecal microorganisms. Based on the PCA of OTU abundance, the points of different colors represent different sample grouping situations. The closer the sample points, the higher the sample similarity. The dilution values of PC1 and PC2 for the difference in sample composition were 20.98 and 12.23%, respectively. The R value of ANOSIM was 0.2160, and the P-value was 0.02 (Figure 4).

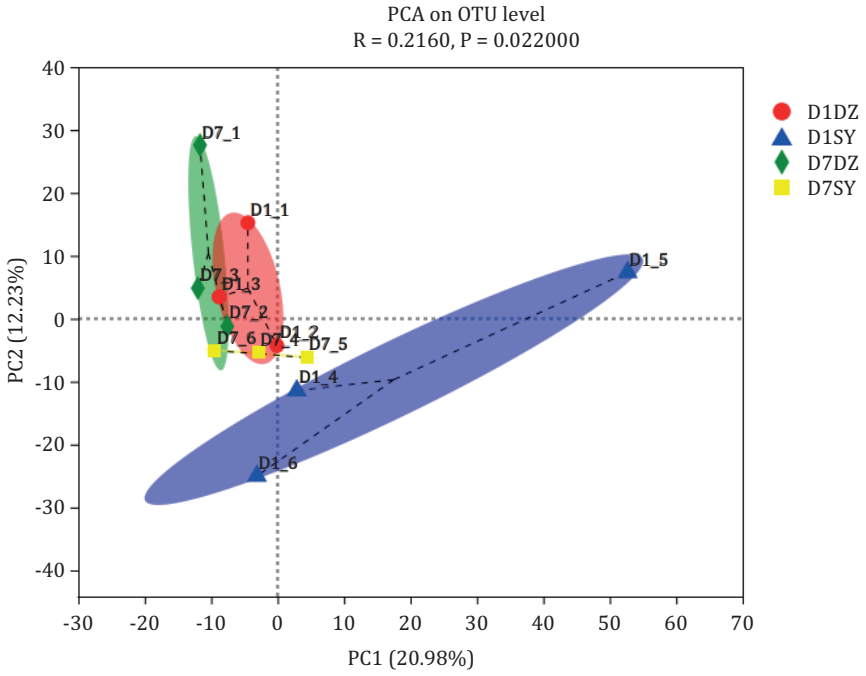
At phylum level (Figure 5), Firmicutes is the dominant bacteria, followed by Bacteroidota and Actinobacteriota. The remainder consists of Patescibacteria, Verrucomicrobiota, Spirochaetota, Proteobacteria, Cyanobacteria, unclassified_k__norank_d__Bacteria, and Campilobacterota (Table 3).

Only the dominant bacteria (with an abundance of more than 5%) and subdominant bacteria (with an abundance of 0.5–5%) are listed in Table 3. The relative abundance of Firmicutes and Patescibacteria varied significantly (P<0.05; Table 3). The relative abundance of Firmicutes was significantly lower in the D7DZ group (P<0.05), while Bacteroidota and Actinobacteriota were higher in the other groups.



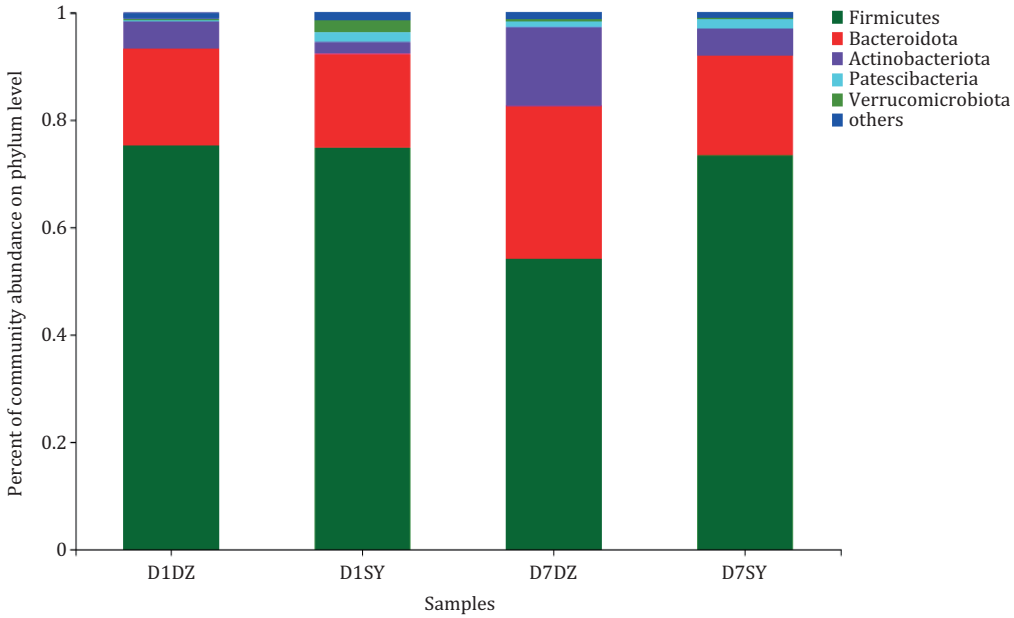
D1DZ - control group on the first day; D1SY - experimental group on the first day; D7DZ - control group on day 7; D7SY - experimental group on day 7.

