

Impact of a Bacterial Consortium Probiotic Derived from Tropical Calves on the Growth rate, Incidence of Diarrhea, Immune Response, and Fecal Microbiota of Holstein Calves

Impacto de un probiótico de consorcio bacteriano derivado de terneros del trópico sobre la tasa de crecimiento, Incidencia de diarreas, respuesta inmune y microbiota fecal de terneros Holstein

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ABSTRACT

Calf survival is a relevant issue in animal health and production, since newborns do not have robust immune protection, and the first 30 days of life are critical. Infectious diseases, especially diarrhea, are the main cause of mortality. The immune response is strongly associated with the composition and metabolism of intestinal microbes. The bacterial consortium A1D42 obtained from the rumen of tropical calves has been shown to have excellent probiotic characteristics. Our aim was to evaluate the impact of probiotic administration on key parameters for the survival of Holstein calves. One group of newborns was treated with a Bacterial Consortium Probiotic (BCP) for 30 days, and another was considered a control group. Calves that received A1D42 had greater weight gain and a lower incidence of diarrhea compared to the control. They also presented an increase in the concentration of IgG, as well as an increase in the bacterial phylum Bacteroidetes. The administration of BCP derived from the same species improves the health parameters of calves and could reduce their mortality.

Keywords: Diarrhea; Immunoglobulin (Ig); Microbiota; Weight gain.

RESUMEN

La sobrevivencia de los terneros es un tema relevante en la salud y producción animal, ya que los recién nacidos no tienen protección inmunitaria robusta y los primeros 30 días de vida son críticos. Las enfermedades infecciosas, especialmente la diarrea, son la principal causa de mortalidad. La respuesta inmunológica esta importantemente asociada con la composición y metabolismo microbiano intestinal. El consorcio bacteriano A1D42 obtenido del rumen de becerros del trópico, ha demostrado tener excelentes características probióticas. Nuestro objetivo fue evaluar el impacto de la administración del probiótico en parámetros clave para la supervivencia de terneros Holstein. Un grupo de recién nacidos fue tratado con probiótico durante 30 días y otro se consideró control. Los terneros que recibieron A1D42 tuvieron mayor ganancia de peso y menor incidencia de diarrea respecto al

control. También presentaron aumento en la concentración de IgG, así como, un aumento del filo bacteriano Bacteroidetes. La administración de un consorcio bacteriano derivado de la misma especie mejoró los parámetros de salud de los terneros y podría reducir su mortalidad.

Palabras clave: Diarrea; Ganancia de peso; Inmunoglobulina G (IgG); Microbiota.

INTRODUCTION

Probiotics are microorganisms that provide health benefits to their host animal through their nutritional, immunological, bacteriostatic, and antimicrobial characteristics (FAO/WHO, 2016; Guarner *et al.*, 2005; Pineiro and Stanton, 2007). In calves, the early establishment of an appropriate gastrointestinal microbiota is associated with greater weight gain, lower incidence of diarrhea, and improved overall health and growth (Oikonomou *et al.*, 2013; Shen *et al.*, 2024). However, nutritional transitions and environmental changes during the first weeks of life can cause stress and disrupt the colonization of beneficial microorganisms, which increases susceptibility to pathogens, disease like diarrhea, morbidity, and mortality, leading to economic losses in cattle production (Ok *et al.*, 2009; Signorini *et al.*, 2012).

The use of probiotics has been proposed as an alternative effective for improving intestinal microbial development, as well as for enhancing calf health, and therefore its productivity (Uyeno *et al.*, 2015). Other studies have demonstrated that probiotics supplementation early in life can improve growth performance and immune response (Wang *et al.*, 2023). Recently, Rodríguez-González *et al.* (2022a) identified a bacterial consortium, A1D42, isolated from the rumen of tropical calves and cultivated in Man Rogosa and Sharpe culture medium (MRS) with characteristics consistent with probiotic in calves. This consortium includes *Lactocaseibacillus casei*, *Lactocaseibacillus rhamnosus*, *Limosilactobacillus fermentum*, and *Lactobacillus johnsonii*. Additionally, A1D42 exhibits tolerance to bile salts, growth across a range of pH values (5-7), antagonistic activity against *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538),

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and significant adhesion to mucins, important features that support its suitability to use in calves.

