

Evaluation of probiotic supplementation to enhance growth, immune response, intestinal function, and survival in extensively raised neonatal alpacas in the Peruvian Central-southern Andes

Walter Palomino-Guerrera^{a,*}, Wenfil Daisi Mariño Huaraca^{a,b}, Mijail Contreras Huamani^a, Pedro Coila^c, Natalia Verónica Taboada^d

^a Estación Experimental Agraria Canaán, Instituto Nacional de Innovación Agraria, 05000 Ayacucho, Peru

^b South American Camelid Laboratory. Escuela de Medicina Veterinaria. Universidad Nacional de San Cristóbal de Huamanga, 05000 Ayacucho, Peru

^c Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional del Altiplano, Puno 21001, Peru

^d Instituto Multidisciplinario de Salud, Tecnología y Desarrollo (IMSaTeD. UNSE-CONICET), Universidad Nacional de Santiago del Estero, CP 4206, Ruta Nacional No 9 Km 1125, S/N, Villa El Zanjón, Santiago del Estero, Argentina

ARTICLE INFO

Keywords:

Alpaca neonates
Probiotics
Growth rate
Immune response
Intestinal function

ABSTRACT

This study aimed to evaluate the effects of multi-strain probiotics on growth, immune response, intestinal function, and survival in neonatal alpacas in the Peruvian Central-southern Andes. A total of 96 neonatal alpacas were selected, with an average weight of 7.89 ± 0.93 kg, regardless of breed (Huacaya and Suri). The study employed a completely randomized design. The neonates were randomly assigned to three groups ($n = 32$): control (T1), Probiolyte® WS (T2), and Polimeve Solúve (T3). Probiotics were administered orally on days 1, 7, 14, 21, and 30 of age. Body weight gain and size were recorded on days 1, 15, 30, 60, and 90; immune response was assessed on 30 and 90 days; and intestinal functional status was evaluated on day 90. The neonates supplemented with Polimeve exhibited greater thoracic circumference after 60 days of age, and increased height at the withers and rump at 15 days ($p < 0.05$). At 90 days, the Polimeve group had a higher proportion of basophils, while the Probiolyte and control groups showed higher proportions of monocytes ($p < 0.05$). IgG concentrations at 30 and 90 days did not differ significantly among groups. Probiotic supplementation did not affect ileal villus length; however, it influenced the depth of jejunal and cecal crypts ($p < 0.05$). Morbidity and mortality rates did not differ significantly between groups. In conclusion, probiotics did not significantly influence growth, immune response, or intestinal function; however, Polimeve may improve weight gain in neonates, while Probiolyte can increase IgG concentrations during the first 30 days of life.

1. Introduction

Approximately 87% of the global alpaca population (4491,671 head) is concentrated in Peru, primarily in the departments of Puno (2030,675 alpacas), Cusco (677,810 alpacas), Arequipa (470,840 alpacas), and Ayacucho (295,856 alpacas) (MIDAGRI, 2025). Alpacas are predominantly raised in high-Andean ecosystems under extensive production systems and play a crucial role in the cultural, social, and economic sustainability of rural communities (Mallma et al., 2021). In recent years, alpaca have gained increasing recognition as a valuable zoogenetic resource, and their population has grown significantly (Gómez-Quispe et al., 2022; Zarrin et al., 2020). The species exhibits

remarkable adaptations to the harsh environmental conditions found above 3800 m above sea level, including efficient forage utilization, reduced soil degradation due to its footpads, and the presence of unique immunoglobulins with potential therapeutic applications (Mallma et al., 2021; MIDAGRI, 2019). In addition to their ecological adaptability, alpacas provide essential products such as meat, fiber, and leather, as well as by-products like manure (Zarrin et al., 2020).

Despite these advantages, neonatal alpacas are particularly vulnerable during the first weeks of life. Animal between 6 and 12 weeks of age, especially those with low birth weight, are highly susceptible to environmental stressors and colonization by enteric pathogens (Alawneh et al., 2020; Gómez-Quispe et al., 2022; Zarrin et al., 2020).

