Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.315

RESEARCH ARTICLE

Effects of *Lacticaseibacillus paracasei* subsp. *Paracasei* Q-1 Supplementation on Growth Performance, Gut Health and Immune Function in Neonatal Calves

Bo Zhang[1*], Zhengang Wu[2*], Yiran Xu[2], Meishu Tan[2], Yuan Tian[3], Liang Kang[2], Chenxuan Huang[4], Desheng Liu[5], Yinghao Zhou[2#], Lianjie Song[2#] and Jianjun Guo[2#]

[1]College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, China; [2]Chengde Academy of Agricultural and Forestry Sciences, Chengde 067000, China; [3]College of Animal Medicine, Xinjiang Agricultural University, Urumqi 830052, China; [4]College of Animal Science and Technology, Shanxi Agricultural University, Jinzhong 030801, China; [5]Fengshan Agricultural Technology Promotion Regional Station, Fengning Manchu Autonomous County Agricultural and Rural Affairs Bureau, Chengde 068000, China. [*]*These authors contributed equally to this study.*
[#]Corresponding author: gjj730209@126.com; songlianjie1993@163.com; mymyinghao@163.com

ARTICLE HISTORY (25-956)

Received: October 5, 2025
Revised: November 07, 2025
Accepted: November 09, 2025
Published online: December 09, 2025

Key words:
Diarrhea
Growth performance
Gut microbiota
Immune function
Intestinal health
Lacticaseibacillus paracasei subsp. *Paracasei* q-1
Neonatal calves

A B S T R A C T

The health of neonatal calves is critically influenced by early gut health and microbiota development, yet the potential benefits of *Lacticaseibacillus paracasei* strains in calves remain underexplored. This study evaluated bovine-derived *Lacticaseibacillus paracasei* subsp. *paracasei* Q-1(*L. paracasei* subsp. *paracasei* Q-1) supplementation on neonatal calf health. Forty five-day-old neonatal Simmental×local yellow cattle calves were randomly assigned to four groups for a 60-day: control (CON), low-dose (LP, 1× 10^8 CFU/calf/day), medium-dose (MP, 3×10^8 CFU/calf/day), and high-dose (HP, 1×10^9 CFU/calf/day). Growth rate, diarrhoea incidence, serum antioxidant and immune markers, intestinal permeability indices, and ruminal and faecal microbiota profiles were assessed. Relative to CON, probiotic inclusion increased average daily gain, reduced diarrhoea incidence, boosted serum superoxide-dismutase and glutathione-peroxidase activities, elevated IgA and IgG concentrations, and lowered circulating D-lactate and lipopolysaccharide, indicating improved antioxidant status, immunity and intestinal-barrier integrity. Rumen and faecal 16S rRNA profiling revealed greater community evenness and enrichment of fibre-utilising Firmicutes taxa in supplemented calves. Within the tested range, the MP group (3×10^8 CFU/calf/day) yielded the most consistent benefits. Larger, longer-term field trials are required before routine on-farm adoption is recommended.

To Cite This Article: Zhang B, Wu Z, Xu Y, Tan M, Tian Y, Kang L, Huang C, Liu D, Zhou Y, Song L and Guo J, 2025. Effects of *Lacticaseibacillus paracasei* subsp. *paracasei* Q-1 supplementation on growth performance, gut health and immune function in neonatal calves. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2025.315

INTRODUCTION

Neonatal calf diarrhea remains a major constraint in the dairy and beef industries, causing elevated morbidity, mortality, and financial burdens (Jessop *et al.*, 2024; Kim, 2021). Multiple factors such as infectious agents, immature gut barrier function, and an imbalanced intestinal microbiota contribute to this condition (Du *et al.*, 2025; Li *et al.*, 2023). Among non-antibiotic interventions, probiotic supplementation has gained considerable attention for its potential to improve gut health, suppress pathogens, and enhance immune function in diverse rearing systems.

Within numerous probiotic candidates, *Lacticaseibacillus paracasei* subsp. *paracasei* (*L. paracasei*) holds promise due to its documented benefits in intestinal integrity, pathogen inhibition, and immune modulation (Kang *et al.*, 2023; Ma *et al.*, 2025; Yang *et al.*, 2025). Recent studies have shown that *L. paracasei* strains can help alleviate enteric diseases, improve feed conversion efficiency, and promote growth in livestock by modulating the gut microbiome (Kaewarsar *et al.*, 2023; Khan, 2019). However, despite growing interest in using probiotics for neonatal calves, studies focusing on the strain-specific effects of *Lacticaseibacillus paracasei* subsp. *paracasei* Q-1(*L. paracasei* subsp. *paracasei* Q-1) remain limited, particularly regarding rumen development, fecal microbial establishment, and immune maturation. Meanwhile, recent evidence shows that many probiotic strains may help alleviate diarrhea by reducing intestinal permeability markers, including D-lactate and

lipopolysaccharide, while enhancing antioxidant enzyme activity (Kostelac *et al.*, 2022; Liu *et al.*, 2024; Ren *et al.*, 2023). Given that the neonatal gut microbiota substantially affects nutrient utilization and immune development (Kostelac *et al.*, 2022; Liu *et al.*, 2024; Ren *et al.*, 2023), it is worth investigating whether *L. paracasei* subsp. *paracasei* Q-1 supplementation can beneficially modulate both ruminal and fecal microbiota alongside growth-related measures. Nonetheless, in vivo data regarding this strain's impact on growth performance, serum biochemical profiles, and immune parameters in calves remain sparse.

Therefore, this study aimed to determine whether *L. paracasei* subsp. *paracasei* Q-1 supplementation could improve neonatal calf performance and gut health, focusing on growth rate, diarrhea incidence, immune and antioxidant status, and shifts in ruminal and fecal microbiota. By analyzing multiple physiological and microbial outcomes, we seek to address existing gaps in strain-specific knowledge and offer practical guidance on applying *L. paracasei* subsp. *paracasei* Q-1 during the early stages of calf rearing.

MATERIALS AND METHODS

Experimental materials: The probiotic strain *L. paracasei* subsp. *paracasei* Q-1 was isolated from fresh bovine feces and provided by the Hebei Provincial Key Laboratory of Veterinary Preventive Medicine. Previous evaluations confirmed its excellent safety profile and probiotic properties in vitro and in vivo, as well as its potential to prevent *Escherichia coli* K99 infections, suggesting promising applications for calf health management (Wang *et al.*, 2025).

To ensure viability during storage and feeding, the strain was freeze-dried and stored at –20°C in vacuum-sealed pouches with desiccants. Prior to the start of the animal trial, we periodically checked the colony-forming units (CFU) by plating serial dilutions on MRS agar to confirm that the viable count remained above 1×10^9 CFU/g. During feed preparation, liquid spraying was performed under low-temperature (below 40°C) conditions to minimize thermal damage, and the final feed was stored in a dry, cool environment for up to two weeks with periodic CFU.

Experimental design and diets: All animal procedures were approved by the Animal Care and Use Committee of Chengde Academy of Agriculture and Forestry Sciences (Approval No. 2024-02). Forty neonatal Simmental × local yellow cattle crossbred calves (5 days old) with similar birth weight and parity were randomly allocated to four groups (n=10 per group) in a single-factor design. Calves were ear-tagged, and all husbandry staff and laboratory analysts were blinded to treatment codes. The trial lasted 65 days, including a 5-day adaptation period and a 60-day experimental phase. Calves were dam-fed and received pelleted starter feed from day 7. Treatments consisted of a control group (CON, no probiotic), low-dose group (LP, 1×10^8 CFU/calf/day), medium-dose group (MP, 3×10^8 CFU/calf/day), and high-dose group (HP, 1×10^9 CFU/calf/day). Diets were formulated according to the Feeding Standard of Beef Cattle (NY/T 815–2004), with pelleted feed prepared by cold pressing and supplemented with *L. paracasei* subsp. *paracasei* Q-1 via liquid spraying technology after pelletization (Ministry, 2004). Both the pelleted starter feed and the alfalfa hay were designed to be approximately isocaloric (estimated total digestible nutrients) and isonitrogenous (crude protein basis), as shown in Table 1. The same basal diet composition was provided in each treatment group, with only the probiotic supplementation differing among groups. The pelleted feed was offered at 1% of body weight twice daily, with alfalfa hay provided at 2 kg/calf/day, maintaining a forage-to-concentrate ratio of 60:40. To minimize observer bias, the personnel responsible for monitoring fecal consistency and overall health were kept unaware of the specific probiotic treatments allocated to each group. Individual calf IDs were coded so that daily health evaluations remained blinded throughout the trial.

