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Effects of Fecal Microbiota Donor on the Intestinal Microbiome, Health, and Growth of Preweaning Calves

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Abstract

Environmental spraying of microbiota is a novel low-stress strategy for manipulating early microbial colonization. This study aimed to compare the effects of spraying Fecal Microbiota (FM) from calves and lactating cows on the growth, health, and intestinal microbiota of preweaning calves A total of 60 newborn calves were randomly divided into three groups: control group (CON), weaned calf fecal microbiota group (WCFG): sprayed with microbiota from weaned cow feces, and lactating cow fecal microbiota group (LCFG): sprayed with microbiota from lactating cow feces. Growth performance, serum biochemical parameters, immune indicators, and intestinal microbial composition were monitored. Compared with the CON and WCFG, the LCFG exhibited significant impairment in growth performance, including a marked decrease in body weight (BW) on day 60, significantly reduced average daily gain (ADG) during days 15–30 and 45–60, and a significantly higher diarrhea rate. Serum analysis revealed systemic metabolic and immune disorders in the LCFG, characterized by decreased blood glucose (GLU), increased alanine aminotransferase (ALT), and altered inflammatory cytokine profiles. Intestinal microbiota analysis indicated that the LCFG developed a unique and potentially dysregulated microbial structure, featuring a reduction in beneficial bacterial genera and enrichment of other genera. Spraying FM from lactating cows induces a series of negative effects, including intestinal dysbiosis, systemic physiological disorders, and ultimately impaired growth in preweaning calves. These findings highlight that environmental fecal microbiota can affect intestinal microbial colonization in preweaning calves.

Introduction

The successful colonization of intestinal microbiota in newborn ruminants during early life plays a decisive role in their subsequent growth and development, immune system establishment, and lifelong production potential, directly affecting host nutrient metabolism efficiency and health homeostasis (Fu et al. 2024; Zhang et al. 2021). Under natural conditions, young animals gradually construct an intestinal microecosystem adapted to physiological needs through close contact with their mothers and the environment (Chen et al. 2025). However, in modern intensive farming systems, newborn calves are isolated from cows and complex environmental microbial reservoirs, residing in a relatively simple artificial environment. This isolation leads to delayed intestinal microbial colonization and structural disorders, which are important contributing factors to diarrhea, growth retardation, and other issues (Cantor et al. 2019).

To improve early microbial colonization in calves, current main intervention strategies include oral probiotics and traditional fecal microbiota transplantation (Bu et al. 2020; Zhang et al. 2026). The efficacy of probiotic preparations is highly dependent on specific strains, and their colonization ability and persistence in exogenous environments are questionable (Zhang et al. 2025). Although traditional FMT can provide complex microbial communities, the invasive procedures of gavage or rectal infusion cause significant stress and potential injury risks to newborn calves (Slanzon et al. 2022). As a low-stress passive intervention approach, environmental microbiota spraying simulates the natural process of young animals contacting environmental microorganisms. It enables microbial transmission without invasive operations, combining the ecological integrity of natural fecal microbial communities with animal welfare friendliness (Reveles et al. 2025). This method has demonstrated potential in regulating intestinal flora and improving growth performance in monogastric animals (Huang et al. 2023).

However, its effectiveness is highly dependent on the compatibility between the functional characteristics of the administered microbial community and the host's physiological status (Cangiano et al. 2024). Compared with fecal microbiota from lactating cows, that from weaned calves is more likely to achieve benign colonization of intestinal flora in preweaning calves after environmental spraying due to better alignment with their nutritional and metabolic needs and intestinal microenvironment (Haley et al. 2020; Kim et al. 2021). This study hypothesizes that environmental fecal microbiota can modulate intestinal microbial colonization in preweaning calves. The aim is to compare the effects of fecal microbiota from lactating cows and weaned calves on growth performance, health, and intestinal microbiota of preweaning calves.

Materials and Methods

Experimental Animals and Management

A single-factor completely randomized experimental design was adopted in this study to explore the effects of environmental FM spraying on the growth performance, blood indicators, and intestinal microbiota of preweaning calves. The experiment was conducted at a commercial dairy farm in Horinger County, Hohhot City, Inner Mongolia Autonomous Region. A total of 60 healthy newborn Chinese Holstein calves with a BW of 41.72 ± 2.81 kg were selected and randomly divided into three treatment groups according to the principle of similar BW, with 20 calves in each group. The three treatment groups were the control group (CON; sprayed with normal saline), weaned calf fecal microbiota group (WCFG; sprayed with FM from weaned calves), and lactating cow fecal microbiota group (LCFG; sprayed with FM from lactating cows) (Fig. 1). The experiment lasted for 60 days, and data collection ended when the calves were 60 days old. Within 1 hour after birth, each calf was fed colostrum equivalent to 10% of its birth weight, and 2 L of pasteurized colostrum was fed 6 hours later, followed by feeding pasteurized milk. The daily milk feeding amount per calf was: 2 L from days 1 to 4, 3 L from days 5 to 10, 3.5 L from days 11 to 15, 4 L from days 16 to 20, and 5 L from days 21 to 30. Calves were fed pasteurized milk twice a day (05:00 and 15:00 daily). Calves were housed individually in outdoor calf hutches (1.5 × 2.2 m) with sand as bedding, and operations such as manure removal and bedding replacement were performed daily. No hay was provided during the experiment, and starter feed and water were available ad libitum.