* Corresponding author.

E-mail address: mvpalomino92@gmail.com (W. Palomino-Guerrera).

<https://doi.org/10.1016/j.vas.2026.100660>

Available online 15 April 2026

2451-943X/© 2026 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

These factors compromise gut health, leading to diarrhea a potentially fatal condition due to excessive loss of electrolytes and water (Rojas et al., 2016). Diarrhea-related nutritional deficiencies contribute significantly to neonatal morbidity (23%) and mortality (7%), generating substantial economic losses for alpaca producers. Estimated losses reach approximately 23 soles per neonatal alpacas and at least 5500 soles annually, disproportionately affecting of small and medium-scale farmers (Caman, 2018; Hanco, 2018).

Several pathogens have been associated with enteric disorders in alpacas. Epidemiological studies have reported a high prevalence of *Eimeria* spp. (80%), followed by coronavirus (40%), *E. coli* (34%), rotavirus (32%), *Clostridium* spp. (22%), and *Cryptosporidium* spp. (20%) (Rojas et al., 2016). Additional bacterial taxa linked to intestinal disease include *Clostridium spiroforme*, *Blautia*, and *Bacteroides fragilis* (Zapata et al., 2024). In many production systems, antibiotics are frequently used to control diarrheal diseases (Lorenz et al., 2011); however, increasing concerns regarding antimicrobial residues and the emergence of antimicrobial resistance have raised important challenges for animal production and public health (Ali et al., 2024; Kelsey & Colpoys, 2018; Lee et al., 2021). Consequently, alternative strategies to maintain gut health and prevent enteric diseases in livestock are increasingly being explored. As a result, the use of antimicrobials has been banned in many countries (Lee et al., 2021).

Among these alternatives, probiotics have attracted considerable attention due to their capacity to modulate intestinal microbiota and improve host health. Probiotics are defined as live microorganisms, including bacteria and fungi, administered individually or as multi-strain formulations to confer health benefits to the host (Wang et al., 2019a). Their use is well established in humans and monogastric livestock species such as pigs and poultry (Guo et al., 2024; Konieczka et al., 2022; Plaza-Díaz et al., 2019). Meanwhile, their application in ruminants has only recently expanded (Ali et al., 2024; Taboada et al., 2022). The most commonly used probiotic strains in livestock production include *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Saccharomyces*, and *Streptococcus* (Guo et al., 2022; Konieczka et al., 2022; Piewngam et al., 2018; Wang et al., 2022a).

Growing evidence suggests that multi-strain probiotics (MSPs) may provide more consistent and synergistic effects than single-strain formulations (Kumar et al., 2025; Wang et al., 2022b). The reported benefits include improved weight gain and growth, as well as higher milk production of good quality (Lele et al., 2019; Wang et al., 2019b); enhanced stimulation of the immune system through increased production of IgG and IgM (Barba-Vidal et al., 2019; Konieczka et al., 2023); improved gastrointestinal health through the modulation of beneficial microbiota, as well as the prevention and correction of digestive disorders by promoting a protective microflora, and stimulation of goblet cells (Wang et al., 2022b). For optimal functionality, probiotic strains must tolerate gastrointestinal conditions, including acidic pH, digestive enzymes, and bile salts, while also exhibiting antagonistic activity against enteric pathogens (Plaza-Díaz et al., 2018; 2019).

Different probiotic formulations have demonstrated beneficial effects in livestock. *Bacillus*-based probiotics have been associated with improvements in animal performance and gut health (Piewngam et al., 2018; Varada et al., 2022). Similarly, *Lactobacillus* and *Bifidobacterium* species have been shown to enhance immune responses increasing cytokines such as IL-2, IL-4, IL-6, and IL-10 and immunoglobulins IgG, IgE, and reduced tumor necrosis factor-alpha (TNF- α) (Kumar et al., 2022). *Saccharomyces cerevisiae*-based probiotics contribute to maintaining intestinal barrier integrity (Ali et al., 2024). Nevertheless, the effectiveness of probiotics in vivo may be limited by factors such as environmental complexity, rapid gastrointestinal transit, and reduced intestinal colonization, which can affect their bioavailability and persistence in the host (Wang et al., 2021).