Table 1: Composition and nutrient levels of pellet diets (DM basis)

Item	Content %
Ingredient composition (% DM)	
Corn	52
Cottonseed meal	28
Soybean meal	10
Bran	5
Premix[1])	5
Total	100
Nutrient levels[2])	
CP	21.92
EE	5.05
NDF	25.73
ADF	15.29
Ash	7.12
Ca	0.60
P	0.40
Nutrients in alfalfa hay	
DM/%	92.75
CP/% DM	8.43
Ee/% DM	2.49
Ash/% DM	5.42
NDF/% DM	53.71
ADF/% DM	25.89
Ca/% DM	0.57
P/% DM	0.29
GE / (MJ/kg DM)	19.97

1) The premix provided the following per kg of pellet diets: VA 250 000 IU,VD 60 000 IU,VE 1 000 IU, Fe 80 mg,Cu 12 mg,Zn 80 mg,Mn 70 mg,Se 0.40 mg,I 1 mg,Co 0.80 mg. 2) Nutrient levels were measured values.

Sample collection: On day 60, eight calves from each group were randomly selected for sample collection. Jugular blood was drawn before morning feeding using vacuum tubes without anticoagulant, allowed to clot at room temperature for 20 min, and centrifuged at 3 000 rpm for 15 min. Serum was separated into sterile 1.5 mL tubes and stored at –80°C. Rumen fluid was collected using a rumen tube after discarding the initial portion to avoid saliva contamination, filtered through sterile gauze, snap-frozen in liquid nitrogen, and stored at –80°C. Fresh fecal samples were obtained rectally, transferred into sterile tubes, sealed, and stored at –80°C.

Growth performance, diarrhea incidence, and fecal scoring: Body weight was recorded at the start and end of the experiment to calculate average daily gain (ADG). Fecal consistency was assessed daily by trained personnel using a four-point scoring system (1=normal soft, 4=watery) according to Renaud *et al*. (2018) and calves with scores ≥3 were considered diarrheic. Diarrhea

incidence was calculated as the number of diarrheic calves per day divided by the total number of calves and observation days, expressed as a percentage.

Serum biochemical, antioxidant, and immune indices: Serum samples were analyzed by Ningxia Saisijuxin Biotechnology Co., Ltd. Biochemical indicators (ALT, AST, TC, TG, HDL-C, LDL-C, BUN, TP, ALB, GLB) were measured with an automatic biochemical analyzer (KHB-1280, Shanghai Kehua Bio-Engineering Co., Ltd.). DAO, GSH-Px, SOD, T-AOC, D-LA, LPS, MDA, IgA, IgG, and IgM were determined using a semi-automatic analyzer (L-3180, Shanghai Kehua), and IL-1β, IL-4, IL-6, IL-10, and TNF-α were quantified with a microplate reader (ST-360).

16S rRNA gene sequencing: Rumen fluid and fecal DNA were extracted using the CTAB method, verified by 1% agarose gel electrophoresis, and diluted to 1 ng/μL. The V3–V4 regions of the 16S rRNA gene were amplified using primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3'), and barcodes were incorporated for multiplexing. PCR products were purified and pooled in equimolar ratios, and sequencing libraries were constructed using the NEBNext® Ultra DNA Library Prep Kit (Illumina, USA), then quantified on an Agilent 5400 (Agilent Technologies Co. Ltd., USA). Negative controls (sterile water instead of DNA template) were included in PCR setups to check for contamination. Sequencing was conducted on an Illumina platform to obtain 250 bp paired-end reads. An average sequencing depth of ~50,000 reads per sample was targeted to ensure coverage of rare taxa.

Bioinformatic Processing and Normalization: Raw FASTQ files were imported into QIIME2 (v.2019.1) and processed following the QIIME2's Atacama soil microbiome tutorial. The DADA2 pipeline was used for quality filtering, trimming, denoising, merging of paired reads, and chimera removal (Callahan *et al.*, 2016). A feature table of amplicon sequence variants (ASVs) was generated, and taxonomic classification against the SILVA (or GREENGENES) database was performed using the QIIME2 feature-classifier plugin. Mitochondrial and chloroplast reads were filtered out before downstream analysis. To account for differences in sequencing depth, we performed rarefaction to a uniform depth based on the lowest sample read count with the QIIME2 core-metrics pipeline. Alpha diversity indices (observed ASVs, Shannon, Chao1, Faith's PD) and beta diversity metrics (Bray–Curtis, weighted/unweighted UniFrac) were then computed, and principal coordinate analysis (PCoA) was used for visualization.

Statistical analysis: Prior to all statistical analyses, datasets were tested for normality (Shapiro–Wilk) and homogeneity of variances (Levene's test). For growth performance, serum parameters, and other biochemical/immune indices, one-way ANOVA was performed using SPSS 25.0, followed by Duncan's multiple range test for post hoc comparisons. Non-normal data were log-transformed as needed. In the microbiome analysis, alpha-diversity indices were compared using Kruskal–Wallis tests with Benjamini–Hochberg corrections for multiple comparisons, while beta-diversity differences among groups were assessed using PERMANOVA. Linear discriminant analysis effect size (LEfSe) or ANCOM were used to identify differentially abundant taxa. Effect sizes were computed where relevant. Statistical significance was declared at $P<0.05$. Results were expressed as mean ± SEM throughout.

RESULTS

Effects of *L. paracasei* subsp. *paracasei* Q-1 on growth performance: Initial body weights did not differ significantly among groups (Table 2). After 60 days, calves supplemented with *L. paracasei* subsp. *paracasei* Q-1 (LP, MP, HP) exhibited significantly higher final body weights compared with CON ($P=0.005$). ADG was enhanced by 11.39–13.92% in probiotic-treated groups relative to CON ($P=0.001$), while no differences were observed among LP, MP, and HP ($P>0.05$).

Table 2: Effects of L. paracasei subsp. paracasei Q-1 on growth performance of calves

Items	Groups				*P*-value
	CON	LP	MP	HP	
IBW/(kg)	39.11±0.71	38.77±0.76	39.31±0.45	38.24±0.57	0.652
FBW/(kg)	86.33±1.54^{b}	91.81±1.18^{a}	92.29±1.38^{a}	92.42±1.08^{a}	0.005
ADG/ (kg/d)	0.79±0.03^{b}	0.88±0.02^{a}	0.88±0.02^{a}	0.90±0.02^{a}	0.001

Mean values with distinct superscript alphabets ($^{a, b, c}$) indicate significant differences ($P<0.05$) within the same row.

Effects of *L. paracasei* subsp. *paracasei* Q-1 on diarrhea incidence and fecal score: Calves receiving *L. paracasei* subsp. *paracasei* Q-1 had significantly lower fecal scores compared with CON throughout the study ($P<0.001$; Fig. 1), with MP showing the lowest values and differing significantly from all other groups. Diarrhea incidence was markedly reduced in probiotic groups, with the lowest rate observed in MP (10.50%), followed by LP and HP (16.17% & 15.67%), all significantly lower than CON ($P<0.001$). Although the differences in ADG among probiotic groups were not significant, the lower diarrhea incidence in MP may reflect better gut health support at this dose.

Effects of *L. paracasei* subsp. *paracasei* Q-1 on serum biochemical parameters: No significant differences were detected among groups in serum AST, ALT, TC, TG, HDL, LDL, BUN, TP, ALB, or GLB ($P > 0.05$, Fig. 2), These results suggest that *L. paracasei* subsp. *paracasei* Q-1 is biologically safe and does not negatively affect basic metabolic homeostasis in neonatal calves.

Effects of *L. paracasei* subsp. *paracasei* Q-1 on serum immune and antioxidant indicators: Probiotic supplementation significantly enhanced antioxidant capacity, as evidenced by higher GSH-Px activity in all supplemented groups compared with CON ($P<0.001$), and significantly elevated SOD activity in MP and HP compared with CON and LP ($P<0.001$; Fig. 3 and Fig. 4). No differences were observed for T-AOC or MDA ($P>0.05$). Immunoglobulin concentrations (IgA, IgG, IgM) also increased with probiotic dose ($P<0.001$). Elevated antioxidant enzyme activity and immunoglobulin levels may help newborn calves combat oxidative stress and

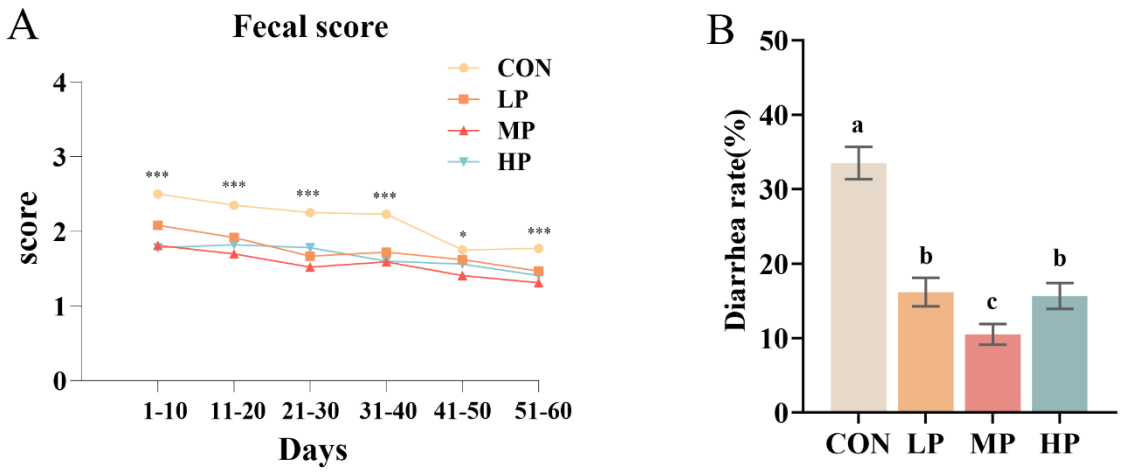

Fig. 1: Diarrhea statistics in neonatal calves supplemented with *Lacticaseibacillus paracasei* subsp. *paracasei* Q-1 at different doses (CON, LP, MP, HP). (A) Fecal scores over 60 days. Values are mean ± SEM (n=10). Significant differences (***P<0.001, *P<0.05) are indicated. (B) Diarrhea rate (%) over 60 days. Values are mean ± SEM (n=60). Groups with different letters (a, b, c) differ significantly (P<0.05).