Experimental Materials

Normal saline, donor cow FM, beakers, 100 mL centrifuge tubes, centrifuges, cryopreservation tubes, tape measures, weight scales, and measuring rods were used, and regular disinfection was performed after use.

Preparation of Fecal Microbiota Suspension

Through the ranch's intelligent cattle breeding system, weaned calves aged 60–120 days with no history of illness were selected as donors for the WCFG. High-yield lactating cows within 3 months of no illness and 200–220 days after calving for the first time were selected as donors for the LCFG. The collected feces were tested using a calf diarrhea five-in-one antigen rapid detection card, and the FM suspension was prepared only when all indicators of rotavirus, coronavirus, Escherichia coli F5 (K99), Cryptosporidium, and Clostridium perfringens in the calf antigen detection card were negative. The FM suspension was prepared at a ratio of 10% (1 part feces: 9 parts normal saline).

Preparation method: Fresh fecal samples were added to sterile normal saline for dilution, thoroughly shaken, and stirred with a glass rod for 5 minutes to fully mix the fecal samples in sterile normal saline. After standing for 5 minutes, the supernatant was collected by filtration through 4 layers of sterile defatted gauze and dispensed into 100 mL centrifuge tubes as FM suspension. The obtained suspension was stored at -20℃. Frozen storage facilitates subsequent experiments without affecting the bacterial solution.

Spraying method: The suspension was thawed in a water bath at 37℃ one hour before spraying. After thawing, 100 mL of FM suspension was uniformly sprayed inside and around the calf hutches using a spray bottle at around 12:00 noon. Spraying was conducted continuously for 30 days; the WCFG and LCFG were sprayed with FM suspension, while the CON was sprayed with 100 mL of normal saline.

Data Collection and Sample Determination

Growth Performance

BW and ADG: Calves were weighed at the start of the experiment (1 day old) and every 15 days thereafter until the end of the experiment (60 days old), and the ADG per week for each calf was calculated.

Body measurements and body measurement indices: Body measurements of calves were taken at the start of the experiment (1 day old) and every 15 days thereafter until the end of the experiment (60 days old).

Body height (withers height), chest circumference, and body length. Measurement methods are as follows:

Body height: The vertical height from the highest point of the cow's withers to the ground, measured with a measuring rod; Chest circumference: A perpendicular line was drawn at the posterior edge of the scapula, and the circumference was measured with a tape measure; Body length: The distance from the anterior edge of the scapula to the posterior edge of the ischial tuberosity on the same side, measured with a measuring rod.

Body length index = (Body length / Body height) × 100. It reflects the relative development of body length and height.

Chest circumference index = (Chest circumference / Body height) × 100. It reflects the relative development of the body trunk.

Body trunk index = (Chest circumference / Body length) × 100. It reflects the relative development of body trunk capacity.

Blood samples were collected at the start of the experiment (1 day old) and every 15 days thereafter until 60 days old. After 4 hours of fasting post-morning feeding, healthy calves of similar age were selected from each treatment group, and 10 mL of blood was collected from the jugular vein of calves using vacuum blood collection tubes without anticoagulant, stored in an incubator containing ice packs, allowed to stand for 2 hours, then centrifuged at 1800×g for 10 minutes to separate the supernatant to obtain serum, which was stored at -20℃ for subsequent determination.

Serum Biochemical Indicators

Serum biochemical indicators: GLU, total protein (TP), albumin (ALB), triglyceride (TG), total cholesterol (TC), blood urea nitrogen (BUN), ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP); Determination method: Corresponding kits (Nanjing Aoqing Biotechnology Co., Ltd.) were purchased, and determinations were performed using an automatic biochemical analyzer according to the operating steps specified in the kits.

Growth and development-related hormones: Growth hormone-releasing hormone (GHRH), somatostatin (SST), growth hormone (GH), insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-2 (IGFBP-2), insulin-like growth factor binding protein-3 (IGFBP-3), insulin (INS), glucagon-like peptide-2 (GLP-2); Determination method: Corresponding kits (Nanjing Aoqing Biotechnology Co., Ltd.) were purchased, and determinations were performed using enzyme-linked immunosorbent assay (ELISA) according to the operating steps specified in the instructions. Antioxidant indicators: Malondialdehyde (MDA), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (T-AOC); Determination method: Corresponding kits (Nanjing Aoqing Biotechnology Co., Ltd.) were purchased, and determinations were performed using ELISA according to the operating steps specified in the instructions. Immune function indicators: Immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α); Determination method: Corresponding kits (Nanjing Aoqing Biotechnology Co., Ltd.) were purchased, and determinations were performed using ELISA according to the operating steps specified in the instructions.