Despite growing evidence supporting the use of probiotic supplementation in various ruminants (Ali et al., 2024; Konieczka et al., 2023; Singh et al., 2021; Varada et al., 2022), there is insufficient information

on the effect of probiotics in neonatal alpacas. Given the beneficial properties of probiotics, it would be important to incorporate them into the feeding programs for neonatal alpacas as a preventive strategy to improve health indicators, since in the first days of life they are prone to contracting diseases such as clostridiosis and coccidiosis, which are the leading causes of mortality. Therefore, the objective of the present study was to evaluate the effect of MSPs on growth, survival, immune response, and intestinal functional status in neonatal alpacas raised under extensive systems in the Andean region of Central-southern Peru.

2. Materials y methods

2.1. Animal ethics statement

This experiment was approved by the Research Ethics Committee of the Vice-Rectorate for Research at the Universidad Nacional de San Cristóbal de Huamanga, Ayacucho, Peru (No. 003-2025/CEI-VRI-UNSCH).

2.2. Location

The study was led in the Chuschi district, Ayacucho, Peru (Fig. 1), between March and July 2024. The area is characterized by a cold, dry climate, with a distinct rainy season (December to March) and dry season (May to October). Average temperatures range from 0.1 °C in July to 28.7 °C in November, with monthly precipitation between 5.5 and 154.7 mm (SENAMHI, 2018). The ecosystem features gentle slopes, small wetland areas, and extensive grasslands dominated by native vegetation including *Phoaceae*, *Ciperaceae*, *Rosaceae*, *Asteraceae*, and *Fabaceae* (Trillo et al., 2021).

2.3. Experimental design

The study employed a completely randomized design. The Ninety-six (96) experimental units were randomly assigned to one of three groups of $n = 32$ neonates each. The groups consisted of two probiotic supplementation treatments and a control. No treatment was administered to the control group (T1), while the supplemented groups received either Probiolyte® WS (Agrovet Market Animal Health, Peru) (T2) (Table 1) or Polimeve Solúve® (Imeve Laboratory, Brazil) (T3) (Table 2), both composed of quantified colony-forming units (CFUs). The activation of the product's additives was verified in accordance with the manufacturer's instructions.

2.4. Animals and supplementation

Neonatal alpacas with an average live weight 7.89 ± 0.92 kg were included, both males and females, regardless of coat color or breed (Huacaya and Suri). With between 1 and 3 births per day in the herds, the 96 experimental units were completed within one month. All neonates were confirmed to have consumed colostrum before being randomly allocated to one of the three groups and monitored for 90 days.

Probiotic products were administered orally at 1, 7, 14, 21, and 30 days of age: 10 g (1×10^7 CFU/g) of Probiolyte® WS and 5 g (1.85×10^9 CFU/g) of Polimeve Solúve®, according to the assigned treatment. The dose was determined based on the manufacturer's instructions and previous studies by Hanco (2018); Wang et al. (2022) y Konieczka et al. (2023). The product was dissolved in 15 mL of boiled non-potable water cooled to 38–40 °C and administered orally using a 30 mL dosing syringe equipped with a cannula. Doses were given between 6:00 and 8:00 a.m., while the herds were still in their resting pen. Neonates were either allowed to graze freely with the herd or kept in fenced native pastures with their dams for approximately 10 days before being released to join the herd. The alpacas access to water has been thru local springs and rivers. It is worth noting that during the study, two neonates from the