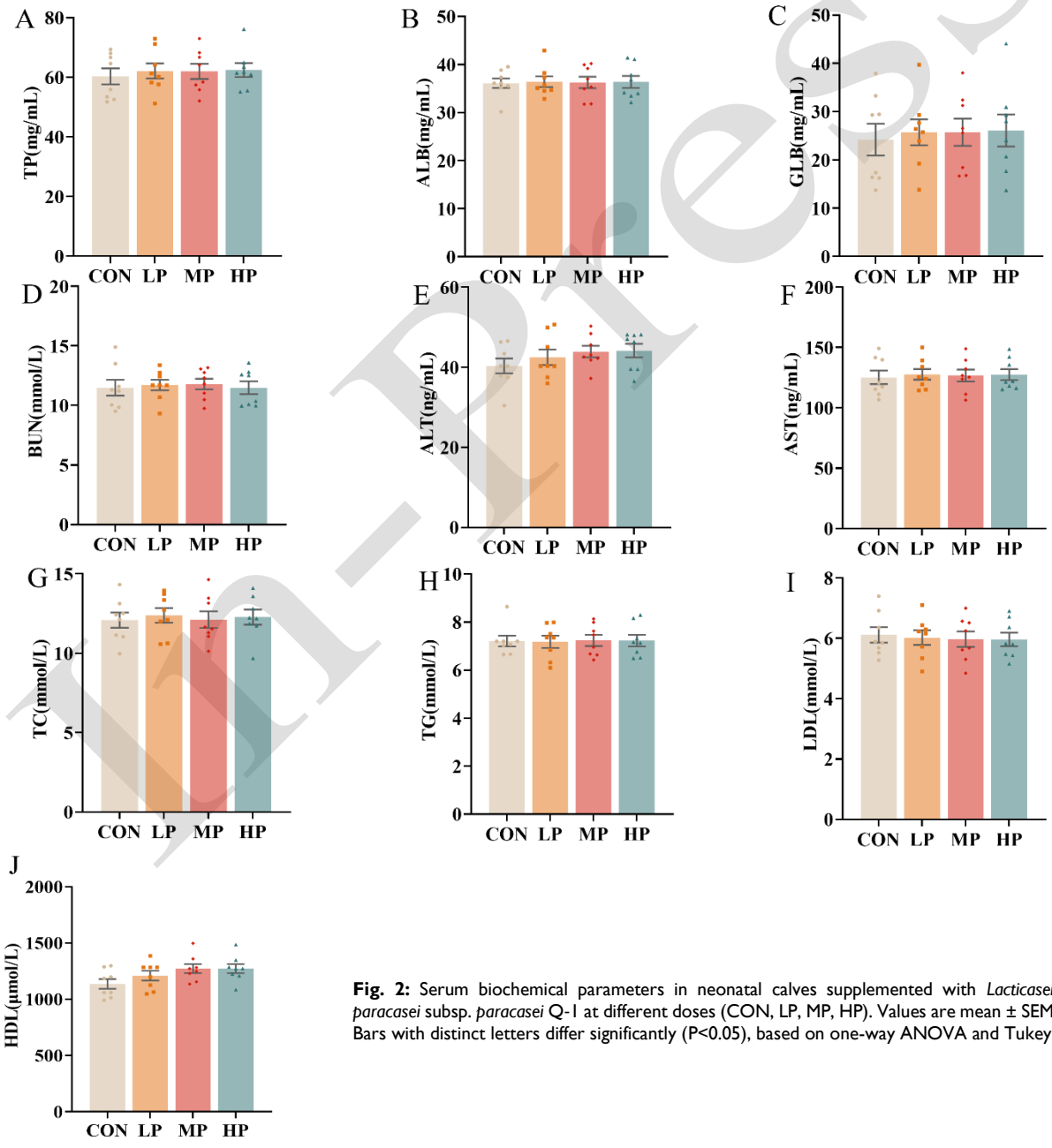

Fig. 2: Serum biochemical parameters in neonatal calves supplemented with *Lacticaseibacillus paracasei* subsp. *paracasei* Q-1 at different doses (CON, LP, MP, HP). Values are mean ± SEM (n=8). Bars with distinct letters differ significantly (P<0.05), based on one-way ANOVA and Tukey's test.

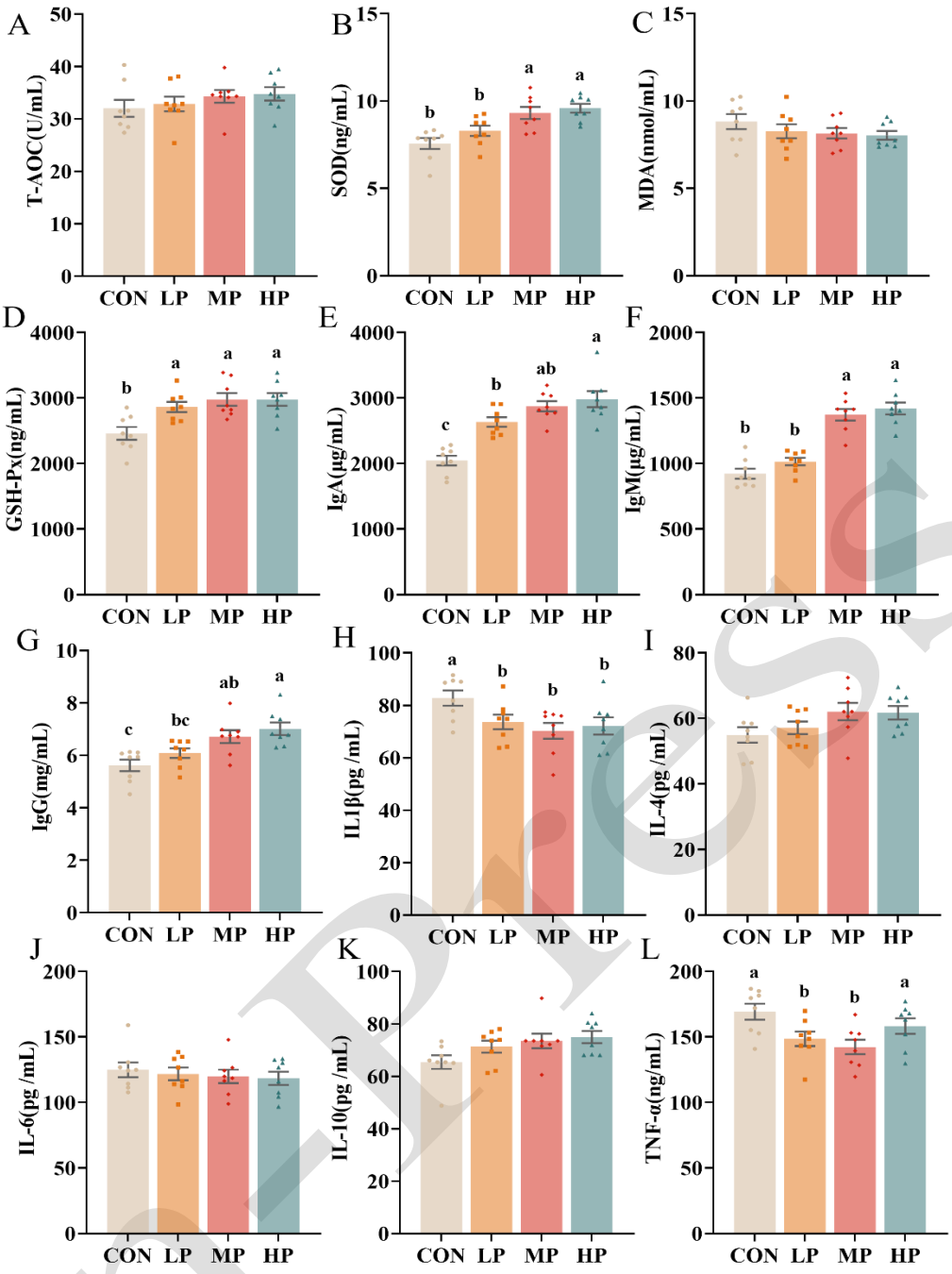

Fig. 3: Serum antioxidant capacity, immunoglobulins, and select cytokines in neonatal calves supplemented with *Lacticaseibacillus paracasei* subsp. *paracasei* Q-1 (CON, LP, MP, HP). Values are mean ± SEM (n=8). Different letters indicate statistically significant differences (P<0.05) by one-way ANOVA (Tukey's test).