Figure 3 - Venn diagram of microorganism operational taxonomic units among groups.



D1DZ - control group on the first day; D1SY - experimental group on the first day; D7DZ - control group on day 7; D7SY - experimental group on day 7; OTU - operational taxonomic unit.
x- and y-axes represent the two selected principal component (PC) axes, and the percentage represents the explanatory value of the principal component to the differences within the sample composition.

Figure 4 - Principal component analysis (PCA).



D1DZ - control group on the first day; D1SY - experimental group on the first day; D7DZ - control group on day 7; D7SY - experimental group on day 7.
The y-axis represents species names at taxonomic level, and the x-axis represents the average relative abundance of species in the different groups.

Figure 5 - Relative abundance of fecal microbial communities at phylum level.

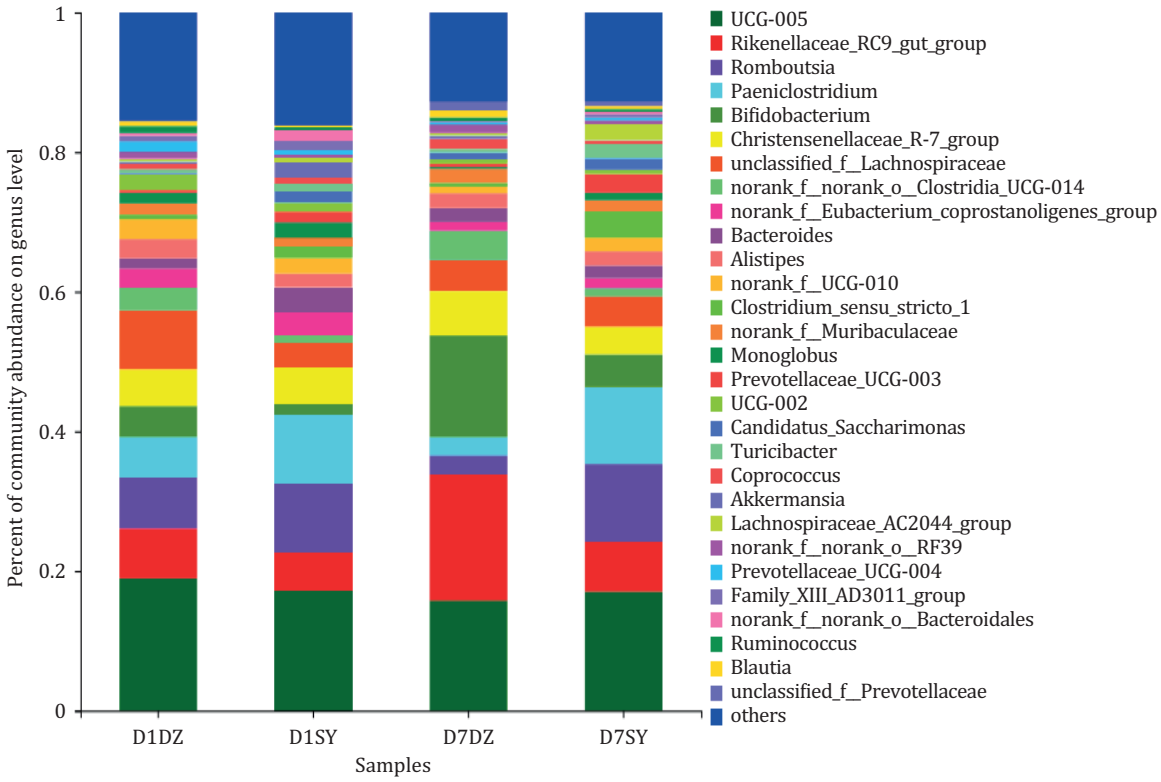
Table 3 - Relative abundance of fecal microbial communities at phylum level (%)