Despite these characteristics, the effect of A1D42 on growth performance, immune biomarkers, and fecal microbiota composition in newborn Holstein calves have not yet been evaluated. Therefore, the aim of this study was to assess the impact of administering the A1D42 bacterial consortium for 30 days on the growth rate, diarrhea incidence, serum IgG concentrations, and fecal microbiota in newborn Holstein calves.

MATERIAL AND METHODS

Bacterial Consortium Probiotic development

Rodríguez-González *et al.* (2022a) developed a bacterial consortium probiotic (A1D42) from rumen fluid of clinically healthy Holstein × Zebu calves. This consortium was characterized, and its probiotic qualities were demonstrated, such as resistance to gastric juices, bile salts, adhesion to mucins, competence against pathogens, etc. Furthermore, by 16S ribosomal gene sequencing, they demonstrated a marked abundance of the Firmicutes phylum, mainly represented by the Lactobacillales order, and by conventional PCR, they identified the presence of important probiotic strains such as *L. casei*, *L. rhamnosus*, *L. fermentum* and *L. johnsonii*.

The Rodríguez-González *et al.* (2022a) bacterial consortium probiotic (BCP) A1D42 was used in the current study and was prepared as follows. A pre-inoculum of the A1D42 was prepared from a 2.5 mL frozen stock in 75 mL of MRS (Man Rogosa and Sharpe) culture medium and incubated overnight at 37 °C in anaerobic conditions. Subsequently, the pre-inoculum was transferred into 2.4 L of MRS at 37 °C, pH 6.8 in an autoclavable 3 L bioreactor (my-Control, Applikon, Denmark) with a pH sensor (AppliSens), dissolved oxygen (DO) sensor (AppliSens), and biomass sensor (ABER Futura), and incubated for 24 h at the Laboratorio de Rumiología y Metabolismo Nutricional (RuMeN), Facultad de Estudios Superiores-Cuautitlán (FESC), Universidad Nacional Autónoma de México (UNAM), Querétaro, Querétaro, México. Colony-forming units per mL (CFU/mL) were measured with the biomass sensor, and a sample of the culture was also plated on MRS agar, and CFU/mL were determined to ensure 100 % cell viability.

Rodríguez-González *et al.* (2022b) determined that a dose of the probiotic A1D42 of 4×10^{11} to 4×10^{12} CFU/g showed a digestibility greater than 60 % in the DAISYII digester with ruminal fluid, compared to almost 50 % in the control without probiotic. Therefore, the necessary volume for the dose of 4×10^{11} CFU/mL per calf was centrifuged at 4000 x g for 15 min, and the sediment was transferred to a clean 15 mL polypropylene tube. Each tube contained the daily dose for each calf, was stored at 4 °C protected from light, and was prepared weekly for administration throughout the experiment. In addition, a sample of the probiotic was grown on MRS agar to ensure 100 % cell viability for each batch of culture.

Animals and diet

The experimental protocols were approved by the Internal Committee for the Experimental Animal Care and Use of the National Autonomous University of Mexico (CICUAE, DC.C24_01) by NOM-062-ZOO-1999.

The study involved a total of eight newborn Holstein Friesian calves (four males and four females) born between October and November 2023, with an average weight of 36.46 kg (± 6.19). At birth, calves were separated from their mothers and housed in individual pens (1.20 x 2.15 m). They were fed colostrum at days 1-3, based on the mother's production. Starting on day 2, the calves were fed daily with 6 L of colostrum/milk (3 L in both, 8:00-9:00 am and 2:00-3:00 pm). Starting from day 7, the calves were provided with water. After day 15, all calves in the two assigned treatment groups were given ad libitum access to a nutritional supplement developed at the FESC. Since maternal milk provides the basic nutritional requirements only during the first two to three weeks of life, this supplement was formulated to contain 19 % crude protein and 2.8 Mcal of metabolizable energy, following the NRC recommendations (2001). The supplement consists of a mixture of alfalfa, rolled corn, soybean meal, and minerals. All animals had equal access to the same resources, including water, colostrum, and milk.