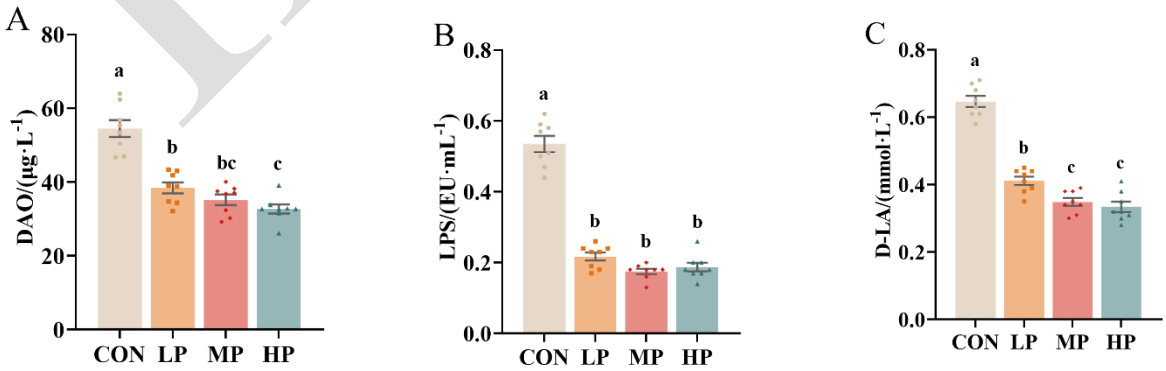

Fig. 4: Intestinal permeability markers (D-LA, LPS, DAO) in neonatal calves receiving *Lacticaseibacillus paracasei* subsp. *paracasei* Q-1 supplementation. Values are mean ± SEM (n=8). Groups with different letters are significantly different (P<0.05).

strengthen mucosal defenses during early life. In the inflammatory profile, IL-1β levels were reduced in all probiotic groups (P=0.032), while TNF-α was lower in LP and MP than in CON (P=0.015). These changes in cytokine patterns support the notion that *L. paracasei* subsp. *paracasei* Q-1 may modulate pro-inflammatory pathways, potentially promoting a more balanced immune response.

Effects of *L. paracasei* subsp. *paracasei* Q-1 on intestinal permeability indicators: Markers of gut barrier function showed improvement in probiotic groups. D-LA concentrations were lowest in MP and HP, significantly below LP and CON (P<0.001; Fig 4). Serum LPS was significantly reduced in all probiotic groups compared with CON (P<0.001). DAO activity displayed a dose-dependent decline, reaching the lowest values in HP (P<0.001). Reduced D-LA, LPS, and DAO collectively suggest enhanced intestinal integrity, which may partly explain the lower diarrhea incidence and improved health outcomes.

Effects of *L. paracasei* subsp. *paracasei* Q-1 on rumen microbiota: High-throughput 16S rRNA sequencing of rumen fluid confirmed adequate sequencing depth. A total of 20,784 ASVs were identified, with 1,288 shared across all groups (Fig 5a). PCoA based on Bray-Curtis and UniFrac distances revealed distinct clustering among groups (Fig 5b), although alpha diversity indices (Shannon, Simpson, ACE, Chao1) showed no significant differences (P>0.05; Fig 5c). The rumen microbiota was dominated by Bacteroidota, Firmicutes_A, Firmicutes_C, and Patescibacteria (Fig 5d). Although these major phyla remained constant, significant shifts were noted in minor phyla, such as an increase in Firmicutes_D abundance in HP (P=0.009) and a decrease in Eremiobacterota in all supplemented groups (P=0.007). LEfSe analysis revealed treatment-specific microbial biomarkers (Fig 6a–b): CON was enriched with UBA4334, HP with unclassified taxa (Bact_11, UBA2450, UBA3207), LP with Catonella, and MP with Firmicutes-associated taxa (e.g., RUG12438, Firmicutes_D). Correlation analyses showed Firmicutes_D positively associated with IgM and IL-10 but negatively correlated with DAO, while Cyanobacteria negatively correlated with LPS and DAO. UBA4334 exhibited negative correlations with D-LA, LPS, DAO, and IL-10 (Fig 6c).

Effects of *L. paracasei* subsp. *paracasei* Q-1 on intestinal microbiota: Fecal 16S rRNA sequencing yielded 10,036 ASVs, with 664 shared across all groups (Fig 7a). PCoA revealed clear separation of groups, with PC1 and PC2 explaining 31.6 and 18.1% of variance, respectively (Fig 7b). Alpha diversity metrics (Shannon, Simpson, Pielou evenness) were significantly higher in MP and HP than in CON (P>0.05, Fig 7c), indicating a more balanced fecal microbial community in those groups. At the phylum level, Spirochaetota was significantly reduced in LP and HP (P=0.017), Proteobacteria decreased in HP relative to LP (P=0.033), Firmicutes_D increased in LP (P=0.001), Actinobacteriota was depleted in MP and HP (P=0.021), and Elusimicrobiota was enriched in HP (P=0.015) (Fig 7d). Genus-level analysis revealed consistent patterns, including significant reductions in *Treponema_D* in LP and HP (P=0.009) and enrichment of *Cryptobacteroides* in HP (P=0.001). *Paraprevotella* and *Psychrobacter* decreased in MP and HP (P=0.026, P=0.040), RF16 was reduced in MP (P=0.001), while *Faecalimonas* was elevated (P=0.036). Additional enrichments included CAG_41 and *Clostridium_T* in HP (P=0.010, P=0.004) and CAG_603 and *Succinivibrio* in LP (P=0.019, P=0.014) (Fig 7e.). LEfSe identified group-specific biomarkers (Fig 8a), with CON enriched in Spirochaetota and *Treponema_D*, HP in *Eubacterium_F*, LP in Proteobacteria and *Psychrobacter,* and MP in *Succinivibrio*. Correlation analysis (Fig 8b) revealed that Spirochaetota, Actinobacteriota, *Treponema_D*, and Firmicutes_D were positively correlated with SOD and immunoglobulins (IgA, IgG). *Psychrobacter* correlated positively with IgG, whereas RF16 was negatively associated with IgM and LPS but positively correlated with TNF-α, IL-6, and antioxidant activity. *Faecalimonas* correlated positively with IL-4 and negatively with IL-1β, suggesting potential anti-inflammatory effects. Although these associations offer clues to how specific microbial shifts may interact with antioxidant or immune responses, additional functional assays or metagenomic analyses would help clarify the underlying mechanisms.

DISCUSSION

The present study provides comprehensive insights into the effects of *L. paracasei* subsp. *paracasei* Q-1 supplementation on neonatal calf health. Our results demonstrate that *L. paracasei* subsp. *paracasei* Q-1 not only promotes growth performance but also improves intestinal health, enhances immune and antioxidant responses, and modulates the gut and rumen microbiota. However, it is important to note that multiple factors—such as dietary composition, environmental conditions, and maternal immunity—may also influence these outcomes. Our inferences focus on the role of L. paracasei subsp. paracasei Q-1 within the scope of this pilot trial, but further research is needed to elucidate complex interactions and confirm causality. These findings underscore the potential of *L. paracasei* subsp. *paracasei* Q-1 as a beneficial probiotic in neonatal calf management, with significant implications for animal health and livestock productivity.

Growth performance and diarrhea incidence: The growth-promoting effects of *L. paracasei* subsp. *paracasei* Q-1 are consistent with findings from previous studies on probiotics in livestock, particularly in terms of enhanced ADG (Li *et al.*, 2022; Lin *et al.*, 2022). The observed 11.39 to 13.92% improvement in ADG in the probiotic-treated groups compared to the control group suggests that *L. paracasei* subsp. *paracasei* Q-1 has the potential to improve feed conversion efficiency and nutrient absorption. While higher doses did not further improve growth performance, the results indicate that a lower dose (1×10^8 CFU/head/day) is sufficient to exert a significant effect. This supports the growing body of evidence suggesting that probiotics do not necessarily need to be administered in high doses to be effective, and that optimizing the dose may lead to more cost-effective supplementation strategies (Alqahtani *et al.*, 2024; Suez *et al.*, 2019).