Health Status

Diarrhea rate: The fecal status of each calf was observed at 09:00, 13:00, and 20:00 daily until the end of the experiment. Fecal appearance was scored as follows: 1 point for strip-shaped or granular feces; 2 points for soft feces that can form a thick shape; 3 points for unformed feces without separation of

feces and water; 4 points for liquid, unformed feces with separation of feces and water; The criteria for judging diarrhea (score ≥ 3 points for 2 consecutive days or more).

Calculation formula: Diarrhea rate = (Number of diarrhea calves / Total number of calves) ×100%

DNA Extraction, PCR Amplification, and 16S rRNA Sequencing

Genomic DNA was extracted from fecal samples using the hexadecyl trimethyl ammonium bromide (CTAB) method. Post-extraction, the quality assessment of the DNA samples was conducted on 1% agarose gels, while their concentration and purity were quantified utilizing a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Waltham, MA, USA). To examine the microbial community composition, the hypervariable V3-V4 regions of the 16S rRNA genes were targeted for amplification, followed by sequencing. Sequencing was conducted by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China), located in Shanghai, China, utilizing the Illumina MiSeq PE300 platform. The primer pair used consisted of 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR conditions adhered strictly to those outlined previously by Wu et al(Wu et al. 2022). The raw sequences of the 16S rRNA gene underwent preliminary processing utilizing the fastp tool, specifically version 0.20.0, which involved demultiplexing and stringent quality filtering. Following this, FLASH, version 1.2.7, was employed to merge these sequences. Further refinement was applied based on well-established criteria previously outlined by Wu et al(Wu et al. 2022). Operational taxonomic units (OTUs) were grouped by Uparse, applying a 97% similarity threshold for clustering. To guarantee the accuracy of the data, chimeric sequences were detected and excluded using Usearch (version 7.0), following the procedures outlined by Edgar(Edgar 2013). The (Ribosomal Database Project) RDP Classifier algorithm was utilized to assign taxonomic classifications to each OTU by comparing their representative sequences against the Silva 16S rRNA database (specifically version 138). A threshold of 70% confidence was applied, in accordance with the methodology previously described by Wang et al(Wang et al. 2007). The gut microbiota was analyzed bioinformatically using the Majorbio Cloud platform,which is accessible via the web at https://cloud.majorbio.com. Utilizing Mothur version 1.30.1 and the curated OTU data, various Alpha diversity metrics were computed, including the number of observed operational taxonomic units (OTUs), the Chao1 estimator of richness, the Shannon diversity index, and Good's coverage. To evaluate the similarities among microbial communities across different samples, principal coordinate analysis (PCoA) was conducted based on Bray-Curtis dissimilarity, using the Vegan package in its version 2.5-3. LEfSe (Linear Discriminant Analysis Effect Size)(Segata et al. 2011) was utilized to detect bacterial taxa, ranging from phyla to genera, that exhibited significant abundance differences among the various groups. This analysis was conducted using an LDA score threshold of greater than 2 and a statistical significance level of $P < 0.05$.

Statistical Analysis

Various indicators were analyzed at 15 days old and every 15 days thereafter. Single-factor analysis of variance (ANOVA) was performed using SPSS 23.0 software, and multiple comparison tests were conducted using Tukey-Kramer. $P < 0.01$ was used as the criterion for extremely significant difference, $P \le 0.05$ as the criterion for significant difference, and $0.05 < P \le 0.10$ as the criterion for tendency of significant difference.

Results

Calf Growth Performance and Diarrhea Rate

Spraying fecal microbiota from lactating cows had a significant negative impact on the growth performance of calves. However, environmental spraying of FM from different sources had no significant effect on body measurement indicators (body length, body height, chest circumference, body length index, body trunk index, and chest circumference index) ($P > 0.05$). As shown in Fig. 2, compared with the CON and WCFG, the 60-day BW and ADG from days 1 to 60 of calves in the LCFG were significantly decreased ($P < 0.05$), and the diarrhea rate from days 1 to 60 of calves in this group was also significantly increased ($P < 0.05$). These results indicate that spraying FM from lactating cows inhibits calf growth and increases the risk of diarrhea.

Serum Metabolic and Immune Indicators

In terms of nutrition and metabolism-related indicators (Fig. 3A), the GLU of calves in the WCFG at 15 days of the experiment was significantly higher than that in the CON and significantly lower than that in the LCFG; the TG at 30 days, ALB at 60 days, ALT at 30 days, and AST at 60 days were significantly higher than those in the CON and significantly lower than those in the LCFG.