Item	D1DZ	D1SY	D7DZ	D7SY	P-value
Firmicutes	75.21±6.13A	75.32±8.81A	54.00±1.12B	73.57±3.41A	0.004
Bacteroidota	18.14±6.18	17.20±5.96	26.88±16.30	18.54±7.06	0.865
Actinobacteriota	5.00±2.57	2.24±1.57	16.64±16.50	5.03±7.12	0.423
Patescibacteria	0.17±0.16a	1.71±0.55a	0.84±0.81ab	1.66±0.83a	0.049
Verrucomicrobiota	0.33±0.35	2.15±3.65	0.36±0.58	0.11±0.12	0.641
Spirochaetota	0.61±0.76	0.39±0.37	0.12±0.04	0.50±0.03	0.567
Proteobacteria	0.13±0.04	0.25±0.23	0.62±0.34	0.20±0.01	0.169
Cyanobacteria	0.20±0.10	0.56±0.26	0.05±0.04	0.15±0.19	0.109
unclassified_k__norank_d__Bacteria	0.17±0.11	0.06±0.06	0.43±0.43	0.18±0.24	0.425
Campilobacterota	0.01±0.01	0.06±0.05	0.03±0.05	0.02±0.01	0.573

D1DZ - control group on the first day; D1SY - experimental group on the first day; D7DZ - control group on day 7; D7SY - experimental group on day 7.
Data are presented as mean ± standard error.
Different letters in the same row are statistically different (a,b - P<0.05; A,B - P<0.01).

At genus level (Figure 6), UCG-005 had the most relative abundance, with genera greater than 5% that included the Rikenellaceae_RC9_gut_group, Romboutsia, Paeniclostridium, Bifidobacterium, Christensenellaceae_R-7_group, and unclassified_f__Lachnospiraceae.

The relative abundance of Rikenellaceae_RC9_gut_group and Bifidobacterium increased in the D7DZ group, and the relative abundance of Romboutsia and Paeniclostridium decreased (Table 4). In the D7SY group, the Christensenellac-eae_R-7_group richness was lower (P = 0.355) than in the other groups; both Clostridium_sensu_stricto_1 (P = 0.084) and Prevotellaceae_UCG-003 (P = 0.681) richness was higher than in the other groups; however, this difference was not significant.



D1DZ - control group on the first day; D1SY - experimental group on the first day; D7DZ - control group on day 7; D7SY - experimental group on day 7.
The y-axis represents species names at taxonomic level, and the x-axis represents the average relative abundance of species in different groups.

Figure 6 - Relative abundance of fecal microbial communities at genus level.

Table 4 - Relative abundance of fecal microbial communities at genus level (%)

Item	D1DZ	D1SY	D7DZ	D7SY	P-value
UCG-005	18.91±1.21	16.67±6.96	15.52±2.80	16.97±3.88	0.468
Rikenellaceae_RC9_gut_group	7.06±4.08	5.30±2.22	16.66±13.83	7.16±1.34	0.566
Romboutsia	7.16±4.57	10.40±5.71	3.09±2.80	11.15±2.55	0.114
Paeniclostridium	5.74±1.95	10.15±5.92	2.95±2.42	11.09±3.17	0.119
Bifidobacterium	4.32±2.13	1.68±1.52	16.38±16.43	4.56±6.94	0.379
Christensenellaceae_R-7_group	5.27±1.16	5.23±1.15	6.48±2.46	4.00±0.74	0.355
unclassified_f__Lachnospiraceae	8.62±2.81	3.38±1.26	4.37±1.81	4.40±0.84	0.227
Clostridia_UCG-014	3.21±2.18	1.19±0.32	3.55±3.93	1.11±0.32	0.494
Eubacterium_coprostanoligenes_group	2.81±0.67	3.17±1.19	1.26±0.08	1.46±0.20	0.062
Alistipes	2.76±1.56	1.98±0.32	1.78±2.00	2.06±1.12	0.902
Bacteroides	1.49±0.77	3.29±3.97	1.96±1.23	1.80±0.41	0.870
Ruminococcaceae_UCG-010	2.84±0.81	2.28±0.28	0.90±0.43	2.01±1.29	0.052
Clostridium_sensu_stricto_1	0.60±0.54	2.03±2.43	0.56±0.45	3.69±1.24	0.084
norank_f__Muribaculaceae	1.64±0.63	1.11±0.84	2.17±1.50	1.60±0.26	0.787
Prevotellaceae_UCG-003	0.40±0.25	1.80±2.24	0.41±0.58	2.64±3.73	0.681