Experimental design and sample collection

The animals were randomly divided into two groups (n = 4 per group): one with the administration of BCP for 30 days, and one without BCP administration as a control. In addition, to reaffirm the dosage and the 30 days administration period of probiotic supplementation, schemes previously reported were considered (Cai *et al.*, 2024; Wu *et al.*, 2021; Guo *et al.*, 2022; Varada *et al.*, 2022; Ruvalcaba-Gómez *et al.*, 2023).

The BCP was administered to calves in liquid form at a dose of 4×10^{11} CFU/day, stored in 15 mL tubes at 4 °C, during their morning feeding. It was mixed with 500 mL of milk in 2 L bottles. Once the BCP was administered, the calves received the rest of the milk. The calves in the control group were fed milk without any additives. In the afternoon, all calves from the two treatment groups were given plain milk without any additives.

Blood samples were collected from each calf in both treatment groups on the day of birth (day 0) and on day 30. Between 5 and 20 mL of blood were drawn directly from the jugular vein using a syringe and an 18G needle. These samples were then transferred into 10 mL vacuum tubes without anticoagulant (Smith and Sherman, 2009b) and transported at 4 °C to the laboratory. Similarly, fecal samples of approximately 50 g were obtained from each calf in both treatment groups on the day of birth and day 30. These samples were collected directly from the rectum using sterile gloves and placed in sterile containers. Afterwards, they were transported at 4 °C to the laboratory and stored at -20 °C until analysis (Taylor *et al.*, 2015). Both procedures were performed by trained personnel to ensure the safety and welfare of the calves.

Evaluation of growth rate and incidence of diarrhea

From day 15 until the end of the trial, the total feed intake for the two treatment groups was estimated by recording both the amount offered and the amount not consumed. This included daily voluntary feed intake of the supplement (Smith and Sherman, 2009a) and daily milk intake (Heinrichs, 1993), ensuring an intake of between 4 and 6 L of milk and 0.1 to 0.6 kg of supplement per day, in accordance with NRC (2001) recommendations for calves. Daily weight gain was calculated by weighing the animals on days 1, 15, and 30 using a Truper scale, model BAS-200PLA®. Average daily intake and average daily weight gain were then used to calculate the feed conversion ratio (FCR) using the following equation (FAO, 2011):

$$FCR = \frac{\text{average daily intake (kg/d)}}{\text{average daily weight gain (kg/d)}}$$

Observations of fecal consistency in individual pens were assessed daily by a trained individual, as described by Osorio *et al.* (2012). The fecal scoring system classifies consistency as follows: 1 for solid, 2 for soft, 3 for loose, and 4 for liquid. In addition, the incidence of diarrhea was calculated using the formula described by Wu *et al.* (2021), as follows:

$$\text{Incidence of diarrhea (\%)} = \frac{\text{number of calves with diarrhea} \times \text{diarrhea days}}{\text{total calves} \times \text{experimental days}} \times 100$$

Analysis of IgG in serum by ELISA

Blood collected from the jugular vein was centrifuged at 3500 x g for 20 minutes. The serum was collected in 1.5 mL microtubes and frozen at -70 °C until further processing. IgG serum concentration was determined by a commercial Bovine IgG ELISA kit (ab-205078 Abcam, Cambridge, UK) at the Laboratorio RuMeN, FESC-UNAM. Samples were diluted 1:400000, and a sandwich ELISA assay was performed according to the manufacturer's instructions.