Among growth hormone-related indicators (Fig. 3B), the GHRH of calves in the WCFG at 45 days of the experiment was significantly higher than that in the CON and significantly lower than that in the LCFG; the IGF-1 at 60 days, IGFBP-2 at 60 days, and GLP-2 at 15 days were significantly higher than those in the CON and significantly lower than those in the LCFG.

In antioxidant indicators (Fig. 3C), the MDA at 60 days, SOD at 45 days, and CAT at 45 days of calves in the WCFG were significantly higher than those in the CON and significantly lower than those in the LCFG.

In terms of immune indicators (Fig. 3D), the IgG at 60 days of calves in the WCFG was significantly higher than that in the CON and significantly lower than that in the LCFG; the IL-6 at 60 days, IL-10 at 60 days, and TNF-α at 45 days were significantly higher than those in the CON and significantly lower than those in the LCFG. There were no significant differences in other indicators ($P > 0.05$).

Structure and Function of Calf Intestinal Microbial Communities

As shown in Fig. 4a, the fecal bacterial composition of lactating cow donors (D_L) at the phylum level was dominated by Firmicutes (59.11%), Bacteroidota (34.05%), Actinobacteriota (10.21%), and Proteobacteria (1.99%). For weaned calf donors (D_P), the dominant phyla were Firmicutes (52.46%), Bacteroidota (15.04%), Actinobacteriota (10.21%), and Proteobacteria (1.99%).

Alpha diversity analysis (Fig. 4b) revealed that compared with the CON group, both the WCFG and LCFG had significantly higher Chao1 and Shannon indices (Wilcoxon rank-sum test, P = 0.0030 for both) and significantly lower Simpson indices (Wilcoxon rank-sum test, P = 0.0025), indicating that fecal microbiota spraying increased intestinal microbial richness and evenness while reducing the dominance of dominant species.

Beta diversity analysis based on Bray-Curtis distances (Fig. 4c) showed that fecal microbiota spraying significantly altered the intestinal microbial β-diversity of calves (R = 0.3233, P < 0.01).

Venn diagram analysis at the genus level (Fig. 4d) indicated 115 core operational taxonomic units (OTUs) shared among the CON, WCFG, and LCFG groups. Additionally, the CON group had 72 unique OTUs, the WCFG had 82 unique OTUs, and the LCFG had 64 unique OTUs.

At the phylum level (Fig. 4e): The top 5 phyla in the CON group were Firmicutes, Bacteroidota, Actinobacteriota, Patescibacteria, and Proteobacteria; in the WCFG, they were Firmicutes, Bacteroidota, Patescibacteria, Actinobacteriota, and Proteobacteria; in the LCFG, they were Firmicutes, Bacteroidota, Actinobacteriota, Patescibacteria, and Spirochaetota.

At the genus level (Fig. 4f): The top 5 genera in the CON group were Prevotella, Faecalibacterium, Lactobacillus, Blautia, and Subdoligranulum; in the WCFG, they were UCG-005, norank_f__Eubacterium_coprostanoligenes_group, Christensenellaceae_R-7_group, Candidatus_Saccharimonas, and Rikenellaceae_RC9_gut_group; in the LCFG, they were UCG-005, Rikenellaceae_RC9_gut_group, norank_f_Eubacterium_coprostanoligenes_group, norank_o__Clostridia_UCG-014, and Christensenellaceae_R-7_group.

Genus-level differential analysis (Fig. 4g) showed that compared with the CON group, UCG-005, norank_f__Eubacterium_coprostanoligenes_group, Christensenellaceae_R-7_group, norank_o__Clostridia_UCG-014, Candidatus_Saccharimonas, and Paeniclostridium were significantly more abundant in both the WCFG and LCFG (P < 0.01), while Prevotella, Faecalibacterium, Blautia, and Lactobacillus were significantly less abundant (P < 0.01).

Linear discriminant analysis (LDA) identified 7 bacterial orders, 8 bacterial families, and 9 bacterial genera as differential taxa. Among the top 15 genera by relative abundance, 10 were identified as key biomarkers distinguishing the three groups (Fig. 4h), including f__Prevotellaceae,g__Prevotella,o__Bacteroidales,c__Bacteroidia,g__UCG-005,f__Oscillospiraceae,g__norank_f__Eubacterium_coprostanoligenes_group,f__Eubacterium_coprostanoligenes_group,c__Clostridia,o__Peptostreptococcale Tissierellales,and g__Christensenellaceae_R-7_group.