D1DZ - control group on the first day; D1SY - experimental group on the first day; D7DZ - control group on day 7; D7SY - experimental group on day 7.
Data are presented as the mean ± standard error.

4. Discussion

The ruminant gastrointestinal tract contains a large number of microflorae. The normal and stable intestinal flora play a vital role in promoting the development of colonic morphology and structure, maintaining normal immune function, and resisting the invasion of exogenous pathogenic factors (Arnold et al., 2021). Research on the ruminant gut microbiota has primarily focused on the fecal microbial flora because it is an excellent representative of the larger gut microbiota found in the intestines (Meale et al., 2017a). Microbial flora diversity reflects the ability of the microorganism to adapt to specific environments and compete for nutrients through the abundance of Alpha and Beta diversities (Qi et al., 2021).

The results of this study showed that D7SY, compared with D7DZ, had an increased Shannon index, decreased Simpson index, and increased Ace and Chao indices, indicating that the community diversity and richness of calf fecal flora increased after seven days of feeding the calves a lactic acid bacterial preparation. Although the results of the Alpha diversity index analysis showed that the fecal flora diversity and richness of the calves increased after seven days of lactic acid bacteria supplementation, it is essential to analyze which flora has changed within the microbial community structure at different taxonomic levels.

D7SY compared with D1SY and D7DZ compared with D1DZ had decreased Shannon, Ace, and Chao indices and an increased Simpson index, indicating reduced community diversity and abundance. This is inconsistent with the results of Meale et al. (2017b), who investigated the calf fecal microbial Chao index over time. The decrease in community diversity and abundance may be achieved by feeding management during the trial. It has been shown that the feed management measures of cattle play an indispensable role in the structure of fecal microbial communities (Shanks et al., 2011).

The PCA shows that the differences between multiple sets of data that are reflected on the two-dimensional coordinate graph and the two characteristic values that can best reflect the differences between samples are taken as the coordinate axes. The scale of the X- and Y-axes in PCA are the relative distance, which has no practical significance. For example, the more similar the composition of the sample species is, the closer the distance will be reflected during PCA. The PCA in this study showed an $R = 0.2160$ and a $P = 0.02$, indicating that feeding lactic acid bacteria preparations for one day affects the microbial species composition of the feces of calves.

The two most abundant phyla in the healthy gut microbiota are Firmicutes and Bacteroides (Li et al., 2020). The results of this test showed that the dominant strain in all groups was Firmicutes, followed by Bacterioides and Actinometry. Zhang et al. (2020) explored the structural composition of fecal microbes in healthy calves as well as calves with diarrhea, and found that Firmicutes and Bacteroides were the two most abundant phyla in the fecal microbiota. Guo et al. (2022) added multiple strains of probiotics into the diets of calves and found that Firmicutes and Bacteroides were also the dominant phylum within the feces of calves. The results of this experiment are consistent with the evidence found by Guo et al. (2022) and Zhang et al. (2020).

The results showed a significant increase in the relative abundance of Firmicutes in the calves that fed the lactobacillus preparations (D7SY) and lower Bacterioides and Actinobacteria compared with the control group on day 7 (D7DZ). The relative abundance of Firmicutes increases after calf weaning and is important in supplying energy within the host gut as well as the development of intestinal epithelial cells (Jami et al., 2013; Li et al., 2021). Bacteroide gates can help degrade complex polysaccharides in the plant cell wall (Meale et al., 2016). Furthermore, multiple actinomycetes comprise the fecal microbiome of healthy humans (Hoyles et al., 2013). Firmicutes have been widely reported to be involved in the degradation of various cellulose and starch (Cholewińska et al., 2021; Yao et al., 2022). We speculate that this phenomenon occurs because the probiotic can manipulate the maturation of intestinal microbial communities and nutrient absorption. Feeding lactic acid bacteria can promote the degradation of beneficial intestinal flora to simple carbohydrates, cellulose, and starch in the diet, and the relative abundance of firmicutes in the intestinal tract and feces increases.