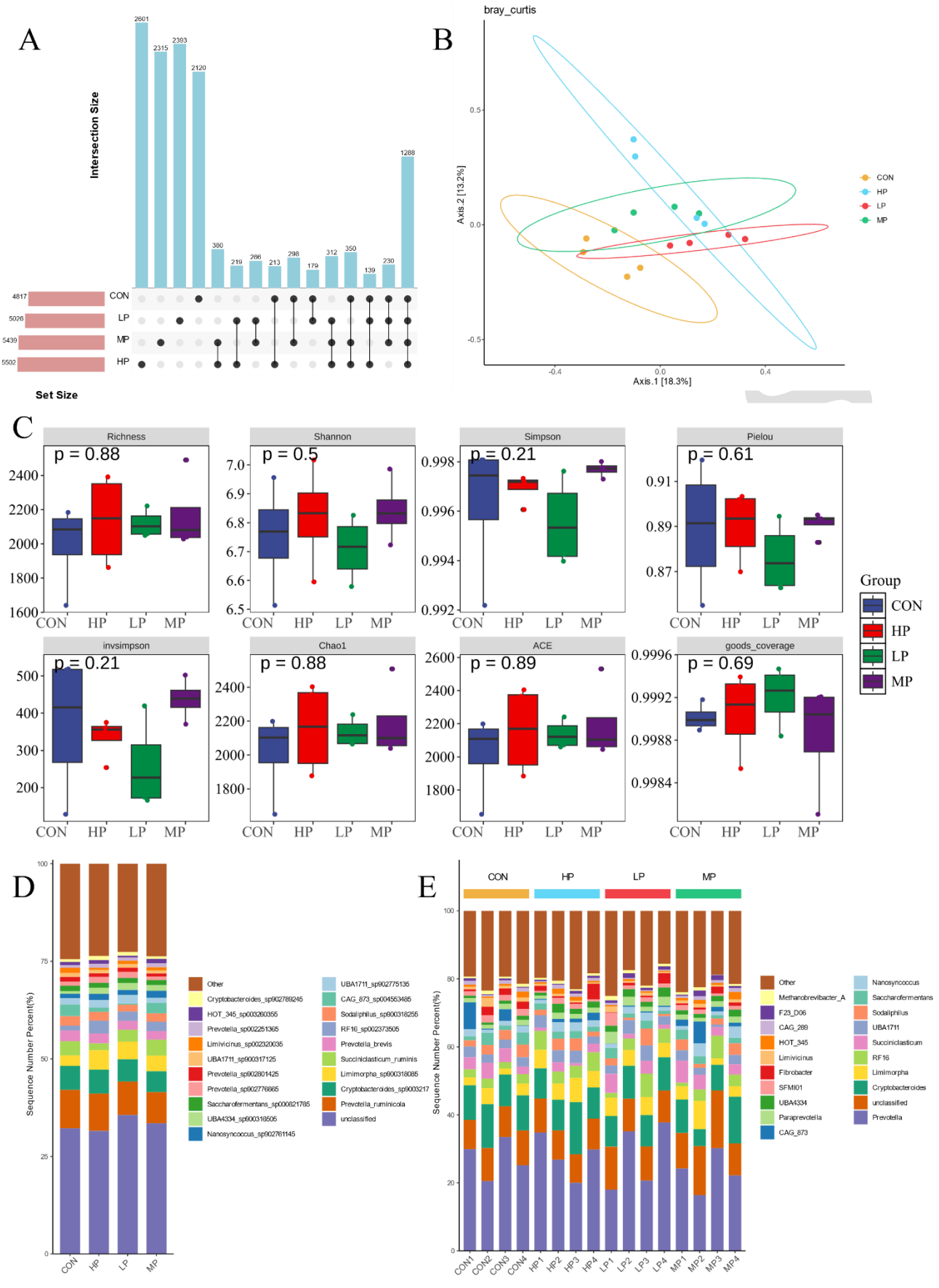

Fig. 5: Rumen microbiota composition and diversity in response to *Lacticaseibacillus paracasei* subsp. *paracasei* Q-1. (A) UpSet plot of ASVs shared among treatment groups. (B) PCoA based on Bray–Curtis distances. (C) Alpha diversity indices. (D) Relative abundance of dominant phyla. (E) Relative abundance of key genera.

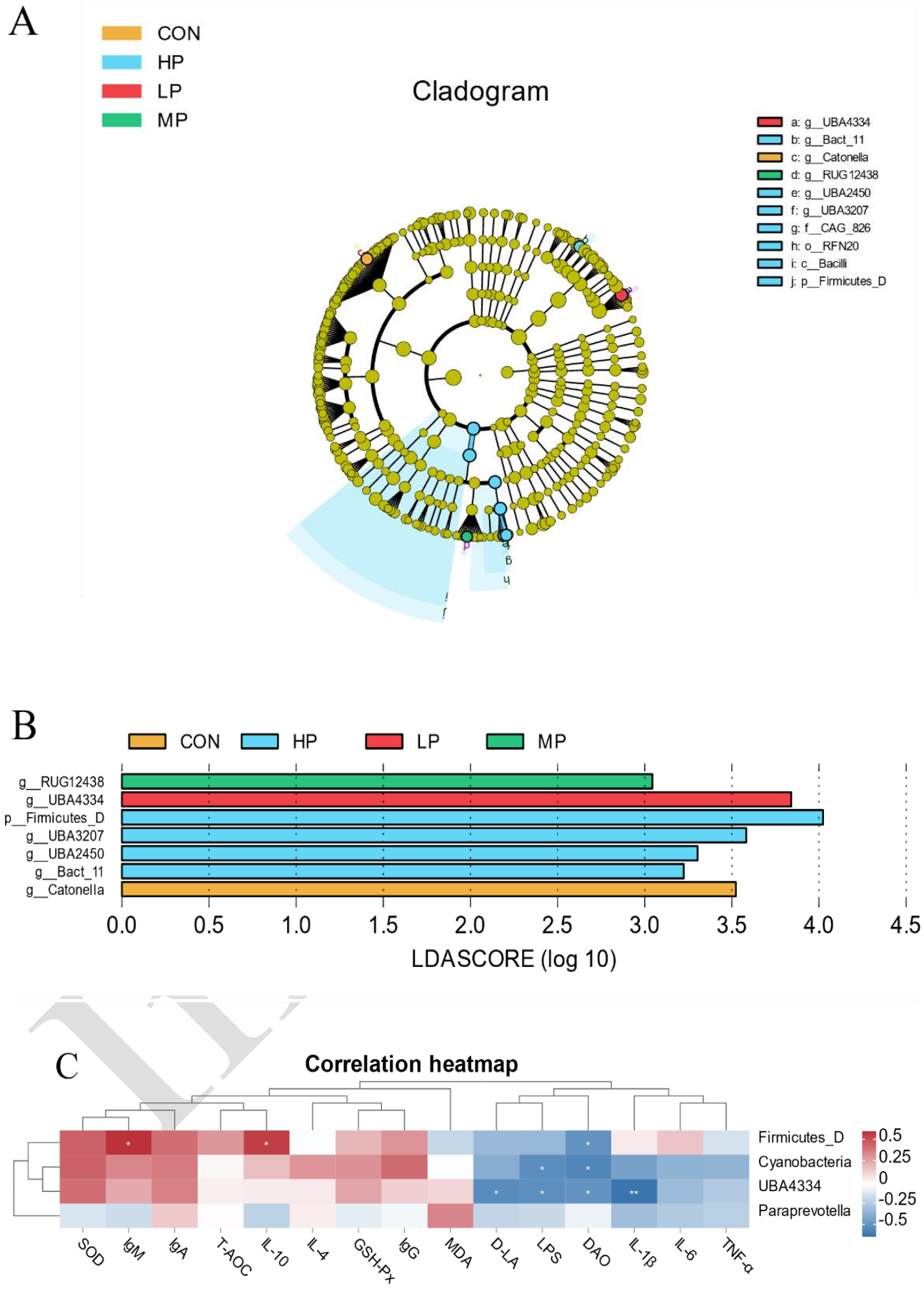

Fig. 6: Differential taxa and associations with serum parameters in the rumen microbiota. (A) Cladogram from LEfSe highlighting enriched taxa per group. (B) LDA scores of discriminant taxa. (C) Spearman correlation heatmap of bacterial taxa and serum measures (red = positive, blue = negative; *P<0.05, **P<0.01).