Correlation heatmap analysis (Figs. 4I and 4J) showed that unclassified_f__Lachnospiraceae was significantly positively correlated with GHRH (P < 0.05); norank_f__UCG-010 was significantly positively correlated with IgG (P < 0.05); Christensenellaceae_R-7_group and Candidatus_Saccharimonas were significantly positively correlated with IgA (P < 0.05); unclassified_f__Lachnospiraceae was significantly positively correlated with MDA (P < 0.05); Subdoligranulum and Faecalibacterium were significantly positively correlated with GSH-Px (P < 0.05); and Monoglobus was significantly positively correlated with GSH-Px (P < 0.05). No significant correlations were observed between BW/ADG and intestinal bacterial genera (P > 0.05).

Discussion

By comparing two naturally occurring early microbial colonization models—"simulating dam-offspring cohabitation" (lactating cow-derived FM) and "simulating peer cohabitation" (weaned calf-derived FM)—this study reveals a conclusion with critical implications for modern farming practices: environmental application of fecal microbiota can effectively modify the calf intestinal microbiome.

The results confirm that environmental spraying effectively modifies the composition and structure of calf intestinal microbiota. Both sprayed groups showed significant changes in Alpha diversity: The extremely significant increase in Chao1 and Shannon indices indicates that exogenous microbial introduction enhanced intestinal microbial species richness and evenness(Huuki et al. 2024); the marked decrease in Simpson indices further suggests weakened dominance of dominant flora and more dispersed species distribution(He et al. 2022). PCoA based on Bray-Curtis distance revealed an extremely significant difference in flora structure between LCFG and CON, with a large clustering distance from the lactating cow donor flora. After entering the calf intestine, the lactating cow-derived microbiota failed to integrate effectively but disrupted the natural succession of the original flora, forming a unique dysregulated community(Li et al. 2025). This shift in diversity patterns directly confirms that environmental spraying successfully introduces exogenous microbiota. However, the donor source determines the direction and nature of this structural change(Park et al. 2022). The impaired growth performance and increased diarrhea rate in LCFG calves are attributed to the fundamental niche mismatch between lactating cow intestinal microbiota and the physiological needs of preweaning calves, which triggers microbial dysbiosis(Alimirzaei et al. 2020).

While the "dam-offspring cohabitation" model, with its direct maternal microbial transfer, is traditionally considered foundational for offspring health(Gomes et al. 2024), our environmental spraying method introduced a critical variable: extended aerial exposure of the microbiota prior to contact. This difference in transmission route may have altered the integrity or composition of the microbial community compared to natural, immediate transfer. Nevertheless, this study challenges the universality of this paradigm in modern high-yield dairy systems. In this study, the lactating cow donor intestine was dominated by Firmicutes and Bacteroidota, with a significant enrichment of fiber-degrading genera at the genus level(Matthews et al. 2019)—microbes whose core function is to efficiently degrade complex carbohydrates in roughage(Williamson et al. 2022). In contrast, preweaning calves rely primarily on "milk + low-fiber starter feed" in early life, and their intestines require colonization by lactose-degrading bacteria and moderate short-chain fatty acid (SCFA)-producing bacteria(Song et al. 2019). When lactating cow-derived microbiota enters the calf intestine, exogenous fiber-degrading bacteria rapidly occupy ecological niches, leading to

a significant reduction in beneficial genera: Lactobacillus decreased by 38.2%, Faecalibacterium by 45.1%, and Prevotella (a key genus for lactose metabolism) declined from 18.7% to 9.3%, resulting in obvious dysbiosis(Arnalot et al. 2025).

This microbial imbalance directly triggers a cascade of physiological responses, serving as a key link between microbiota changes and growth phenotypes(Alawneh et al. 2024). The reduction in Prevotella and Lactobacillus decreases intestinal lactose degradation efficiency by over 30%; undigested lactose ferments in the intestine, increasing osmotic pressure—this not only exacerbates diarrhea(Lobo et al. 2023) but also reduces absorbable glucose, leading to a 15.3% decrease in serum GLU at day 15 compared with CON and subsequent energy deficiencys(Beaver et al. 2021; Cockram et al. 2024)). The reduction in Faecalibacterium reduces butyrate secretion(Scharf et al. 2025), increasing intestinal permeability by 28% and enabling endotoxin translocation into the bloodstream, which activates systemic inflammatory responses(Chen and Vitetta 2022). This is reflected in a 28.6% increase in serum IL-6 at day 60, a 32.1% increase in TNF-α at day 45, and a 19.8% decrease in the anti-inflammatory factor IL-10. Reduced butyrate and SCFA levels also inhibit intestinal GPR43 receptor activation(Wang et al. 2012), impairing hepatic IGF-1 synthesis—serum IGF-1 at day 60 was 21.4% lower than that in CON. Additionally, inflammation-induced "metabolic redistribution" diverts 15–20% of growth-related energy toward immune processes, further weakening anabolic metabolism(Danesh Mesgaran et al. 2022; Zebeli and Metzler-Zebeli 2012). These findings indicate that in contexts with significant physiological and nutritional gaps, blindly simulating the "dam-offspring" microbial environment is not only ineffective but may also act as a stressor that disrupts the intestinal microecological homeostasis of preweaning calves.