Bacterial quantification by qPCR

Fecal DNA extraction was performed with the RBB+C method (Yu and Morrison, 2004) using zirconium beads and lysis buffer for cell disruption, followed by precipitation of nucleic acid using 10 M ammonium acetate, isopropanol, and 70 % ethanol. Subsequently, protein purification and elimination were carried out using columns from the QIAamp

DNA Mini Kit (QIAGEN, Aarhus, Denmark). The DNA samples were quantified using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific Inc. Wilmington, DE) and its purity was evaluated by the 260/280 ratio. DNA concentration and purity were assessed using a Nanodrop spectrophotometer and DNA integrity was confirmed by electrophoresis on a 1% agarose gel. The qPCR reaction was performed using the StepOne Real-Time PCR System (Applied Biosystems). A total of 20 ng of DNA was used for the qPCR analysis and the primers used are listed in Table 1. Reactions were prepared using 5 µL FastStart SYBR Green Master 2x (Roche), 1 mM MgCl₂, 0.25 µM of each primer to a final volume of 10 µL. No-template controls were used as negative controls. The amplification conditions were: 95 °C for 10 min (initial denaturation), followed by 40 cycles of 95 °C for 10 s (denaturation), 56 °C for 15 s (annealing) and 72 °C for 15 s (elongation). The melting curve was obtained starting at 56 °C for 1 min, with temperature increments of 0.3 °C / s until reaching 95 °C. The melt curves were performed to ensure purity of the amplified product, which was confirmed by a single peak after 80 °C and efficiency above 90 % was ensured for each pair of primers. All samples were analyzed in duplicate and the 2^{-ΔΔCt} method was used to determine relative quantification (Livak and Schmittgen, 2001) for phylum Firmicutes and Bacteroidetes, which compares the differences in threshold (Ct) values between the problem sample and an internal reference gene (Universal primers), also normalizing against a control group (day of birth). For *Enterobacteriaceae* and *Lactobacillus* the relative abundance was determined using by the 2^{-ΔCt} method (Livak and Schmittgen, 2001). Changes in relative abundance were considered statistically significant (*p* < 0.05) and were interpreted with caution due to the inherent variability of microbial populations.

Statistical Analysis

The normal distribution of the data was assessed using the Shapiro-Wilk test. Subsequently, analysis of variance (ANOVA) was performed to evaluate statistical significance using a randomized model with SAS PROC GLM, applying a significance threshold of *p* < 0.05. IgG concentrations obtained by ELISA and relative bacterial concentrations were analyzed using a randomized model, considering treatment and sam-

Tabla 1. Primers utilizados en este estudio.

Table 1. Primers used in this study.

Target	Primer Sequence	Annealing T°	References
Universal	F: AAACCTCAAAGAATTGACGG R: CTCACRRCACGAGCTGAC	56 °C	Bachetti De Gregoris <i>et al.</i> (2011)
Firmicutes	F: TGAAACTYAAAGGAATTGACG R: ACCATGCACCACCTGTC	56 °C	Bachetti De Gregoris <i>et al.</i> (2011)
Bacteroidetes	F: CRAACAGGATTAGATACCTT R: GGTAAGGTTCTCGCGTAT	56 °C	Bachetti De Gregoris <i>et al.</i> (2011)
Lactobacillus	F: AGCAGTAGGGAATCTTCCA R: CACCGCTACACATGGAG	58 °C	Rinttila <i>et al.</i> (2004)
Enterobacteriaceae	F: ATGTTACAACCAAAGCGTACA R: TTACCYTGACGCTTAACATGC	60 °C	Takahashi <i>et al.</i> (2017)

pling time as factors. The analysis was performed using the SAS GLM procedure (version 9.3; SAS Institute Inc., Cary, NC, USA), and significant differences were defined as $p < 0.05$. No corrections were applied for multiple comparisons due to the exploratory nature of the study and the small sample size.

RESULTS

Growth Rate and Incidence of Diarrhea

The effect of a 30 days administration of BCP on weight gain, incidence of diarrhea, total feed intake, and feed conversion ratio are shown in Table 2. There were no significant differences in total feed intake among treatments. However, there were noted differences in daily weight gain between the BCP group (490 g) and the control group (310 g) ($p < 0.05$). Feed conversion ratio was better for BCP treatment (12.23) than the control group (19.38). Moreover, the duration of diarrheal episodes was different between treatments; Calves supplemented with BCP exhibited 5-9 diarrhea days, whereas control calves had 9-18 diarrhea days. Therefore, the percentage of incidence of diarrhea was significantly lower in the BCP treatment group, with 24.4 %, than in the control group (47.8 %).