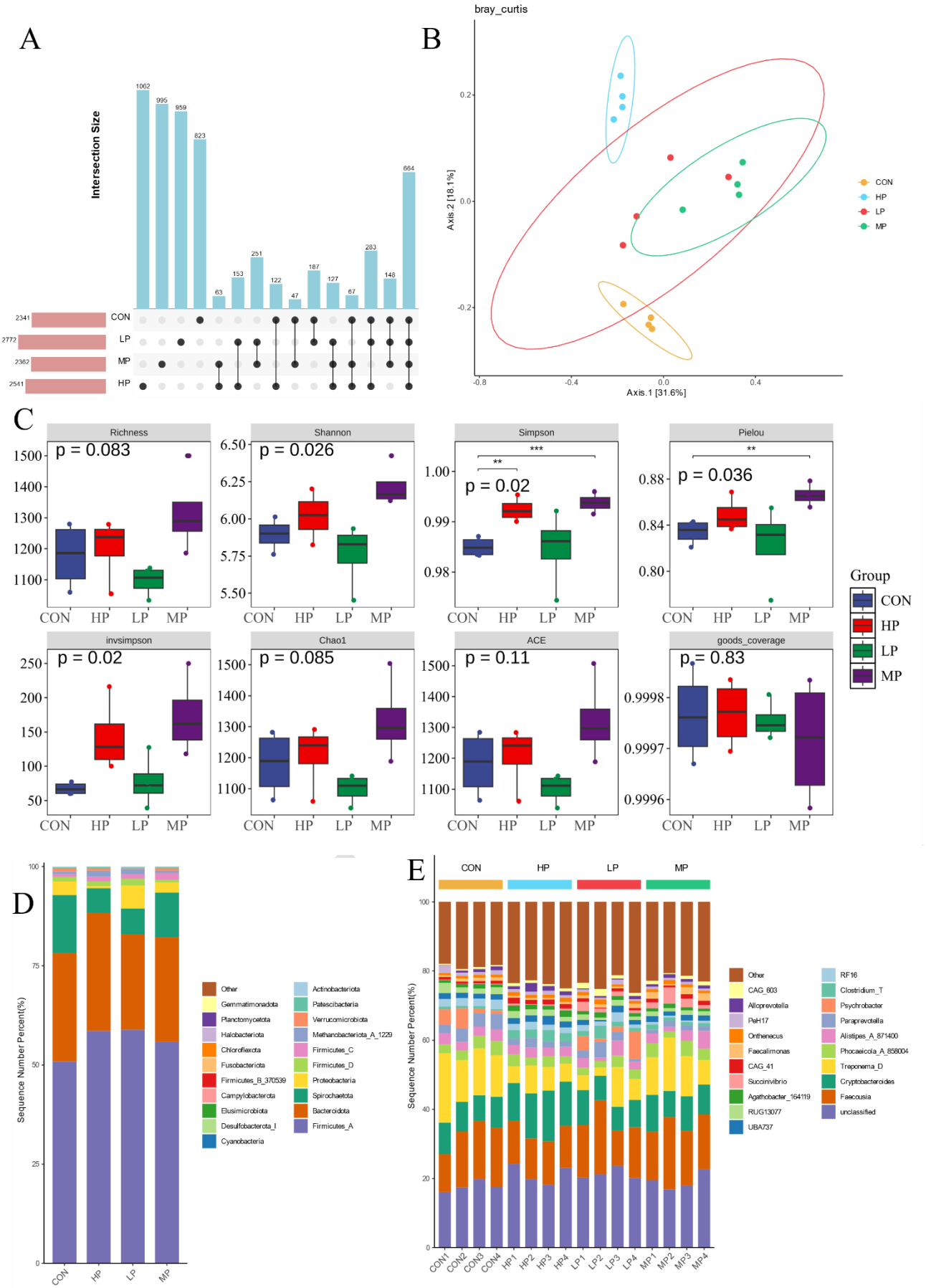

Fig. 7: Fecal microbiota composition and diversity under *Lacticaseibacillus paracasei* subsp. *paracasei* Q-1 supplementation. (A) UpSet plot of ASVs across groups. (B) PCoA of microbial communities. (C) Alpha diversity indices, with MP and HP groups showing significant increases (P<0.05). (D–E) Relative abundances of main phyla and genera.

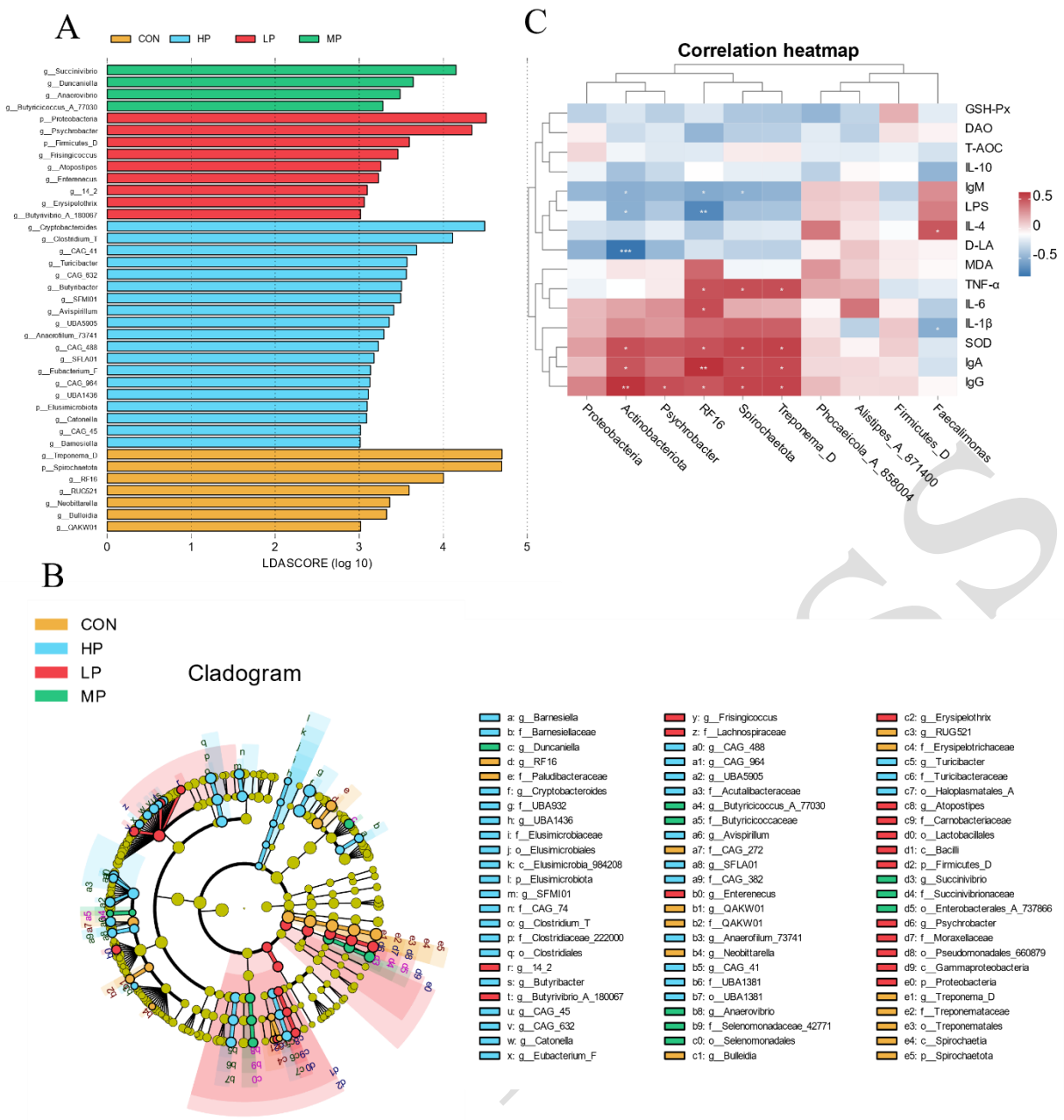

Fig. 7: Taxonomic differences and correlations in fecal microbiota after *Lacticaseibacillus paracasei* subsp. *paracasei* Q-1 supplementation. (A) LDA scores of significantly discriminant taxa. (B) Spearman correlation heatmap between key bacterial taxa and selected serum parameters (red = positive, blue = negative; *P<0.05, **P<0.01). (C) Cladogram from LEfSe highlighting enriched taxa per group.

The reduction in diarrhea incidence observed in the probiotic-supplemented groups is particularly noteworthy, as diarrhea is a leading cause of morbidity and mortality in neonatal calves (Carter *et al.*, 2021). Probiotics have been widely recognized for their ability to prevent or reduce the severity of enteric diseases by enhancing gut barrier function and modulating the gut microbiota (Ghosh, 2004; Zhou *et al.*, 2022). In this study, the most pronounced reduction in diarrhea was observed in the MP group, which suggests a potentially favorable dose for mitigating enteric issues but does not exclude the possibility that other unmeasured variables may also have contributed. Environmental stressors and dietary consistency, for instance, were not strictly controlled in our study, and thus could be confounding factors.