In sharp contrast, the "simulating peer cohabitation" intervention—spraying weaned calf-derived FM—exhibited excellent biological safety(Amin et al. 2023). Weaned calf donors are in the transition stage from milk to solid feed(Ostendorf et al. 2025); their intestinal microbiota already has preliminary solid feed utilization capabilities while retaining a large number of microbes adapted to the "lactose-low fiber" environment(Hao et al. 2021). After spraying, the recipient microbiota showed 72% similarity to the donor, with no enrichment of potential pathogens in unique OTUs. Although the abundances of lactose-degrading bacteria and barrier-protective bacteria were lower than those in CON, the difference was not significant, and no dysbiosis was observed(Li et al. 2023)Serum GLU, IGF-1, IL-6, and TNF-α in WCFG were not significantly different from CON, maintaining metabolic, immune, and growth homeostasis. This result strongly confirms that "physiological stage proximity" between donor and recipient is a more critical ecological principle than "genetic proximity" in early microbial interventions.

In summary, this study demonstrates that environmental spraying of fecal microbiota can effectively alter the intestinal microbial community structure of calves, yet the ultimate effect is entirely dependent on the donor source. Fecal microbiota derived from lactating cows induced a series of negative outcomes, while that from weaned calves proved relatively safe, highlighting the critical importance of matching the physiological stages of donor and recipient. From a practical standpoint, these findings underscore the necessity of implementing careful environmental manure management in calf rearing—specifically, the prompt removal of lactating cow feces to prevent calves from exposure to mismatched microbial communities, thereby safeguarding the healthy colonization of their gut microbiota.

Declarations

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Author Contributions : Song Niu : Conceptualization, Data curation, Formal analysis, Methodology, and Writing – original draft. Yaning Sun: Data curation, Formal analysis, Investigation, and Writing – original draft.Zhanhe Zhang: Data curation, Formal analysis. Donglin Wu: Conceptualization, Writing – review and editing. Ming Xu: Conceptualization, Funding acquisition, Supervision, Validation, Writing – review and editing. All authors were involved in drafting the manuscript or revising it critically for important intellectual content. All authors had full access to the data and approved the manuscript before it was submitted by the corresponding author.

Data Availability : All raw sequence data from the present study are available at NCBI SRA with the BioProject ID: PRJNA1474617.

Ethics Approval: The animal experimental protocol was approved by the Animal Care and Use Committee of Inner Mongolia Agricultural University (Approval No. NND2024038). The present study complied with the basic principles of laboratory animal welfare.