IgG in serum by ELISA

Immune response was evaluated through IgG quantification by ELISA. Serum samples were obtained on days 0 and 30 of the experiment. The average IgG concentration obtained for group BCP was 5.38 ± 0.55 mg/mL and 7.08 ± 0.60 mg/mL on days 0 and 30, respectively. While for the control group, it was 3.49 ± 0.60 mg/mL and 1.72 ± 0.60 mg/mL on days 0 and 30, respectively (Table 2). Serum IgG concentrations in calves on day 0 showed no significant differences between groups ($p > 0.05$), while on day 30 after BCP administration, it showed an increase ($p < 0.05$) compared to the serum IgG concentrations in the control group.

Tabla 2. Variables en terneros sin suplementación (control) y suplementados con un probiótico del Consorcio Bacteriano (BCP) durante 30 días.

Table 2. Variables in calves without supplementation (control) and supplemented with a Bacterial Consortium Probiotic (CBP) for 30 days.

Variables	Control (n=4)	BCP (n=4)
Birth Weight (kg)	37.27 ± 1.37	39.42 ± 1.19
Final Weight on Day 30 (kg)	46.67 ± 1.52^a	54.25 ± 1.31^b
Total Feed intake (kg/Day)	6.30 ± 0.01	6.33 ± 0.005
Growth Rate (kg/Day)	0.31 ± 0.03^a	0.49 ± 0.03^b
Feed Conversion Ratio (kg of feed)	19.38 ± 2.19^a	12.23 ± 1.01^b
Incidence of Diarrhea (%)	47.8 ± 6.76^a	24.4 ± 1.11^b
IgG in serum at Birth (mg/mL)	3.49 ± 0.60	5.38 ± 0.55
IgG in serum on Day 30 (mg/mL)	1.72 ± 0.60^a	7.08 ± 0.60^b

Results are expressed as mean $\pm$ SEM. Means with different letters in a row indicate significant differences ($p < 0.05$).

Bacterial Relative quantification by qPCR

The impact of BCP on the fecal microbiota of calves after 30 days of treatment was assessed by the relative quantification of the Firmicutes and Bacteroidetes phyla using real-time PCR. Bacterial DNA was obtained from the feces of calves, both at birth (day 0) and at the end of the experiment (day 30). The difference in Ct for each phylum (Firmicutes or Bacteroidetes) on day 30 was compared with the Ct obtained with universal primers, designed to amplify conserved bacterial genomic regions that represent the total number of bacteria present. Data were then normalized using the Ct corresponding to each phylum on day 0.

The results showed that, after 30 days of BCP administration, the Firmicutes phylum was not affected in the calves, with a 1.4 ± 0.4 -fold induction, but neither in the control group, with a 1.06 ± 0.14 -fold induction ($p > 0.05$). In contrast, the Bacteroidetes phylum showed an increase in abundance at day 30 in both groups (Figure 1). However, calves with BCP at day 30 showed the highest abundance, 8.77 ± 2 times with respect to the control calves, 3.9 ± 2 times ($p < 0.05$).

On the other hand, the relative abundance of *Enterobacteriaceae* in calves with the BCP was slightly higher than that of the calves of the control group, but not significantly different, 0.6 ± 0.2 and 0.07 ± 0.2 , respectively ($p > 0.05$). Regarding the relative abundance of *Lactobacillus*, similarly to the *Enterobacteriaceae*, it showed an increase in the group treated with the BCP, but with no statistical difference (0.27 ± 0.15 vs 0.05 ± 0.20) ($p > 0.05$). When the comparison was made only for day 30 between the control group and BCP, a higher proportion of *Enterobacteriaceae* was observed in calves receiving BCP (0.93 ± 0.23) compared to control group (0.007 ± 0.32) ($p < 0.05$). However, no changes were observed in the proportion of *Lactobacillus*, with the control group (0.11 ± 0.29) and the BCP group (0.53 ± 0.21) ($p > 0.05$).