Immune modulation and antioxidant responses: In addition to growth performance and diarrhea control, *L. paracasei* subsp. *paracasei* Q-1 supplementation significantly enhanced both immune and antioxidant functions, indicating its potential as a comprehensive support for neonatal calf health. The probiotic-treated groups exhibited higher activities of antioxidant enzymes such as GSH-Px and SOD, suggesting that *L. paracasei* subsp. *paracasei* Q-1 helps mitigate oxidative stress. Oxidative stress is known to contribute to a range of diseases, including enteric infections, by promoting inflammation and damaging cellular components (Bouyahya *et al.*, 2024; Vona *et al.*, 2021). The enhanced antioxidant status observed in this study is consistent with previous reports that probiotics, including *L. paracasei* strains, can modulate redox balance, thereby protecting cells from oxidative damage and supporting better health outcomes (Xu *et al.*, 2023). At a mechanistic level, several studies have shown that *L. paracasei* can activate the Nrf2–Keap1 pathway and up-regulate the expression of

antioxidant genes such as HO-1 and NQO1 in intestinal epithelial cells (Niu *et al.*, 2024; Xu *et al.*, 2022). The concomitant rise in SOD and GSH-Px activities observed here is consistent with that molecular pattern, although confirmation at the transcript or protein level was beyond the scope of this trial.

The increase in immunoglobulin levels (IgA, IgG, IgM) following *L. paracasei* subsp. *paracasei* Q-1 supplementation is another key finding. IgA is particularly important in mucosal immunity, where it prevents pathogens from adhering to the intestinal epithelium. The enhanced production of IgG and IgM in a dose-dependent manner further suggests that *L. paracasei* subsp. *paracasei* Q-1 stimulates both humoral and cellular immunity. The HP group exhibited the highest levels of IgA and IgG, which aligns with previous studies that demonstrated probiotics can enhance systemic immunity (Jinendiran *et al.*, 2021). These immune-enhancing effects are particularly important in neonatal calves, which are more susceptible to infections during the early stages of life (Dinardo *et al.*, 2022; Kim, 2021). Nonetheless, the precise molecular mechanisms underpinning these immunomodulatory and antioxidant effects require further investigation, such as examining cytokine expression pathways, toll-like receptor signaling, or transcriptomic profiles in intestinal tissues. Without such molecular context, our current interpretation remains somewhat speculative.

Intestinal barrier function: Our study highlights the ability of *L. paracasei* subsp. *paracasei* Q-1 to improve intestinal barrier function, which is crucial for preventing the translocation of pathogens and harmful substances into the bloodstream. Intestinal permeability is often increased in neonatal calves suffering from diarrhea, leading to systemic inflammation and impaired immune function (Cangiano *et al.*, 2024; Wu *et al.*, 2022). Reduction in markers of intestinal permeability (D-LA, LPS, DAO) in probiotic-treated groups suggests that *L. paracasei* subsp. *paracasei* Q-1 supports intestinal barrier integrity by promoting tight junction protein production, enhancing epithelial cell proliferation, and reducing inflammation (Mo *et al.*, 2024; Zheng *et al.*, 2022). The ability of *L. paracasei* subsp. *paracasei* Q-1 to modulate gut permeability is particularly important given the critical role of the intestinal barrier in protecting against pathogenic infections and regulating immune responses. By improving gut barrier function, *L. paracasei* subsp. *paracasei* Q-1 may help to reduce the incidence of gastrointestinal diseases, promote better nutrient absorption, and prevent the onset of systemic inflammation, ultimately leading to improved growth and health outcomes in neonatal calves. Although we measured circulating D-lactate, DAO and LPS, we did not directly quantify tight-junction proteins or SCFAs. Given that several *L. paracasei* strains are known producers of acetate and butyrate, metabolites that strengthen barrier integrity through up-regulation of claudin-1 and occludin, future studies incorporating SCFA analysis and intestinal gene expression will be informative.

Microbial composition of the rumen and intestinal microbiota: The effects of *L. paracasei* subsp. *paracasei* Q-1 on the rumen and fecal microbiota provide additional insights into the mechanisms by which this probiotic improves calf health. In the rumen, the probiotic supplementation led to shifts in microbial community composition, with increased abundance of Firmicutes_D in the HP group and a reduction in Eremiobacterota across all probiotic groups. These changes in microbial composition are likely to influence nutrient digestion and fermentation, as Firmicutes are important for the breakdown of fiber and other complex carbohydrates (Sun *et al.*, 2023; Zou *et al.*, 2021). While no significant changes in alpha diversity were observed, the observed shifts in specific microbial taxa suggest that *L. paracasei* subsp. *paracasei* Q-1 promotes a more favorable microbial community in the rumen, which may enhance nutrient utilization and reduce the risk of ruminal dysbiosis.

In the fecal microbiota, *L. paracasei* subsp. *paracasei* Q-1 supplementation significantly altered microbial diversity and richness, particularly in the MP and HP groups. Increases in the Shannon and Simpson diversity indices suggest that probiotic supplementation supports a more balanced and stable microbial community, which is a key feature of a healthy gut. The observed reductions in Spirochaetota and Proteobacteria, coupled with increases in Firmicutes_D, are indicative of a shift toward a more beneficial microbial profile, as these taxa are often associated with better gut health and stability(Bai *et al.*, 2025). Specific genus-level changes, such as the enrichment of *Cryptobacteroides* in the HP group and the depletion of *Paraprevotella* in the MP and HP groups, further suggest that *L. paracasei* subsp. *paracasei* Q-1 modulates the microbiota in a manner that supports both intestinal health and immune function.

Correlation analysis between microbial taxa and health markers revealed significant associations between specific genera and serum biochemical parameters, including immunoglobulin levels and markers of intestinal permeability. These findings underscore the relationship between the gut microbiota and host physiology, suggesting that the beneficial effects of *L. paracasei* subsp. *paracasei* Q-1 on calf health are partly mediated through microbiota modulation. The identification of specific microbial biomarkers associated with probiotic treatment further emphasizes the potential of microbial interventions to improve calf health. Causal inference should nonetheless be made with caution, because microbial shifts could be driven indirectly by changes in feed intake or digesta passage rate rather than by the probiotic itself. Metagenomic or metabolomic profiling would help discriminate primary from secondary effects.

Limitations and future perspectives: Despite the promising outcomes, several limitations need to be addressed. First, the sample size (n=10 per group) and the trial duration (60 days) may limit the power to detect subtle or long-term effects. Additionally, factors like maternal immunity, farm management, and feeding variation were not fully standardized, which could introduce confounding effects. While we attempted to randomize and maintain similar conditions, external variables cannot be completely eliminated. Furthermore, without a mechanistic focus, our discussion of immunomodulatory and antioxidant pathways remains primarily descriptive. Future larger-scale trials with expanded molecular analyses and extended

observation periods will be valuable in clarifying the mode of action and validating the practical feasibility of *L. paracasei* subsp. *paracasei* Q-1 supplementation in diverse farm settings.

Conclusions: In this pilot trial, supplementation with *L. paracasei* subsp. *paracasei* Q-1 appeared to significantly improve the growth performance, help reduce diarrhea incidence, enhance immune function, and strengthen intestinal barrier integrity in neonatal calves. Additionally, *L. paracasei* subsp. *paracasei* Q-1 modulated both rumen and fecal microbiota composition, suggesting its potential as an effective probiotic to promote calf health and productivity. Among the tested doses, 3×10^8 CFU/calf/day provided the most consistent benefits in this preliminary study. However, further large-scale trials, field-scale validation, and dose–response analyses are needed to confirm economic feasibility and fully establish the optimal supplementation strategy before on-farm adoption.

Data availability: The 16S rRNA sequencing data have been deposited in the Sequence Read Archive (SRA) of NCBI, with accession number PRJNA1330506.

Ethics declarations: All animal procedures were approved by the Animal Care and Use Committee of Chengde Academy of Agricultural and Forestry Sciences of Forestry and Agriculture (Approval No. 2024-02). The animal experiments strictly adhered to ethical standards.

Author contribution: JJ, GL, JS conceived and designed the study; BZ, ZW, MT, YX, LK conducted the research; ZW, MT, YH, LS, DS, L analyzed and interpreted the data; BZ, ZW wrote the manuscript; and JJ, G, LJ, YH, CH, YX revised the manuscript. All authors read and approved the final version of the manuscript.

Funding: This work was supported by the Chengde Science and Technology Plan Project "Research and Demonstration Application of Key Technologies for Replacing Antimicrobial Agents in Major Diseases in Calf Green and Healthy Farming" (202305B049), Chengde National Sustainable Development Agenda Innovation Demonstration Zone Construction Science and Technology Special Project "Weichang Yudaokou Smart, Circular Animal Husbandry and Agriculture Demonstration Zone Construction" (202202F016), Chengde Livestock Workstation, and Hebei Province Phase III Modern Agricultural Industry Technology System Meat Cattle Innovation Team Chengde Comprehensive Experimental Station (HBCT2024240402).