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Figures

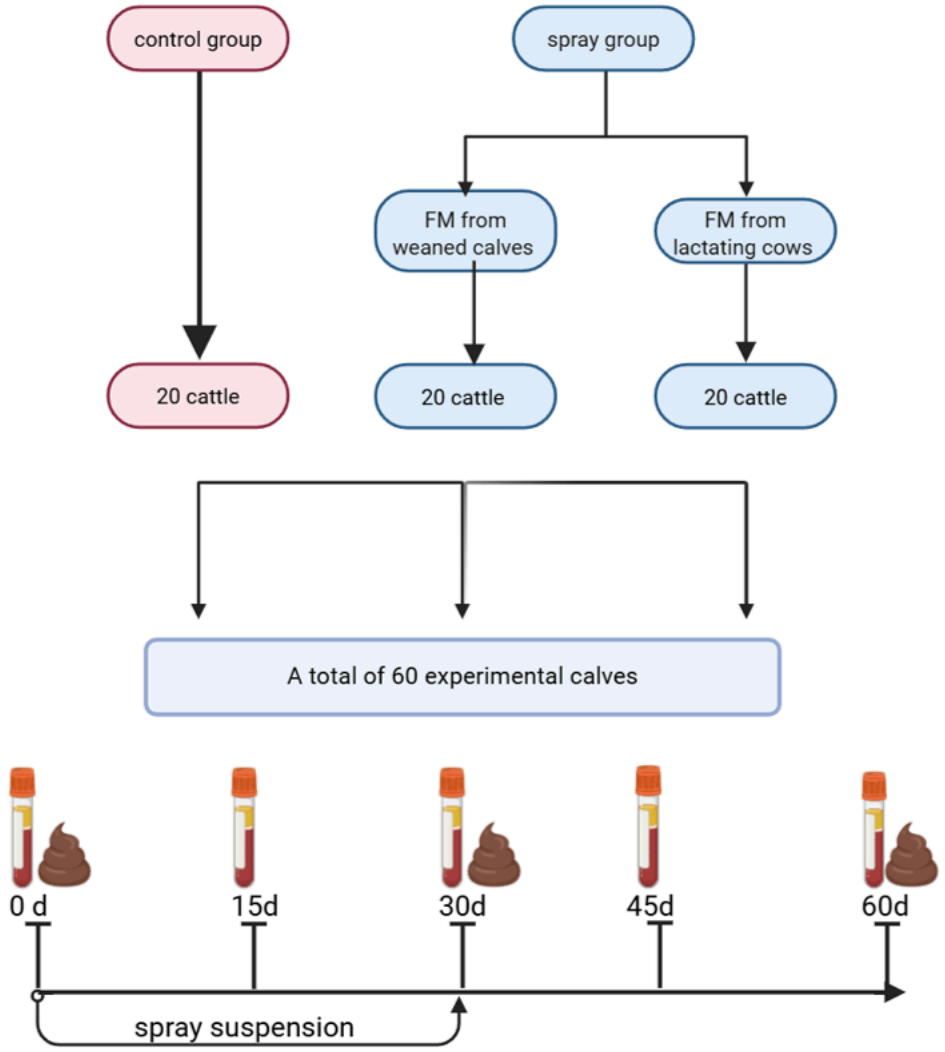


Figure 1

Experimental design, including calf screening, sample collection, and environmental fecal microbiota spraying. A total of 60 calves were assigned to the control group (CON), weaned calf fecal microbiota group (WCFG), or lactating cow fecal microbiota group (LCFG), with 20 calves per group

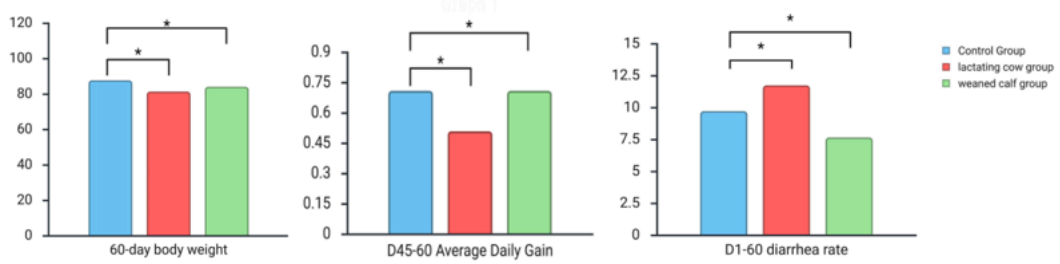


Figure 2

Growth performance and diarrhea rate of preweaning calves. The panels show body weight at day 60, average daily gain from days 45 to 60, and diarrhea rate from days 1 to 60 in the CON, WCFG, and LCFG. $P < 0.05$; $n = 20$