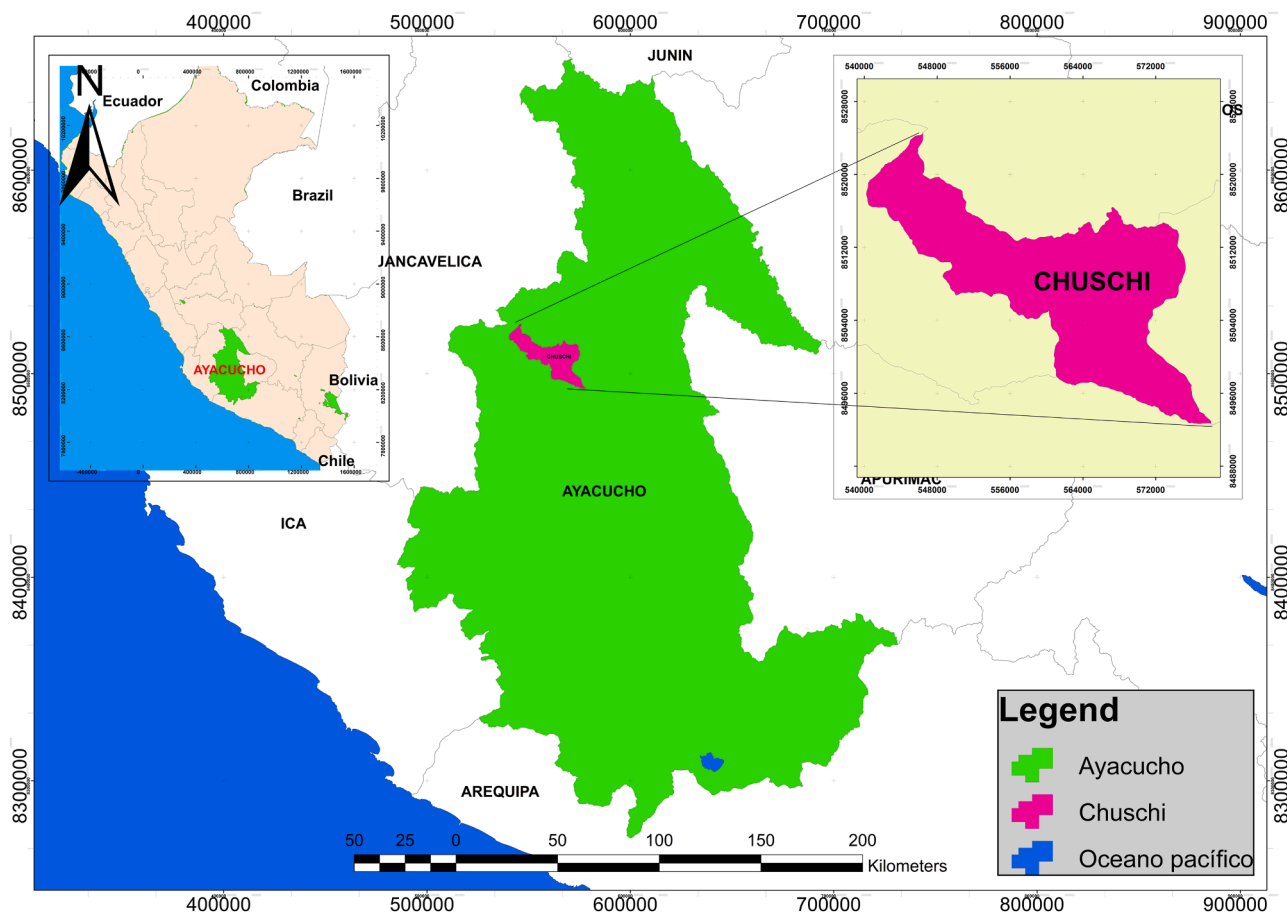


Fig. 1. Geographic location of the Chuschi district, Ayacucho department, Peru.

Polimeve group were preyed upon by predators (foxes), reducing the final number in that group to 30 animals.