REFERENCES

Alqahtani FS, Bahshwan SMA, AL-Qurashi MM, *et al.*, 2024. Impact of dietary bacillus toyonensis m44 as an antibiotic alternative on growth, blood biochemical properties, immunity, gut microbiota, and meat quality of IR broilers. Pakistan Veterinary Journal 44(3): 637-646.

Bai J, Tang L, Liu M, *et al.*, 2025. Effects of substituting alfalfa silage with whole plant quinoa silage on rumen fermentation characteristics and rumen microbial community of sheep in vitro. Frontiers in Veterinary Science 12: 1565497.

Bouyahya A, Bakrim S, Aboulaghras S, *et al.*, 2024. Bioactive compounds from nature: antioxidants targeting cellular transformation in response to epigenetic perturbations induced by oxidative stress. Biomed Pharmacother 174: 116432.

Cangiano LR, Villot C, Guan LL, *et al.*, 2024. Graduate student literature review: developmental adaptations of immune function in calves and the influence of the intestinal microbiota in health and disease. Journal of Dairy Science 107(4): 2543-2555.

Carter HSM, Renaud DL, Steele MA, *et al.*, 2021. A narrative review on the unexplored potential of colostrum as a preventative treatment and therapy for diarrhea in neonatal dairy calves. Animals 11(8): 2221.

Dinardo FR, Maggiolino A, Martinello T, *et al.*, 2022. Oral administration of nucleotides in calves: effects on oxidative status, immune response, and intestinal mucosa development. Journal of Dairy Science 105(5): 4393-4409.

Du X, Maqbool B, Shichiyakh R, *et al.*, 2025. Eubiotics improve gut health and overall production in animals by reducing pathogenic bacteria. The Pakistan Veterinary Journal 45(2): 488-498.

Du Y, Gao Y, Hu M, *et al.*, 2023. Colonization and development of the gut microbiome in calves. Journal of Animal Science and Biotechnology 14(1): 46.

Ghosh S, 2004. Probiotics in inflammatory bowel disease: is it all gut flora modulation? Gut 53(5): 620-622.

Jessop E, Li L, Renaud DL, *et al.*, 2024. Neonatal calf diarrhea and gastrointestinal microbiota: etiologic agents and microbiota manipulation for treatment and prevention of diarrhea. Veterinary Sciences 11(3): 108.

Jinendiran S, Archana R, Sathishkumar R, *et al.*, 2021. Dietary administration of probiotic aeromonas veronii v03 on the modulation of innate immunity, expression of immune-related genes and disease resistance against aeromonas hydrophila infection in common carp (cyprinus carpio). Probiotics and Antimicrobial Proteins 13(6): 1709-1722.

Kaewarsar E, Chaiyasut C, Lailerd N, *et al.*, 2023. Effects of synbiotic lacticaseibacillus paracasei, bifidobacterium breve, and prebiotics on the growth stimulation of beneficial gut microbiota. Foods 12(20): 3847.

Kang X, Li X, Zhou H, *et al.*, 2023. Genome-wide and 16s rRNA sequencing-based analysis on the health effects of lacticaseibacillus paracasei XLK401 on chicks. Microorganisms 11(9): 2140.

Khan M, 2019. Effect of lactobacillus gallinarum PL 53 supplementation on xylose absorption and intestinal morphology in broilers challenged with campylobacter jejuni. Pakistan Veterinary Journal 40(2): 163-168.

Kim S, 2021. Biological factors associated with infectious diarrhea in calves. The Pakistan Veterinary Journal 41(04): 531-537.

Kostelac D, Gerić M, Gajski G, *et al.*, 2022. Probiotic and paraprobiotic derivates exhibit anti-inflammatory and genoprotective effects during induced stress. Journal of Applied Microbiology 133(2): 819-829.

Li L, Chen X, Zhu J, *et al.*, 2023. Advances and challenges in interaction between heteroglycans and bifidobacterium: utilization strategies, intestinal health and future perspectives. Trends in Food Science & Technology 134: 112-122.

Li P, Zheng L, Qi Y, *et al.*, 2022. Dietary lactobacillus fermentum and lactobacillus paracasei improve the intestinal health of broilers challenged with coccidia and clostridium perfringens. Frontiers in Veterinary Science 9: 1025677.

Lin A, Yan X, Wang H, *et al.*, 2022. Effects of lactic acid bacteria-fermented formula milk supplementation on ileal microbiota, transcriptomic profile, and mucosal immunity in weaned piglets. Journal of Animal Science and Biotechnology 13(1): 113.

Liu W, Cheng H, Zhang H, *et al.*, 2024. Effect of lactobacillus paracasei LK01 on growth performance, antioxidant capacity, immunity, intestinal health, and serum biochemical indices in broilers. Animals 14(23): 3474.

Ma W, Wu Y, Sun H, *et al.*, 2025. An in vivo study of the ameliorative effect of supplementation with lacticaseibacillus paracasei glory LP16 in immunocompromised mice. Scientific Reports 15(1): 14932.

Ministry OAOT, 2004. NY/t 815–2004 beef cattle raising standard: China Agricultural Press Beijing.

Mo C, Lou X, Xue J, *et al.*, 2024. The influence of akkermansia muciniphila on intestinal barrier function. Gut Pathogens 16(1): 41.

Niu B, Feng Y, Cheng X, *et al.*, 2024. The alleviative effects of viable and inactive*lactobacillus paracasei* CCFM1120 against alcoholic liver disease*via* modulation of gut microbiota and the nrf2/HO-1 and TLR4/MyD88/NF-κb pathways. Food & Function 15(17): 8797-8809.

Ren S, Wang C, Chen A, *et al.*, 2023. *Lactobacillus paracasei* influences the gut-microbiota-targeted metabolic modulation of the immune status of diarrheal mice. Food & Function 14(9): 4368-4379.

Renaud DL, Duffield TF, LeBlanc SJ*, et al.*, 2018. Risk factors associated with mortality at a milk-fed veal calf facility: a prospective cohort study. Journal of Dairy Science 101(3): 2659-2668.

Suez J, Zmora N, Segal E*, et al.*, 2019. The pros, cons, and many unknowns of probiotics. Nature Medicine 25(5): 716-729.

Sun Y, Zhang S, Nie Q*, et al.*, 2023. Gut firmicutes: relationship with dietary fiber and role in host homeostasis. Critical Reviews in Food Science and Nutrition 63(33): 12073-12088.

Vona R, Pallotta L, Cappelletti M*, et al.*, 2021. The impact of oxidative stress in human pathology: focus on gastrointestinal disorders. Antioxidants 10(2): 201.

Wang F, Chen L, Li Y*, et al.*, 2025. Lacticaseibacillus paracasei subsp. Paracasei q- 1 exhibits good safety and effectively prevents escherichia coli k99-induced diarrhea in mice. Probiotics and Antimicrobial Proteins: 21.

Wu YY, Nie CX, Xu C*, et al.*, 2022. Effects of dietary supplementation with multispecies probiotics on intestinal epithelial development and growth performance of neonatal calves challenged with*escherichia coli* k99. Journal of the Science of Food and Agriculture 102(10): 4373-4383.

Xu J, Xu J, Shi T*, et al.*, 2023. Probiotic-inspired nanomedicine restores intestinal homeostasis in colitis by regulating redox balance, immune responses, and the gut microbiome. Advanced Materials 35(3): 2207890.

Xu J, Zhang X, Song Y*, et al.*, 2022. Heat-killed lacticaseibacillus paracasei ameliorated UVB-induced oxidative damage and photoaging and its underlying mechanisms. Antioxidants 11(10): 1875.

Yang J, Liu Y, Liu J*, et al.*, 2025. Synbiotic formulation of fermented yak milk by lacticaseibacillus paracasei YT805 and inulin and its efficacy on gut microbiota modulation. LWT 230: 118185.

Zeng Z, Guo X, Zhang J*, et al.*, 2021. *Lactobacillus paracasei* modulates the gut microbiota and improves inflammation in type 2 diabetic rats. Food & Function 12(15): 6809-6820.

Zheng J, Ahmad AA, Yang Y*, et al.*, 2022. Lactobacillus rhamnosus CY12 enhances intestinal barrier function by regulating tight junction protein expression, oxidative stress, and inflammation response in lipopolysaccharide-induced caco-2 cells. International Journal of Molecular Sciences 23(19): 11162.

Zhou J, Li M, Chen Q*, et al.*, 2022. Programmable probiotics modulate inflammation and gut microbiota for inflammatory bowel disease treatment after effective oral delivery. Nature Communications 13(1): 3432.

Zou Y, Liang N, Zhang X*, et al.*, 2021. Functional differentiation related to decomposing complex carbohydrates of intestinal microbes between two wild zokor species based on 16SrRNA sequences. BMC Veterinary Research 17(1): 216.