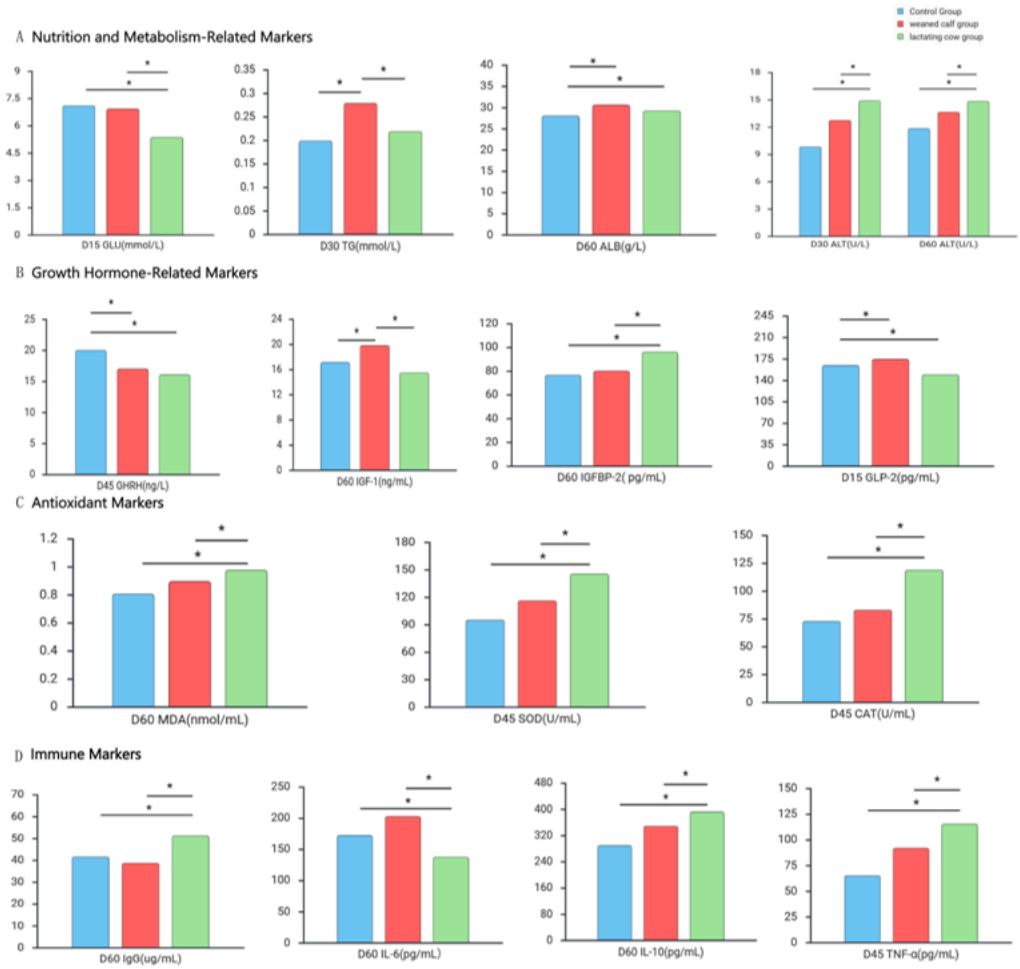


Figure 3

Serum biochemical, hormonal, antioxidant, and immune indicators of preweaning calves. a Nutrition- and metabolism-related indicators: glucose (GLU), triglyceride (TG), albumin (ALB), and alanine aminotransferase (ALT). b Growth-related hormones: growth hormone-releasing hormone (GHRH), insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein 2 (IGFBP-2), and glucagon-like peptide 2 (GLP-2). c Antioxidant indicators: malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT). d Immune indicators: immunoglobulin G (IgG), interleukin 6 (IL-6), interleukin 10 (IL-10), and tumor necrosis factor α (TNF-α). $P < 0.05$; $n = 10$

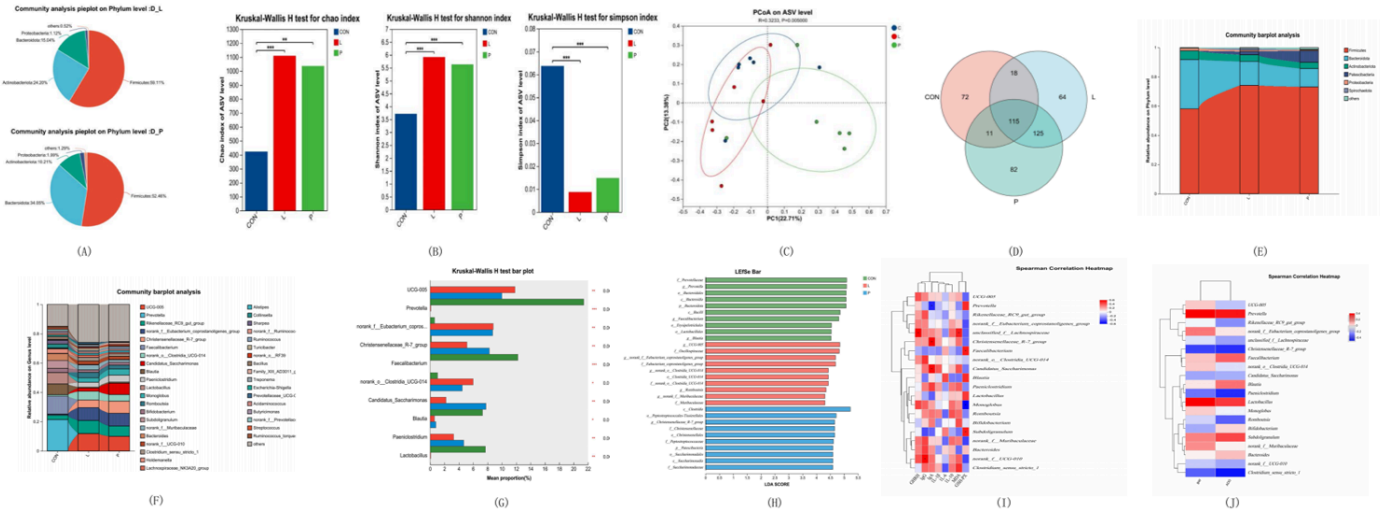


Figure 4

Intestinal microbial diversity and composition of preweaning calves. a Phylum-level bacterial composition of the fecal microbiota from lactating cow and weaned calf donors. b Chao1, Shannon, and Simpson indices. c Principal coordinate analysis based on Bray–Curtis distances. d Venn diagram showing shared and unique operational taxonomic units among the CON, WCFG, and LCFG. e, f Intestinal microbial composition at the phylum and genus levels, respectively. g Genus-level differential taxa identified using the Kruskal–Wallis H test. h Differential taxa identified by linear discriminant analysis effect size. i, j Correlations between intestinal bacterial genera and serum indicators, body weight, and average daily gain. $P < 0.05$; ** $P < 0.01$; $n = 10$