DISCUSSION

Administering probiotics to enhance cattle health, typically using single-strain or combinations of strains from diverse origins, has shown positive results at many stages of cattle production, especially during the first days of calves' lives (Alawneh *et al.*, 2020; Fan *et al.*, 2021; Guo *et al.*, 2021; Wang *et al.*, 2023). In this work, we demonstrate that a probiotic from a bacterial consortium of the same origin as the recipient species is ideal for enhancing growth, digestive health, and the immune response of calves. Additionally, we show the impact of BCP on modulating microbiota, which could be directly related to the effects observed.

In terms of growth rate and incidence of diarrhea, the 30-days administration of BCP to calves, compared to the control group, did not affect total feed intake but improved daily weight gain and feed conversion ratio, while the incidence of diarrhea was lower. Similar results were observed in calves receiving probiotics during the first two to four weeks of age (Wu *et al.*, 2021), the first six weeks of age (Zhang *et al.*, 2019), and for an administration of four weeks (Guo *et al.*, 2021).

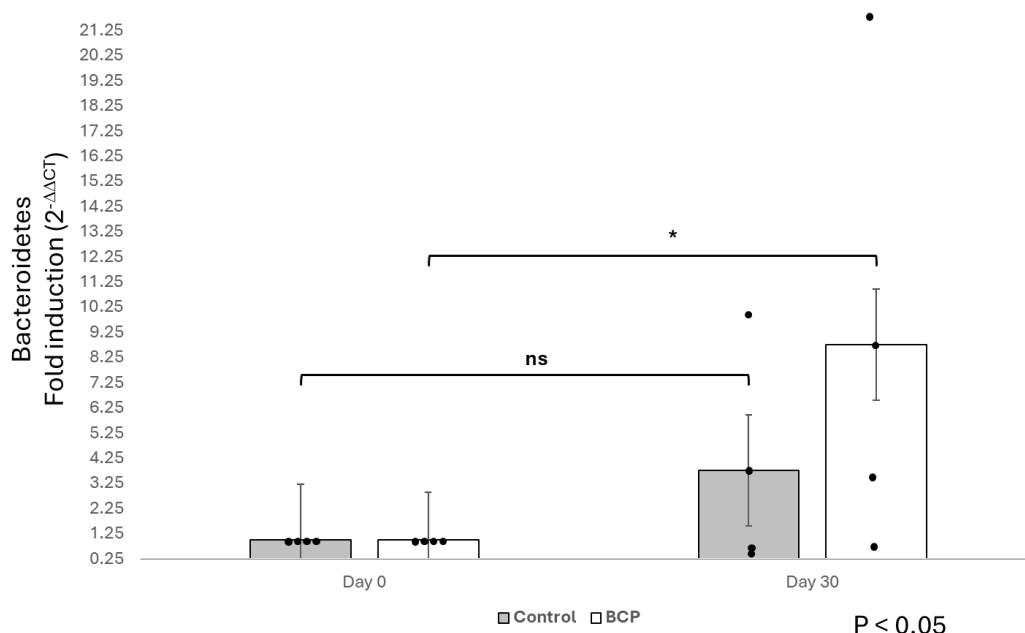


Figura 1. Expresión relativa del filo Bacteroidetes en las heces de terneros suplementados durante 30 días con CBP (blanco) y sin suplementación (gris). Los resultados se expresan como media $\pm$ SEM. * indican diferencias significativas, ns, no diferencias estadísticas ($n = 4$).

Figure 1. Relative expression of the phylum Bacteroidetes in the feces of calves supplemented 30 days with BCP (white) and without supplementation (grey). Results are expressed as the mean $\pm$ SEM. * indicate a significant difference, ns, nonsignificant differences ($n = 4$).