In this study, giving lactobacillus preparations to calves on days 1 and 7 had little impact on the relative abundance of the dominant bacteria within the intestines of calves. However, the relative abundance of Firmicutes in the feces of calves without the lactobacillus preparations changed significantly, thus, inhibiting the relative abundance of Bacteroidota and Actinobacteriota.

Further studies found that UCG-005 had the highest relative abundance at genus level, and at genera level greater than 5%, including Rikenellaceae_RC9_gut_group, Romboutsia, Paeniclostridium, Bifidobacterium, Christensenellaceae_R-7_group, and unclassified_f__Lachnospiraceae. This is more consistent with study results by Wang et al. (2020). UCG-005 is the most abundant population within the colon of weaned calves (Fomenky et al., 2018). The results of this test showed an increase in the relative abundance of the Rikenellaceae_RC9_gut_group, Romboutsia, and Paeniclostridium in calf feces seven days after feeding the lactobacillus preparation (D7SY).

Rikenellaceae_RC9_gut_group is associated with body autoimmunity (Yu et al., 2021), and Romboutsia and Christensenellaceae_R-7_group are potential probiotics (Fan et al., 2020). These results indicate that feeding lactobacillus preparations increased the relative abundance of Rikenellaceae_RC9_gut_group and Christensenellaceae_R-7_group in the feces of calves; therefore, we hypothesized that it might improve the immune performance of calves.

The relative abundance of Bifidobacterium genera increased in both the control and the test groups seven days after feeding. This phenomenon may be caused by the increase in the relative abundance of Bifidobacterium within the feces as the days of feeding the basal diet increases. Kang et al. (2021) showed that supplementing small amounts of fiber to individuals consuming a low-fiber diet increases the relative abundance of gut Bifidobacterium.

Wang et al. (2018) found that Rikenellaceae_RC9_gut_group, Clostridium_sensu_stricto_1, and Paeniclostridium act as signaling bacteria for conventional diarrhea, and their abundance significantly increases or decreases after diarrhea. In this test, the relative abundance of the D7SY groups Paeniclostridium and Clostridium_sensu_stricto_1 increased, suggesting that feeding calves lactic acid bacteria alters the relative abundance of these two genera.

Applying bifidobacterium and lactobacillus to newborn calves during the first week of life increased body weight and feed conversion, while reducing the incidence of diarrhea (Abe et al., 1995). Pinloche et al. (2013) determined the effect of active dry yeast on rumen microbial community structure by clustering 16S rRNA genes. Fiber decomposition flora increased with yeast supplementation, confirming improved fiber decomposition activity of yeast as a putative mode of action. Zhang et al. (2019) fed *Lactobacillus rhamnosus* GG to Holstein calves at the pre-weaning stage, which can diversify the bacterial community composition in the rumen and regulate the balance of rumen and intestinal microbes. These effects were more pronounced in pre-weaning than post-weaning calves, suggesting that probiotic supplements are more effective when the gut microbiome is established and less effective when the microbiome is stable.

Feeding lactobacillus preparations can change the diversity and composition of the fecal flora of calves. The effect of lactobacillus preparations on the functional prediction and metabonomics of the fecal flora of calves can be explored in future research.

5. Conclusions

The lactobacillus preparation affects the composition diversity of the microbial population of calves, therefore, increasing the abundance of Firmicutes, Patescibacteria, Rikenellaceae_RC9_gut_group, Clostridium_sensu_stricto_1, and prevotellaceae_UCG-003 within the feces of calves.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: C. Dong and M. Wei. Data curation: C. Dong and M. Wei. Formal analysis: C. Dong and H. Bao. Investigation: H. Bao. Methodology: H. Bao. Project administration: M. Wei and F. Sun. Resources: M. Wei and F. Sun. Software: C. Dong. Supervision: C. Dong and M. Wei. Validation: M. Bao, J. Ju and L. Du. Visualization: J. Ju and L. Du. Writing – original draft: C. Dong. Writing – review & editing: C. Dong and M. Wei.

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