According to a review and meta-analysis of data from 67 studies conducted by Alawneh *et al.* (2020), the administration of probiotics to calves follows similar schedules across many protocols: they are typically administered once daily, primarily in the morning feed and immediately after birth. Although the reported duration of administration ranged from 2 to 90 days, the beneficial effects were mainly observed during the first days of life (0-30 days). Some variability observed between trials can be attributed to differences in management and environmental conditions. Although the majority of positive responses were reported when probiotics were administered during stressful periods, some conditions that were mentioned for achieving better results were similar to those in the present trial: the use of whole milk with a multistrain probiotic (Cangiano *et al.*, 2020) and liquid administration during the first four weeks of age (Alawneh *et al.*, 2020), to delivery directly into the abomasum (Wang *et al.*, 2023).

On the other hand, calves, due to their state of immunological immaturity, are prone to constant infections that endanger their development and life. Part of the protection they have at the beginning of their lives is through the passive immunity that colostrum confers on them (Godden, 2008). The absorption of immunoglobulins from colostrum reaches a peak in the first 4 h, while in serum at 24 h after birth, and then decreases until its endogenous production (Lopez *et al.*, 2020; Nielsen *et al.*, 2023). Also, after birth, calf concentrations of B lymphocytes are similar to adults at 20 days, and endogenous production of IgG begins between 36

h and 3 weeks (Barrington and Parish, 2001). However, it is known that the immune system is activated due to exposure to antigens. This coincides with the results obtained at day 0 for BCP and control group treatment, when the IgG concentrations were low, without significant differences between them ($p > 0.05$). Regarding this, we were able to determine that BCP and control calves, after 30 days, present IgG in serum, being the treated calves with BCP, having the highest concentration of all measurements. In the control group, IgG concentration decreased relative to baseline, which is to be expected in the absence of immune stimulation. In contrast, supplementation with A1D42 includes several Lactobacillales species, such as *Lacticaseibacillus casei*, which are capable of modulating mucin production.

This effect has been associated with the ability of certain lactobacilli to strengthen innate immunity by promoting intestinal barrier maturation and, simultaneously, modulate adaptive immunity by promoting increased antibody production (Raabis *et al.*, 2019; Guo *et al.*, 2022). In addition, an association has been observed between immune deficiency and the lack of composition in the intestinal microbiota (Raabis *et al.*, 2019). It is also important to mention that the differences in IgG levels at the beginning of the experiment between conditions, control or treated with BCP, could be associated with the time of birth of the calves, since, for technical reasons, the sample was taken early in the morning, but the calf could have been born during the night and consumed some colostrum during that time. Besides, in a study in which the concentration of IgG was determined

for 112 days in calves fed with colostrum of different quality, it was observed that serum IgG is relatively high in the first 21 days after birth, but the calves experience a significant decrease between days 28 to 63 and subsequently increase (Lopez *et al.*, 2020). Thus, our experiments show that there is a significant difference in the concentration of IgG between the calves treated with BCP and calves in the control group. This is indicative that the BCP treatment is generating a good immunological response. Moreover, the microbiota, the proportion of its phyla, and the presence of certain bacteria that comprise it have been widely related to a series of systemic responses.

In the digestive tract of ruminants, there is a diversity of microbial communities that allow them to digest the forages consumed and satisfy the energy and metabolic demands (Robles-Rodríguez *et al.*, 2023). During the early stages, the gut microbiome starts to colonize, and they are sequentially established before weaning (Kim *et al.*, 2021). Bacteroidetes, Firmicutes, and Proteobacteria are the major phyla found in cows regardless of age (Malmuthuge and Guan, 2016; Gomez *et al.*, 2017; Robles Rodríguez *et al.*, 2022). It has been demonstrated that Bacteroidetes are known to play a crucial role in the degradation of starch, fiber, dietary protein, and absorption of amino acids and peptides in the intestine (Malmuthuge *et al.*, 2015), and Firmicutes are common gut microbes that break down complex carbohydrates that can't be digested by the host's enzymes (Choudhury *et al.*, 2021). An increase in phyla Firmicutes and a decrease in Bacteroidetes abundances are associated with imbalances in the gut microbial ecology (gastrointestinal tract dysbiosis condition), and some of these dysbiosis are linked to diseases like diarrhea in calves (Fan *et al.*, 2021).

The increased relative expression of Bacteroidetes in the feces of calves treated with BCP for 30 days may explain the improved outcomes observed in final weight, growth rate, and feed conversion ratio, as well as the reduced incidence of diarrhea (Table 2). These benefits are likely attributable to the positive effects of this phylum on nutrient digestion, absorption, and the balance of gut microbiota, as evidenced by the shorter duration of diarrhea in the BCP group, lasting 4–8 days compared to 9–18 days in the control group. Also, it was observed that 30 days after administration of the BCP, which is *Lactobacillus*-enriched (Rodríguez-González *et al.*, 2022a), it affected the total proportion of *Bacteroidetes* in the feces ($p < 0.05$). However, we did not observe an effect on the proportion of Firmicutes. These results are consistent with an increase in the relative abundance of Bacteroidetes in buffalo calves supplemented with *Limosilactobacillus reuteri* BF-E7 and *Ligilactobacillus salivarius* BF-17 for 30 days (Fernández-Ciganda *et al.*, 2022; Varada *et al.*, 2022), but contrast with no effect of the administration of a probiotic on Bacteroidetes at 60 days of age of calves (Ruvalcaba-Gomez *et al.*, 2023).

In addition, it has been shown that *Lactobacillus* plays a crucial role in maintaining intestinal health, contributing to a lower pH in the intestinal tract and inhibiting the growth of harmful pathogens (Li *et al.*, 2023). In this work, the relative

abundance of *Enterobacteriaceae* and *Lactobacillus* in calves supplemented with the BCP at day 30 showed no differences ($p > 0.05$), similar to that reported by Ruvalcaba-Gómez *et al.* (2023). Therefore, although the microbiota plays a crucial role in regulating the development of the digestive tract and immune function in mammals, its species composition and abundance typically exhibit dynamic changes. Then, the increased proportion of Bacteroidetes observed in supplemented calves could favor the production of short-chain fatty acids, such as butyrate, which modulate epithelial maturation, reduce local inflammation, and promote immune cell differentiation (Malmuthuge *et al.*, 2015; Cai *et al.*, 2024). These mechanisms offer a possible explanation for the shorter duration of diarrheal episodes and the increased serum IgG concentration in supplemented animals. In addition to probiotic administration, these changes are influenced by various factors, such as diet, antibiotic therapy, and the presence of pathogenic microorganisms (Cai *et al.*, 2024). Stability, recovery, and resilience are essential features of a healthy microbiota that help prevent dysbiosis.

Although probiotics can promote faster recovery of equilibrium after disturbances by enhancing resilience, resistance of a stable community, and functional redundancy to maintain similar functions, the complex interactions within the gut may explain the variable results observed across different studies (Safarchi, 2025).

CONCLUSIONS

This study demonstrates that the supplementation of a Bacterial Consortium Probiotic, of bovine origin, in newborn Holstein calves can significantly improve health outcomes and overall cattle production. The effect of 100 % of viable cells of A1D42 in calves, compared to the control, showed a day weight gain of over 50 % and a decrease in feed conversion ratio of approximately 38 % with no differences in feed intake. In addition, the incidence of diarrhea decreased by half in calves that received probiotics, along with an improvement in their immune response by increasing IgG levels. Finally, in the fecal microbiota, there was an increase in the relative abundance in the phyla Bacteroidetes, which has been related to the processing and absorption of nutrients. This study contributes to the understanding of the importance of fecal microbiota and its modulation for improving the quality of life of livestock and its respective applications.

In practical terms, these results indicate that a species-specific probiotic bacterial consortium could be incorporated as a preventive strategy in calf production systems during the first 30 days of life, a critical survival period. However, this study has limitations, including the small sample size and a lack of functional metagenomic analysis to identify specific metabolic pathways. Future research should include evaluating longer administration periods, different dosages, metagenomic or metabolomic analyses, and post-weaning effects.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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