2.5. Sampling and laboratory analysis

2.5.1. Body weight and growth

At 1, 14, 30, 60, and 90 days of age, five zoometric variables were recorded (Table 3). The variables evaluated withers height (WH), rump height (RH), body length (BL), thoracic girth (TG), and body weight gain (BW), to assess body size. BW was measured using a portable scale, and zoometric measurements were taken using a millimetric measuring tape and a wooden Vernier-type zoometric stick (Ablondi et al., 2023; Espinoza, 2018). Based on the collected data, final body weight (BW; kg), average daily gain (ADG; kg/d), and percentage weight gain (PWG) were calculated (Lee et al., 2024; Wang et al., 2022a).

2.5.2. Immune response

Blood samples were collected from 7 randomly selected neonates per treatment group at 30 and 90 days of age. Blood was drawn from the jugular vein into 10 mL tubes without additives and 6 mL tubes with EDTA, during the morning before the animals went out to graze. Samples were refrigerated for transport to the laboratory. The additive-free tubes were centrifuged at $3500 \times g$ for 15 min (Wang et al., 2022a), and the serum was aliquoted into 1.5 mL Eppendorf tubes and stored at -20°C until analysis. IgG concentration was determined using a radial immunodiffusion kit (Triple J Farms, USA) following the manufacturer's instructions. For leukocyte counts, EDTA blood samples were used. A $0.7 \mu\text{L}$ aliquot of blood was used to prepare two smears on a slide with the aid of another slide at a 30° angle. After air drying for 5 min, the slides were stained with Wright stain for 1 min, treated with buffer (distilled

water) for 10 min, rinsed gently with tap water, and air-dried (Wang et al., 2021). The two stained smears were examined under a microscope (AmScope, China) with immersion oil at $100 \times$ magnification, where a total of 100 leukocyte cells (neutrophils, basophils, eosinophils, lymphocytes, and monocytes) were quantified and calculated in percentage values (Salgado-Schüler, 2017).

2.5.3. Functional state of the intestine

Sample collection followed the guidelines of Páucar et al. (2017). At 90 days of age, following an 8-hour fast, two neonates per treatment group were sedated and euthanized (Parasuraman and Christopher, 2022). It should be noted that social and cultural factors among producers have influenced the limited availability of samples for sacrifice. Four intestinal segments (duodenum, jejunum, ileum, and cecum) were carefully dissected and 4 cm sections from each were collected. Using forceps, the samples were rinsed 5–10 times in saline solution to remove mucosal impurities that could accelerate villi autolysis. Clean samples were preserved in 10% formalin and stored at 4°C until analysis. Histological sections were prepared using an automatic microtome (YIDI model YD-355AT, China). Ileal villi height, crypt depth, muscle layer thickness, and mucosal health status were assessed under a light microscope (Leica ICC50W, Germany) with $4 \times$ magnification and a digital camera (Leica DM500, Germany) (Fig. 2).

2.5.4. Survival rate

The presence of diarrhea was recorded from the beginning of the experiment (first probiotic dose at day 1 of age) until 90 days of age to determine morbidity rates. Mortality due to digestive tract infections was also recorded to determine the mortality rate.

Table 1
Composition per 1 g of PMC Probiolyte® WS ¹.

Components	Quantity	Unit
Bacterial strains		
<i>Lactobacillus plantarum</i>	5.916×10 ³	CFU/g
<i>Lactobacillus acidophilus</i>	11.834×10 ³	CFU/g
<i>Lactobacillus rhamnosus</i>	5.917×10 ³	CFU/g
<i>Bacillus subtilis</i>	473.373×10 ³	CFU/g
<i>Bacillus licheniformis</i>	355.030×10 ³	CFU/g
<i>Bifidobacterium longum</i>	3.55×10 ³	CFU/g
<i>Bifidobacterium bifidum</i>	3.55×10 ³	CFU/g
<i>Streptococcus thermophilus</i>	3.55×10 ³	CFU/g
<i>Saccharomyces cerevisiae</i>	47.337×10 ³	CFU/g
<i>Enterococcus faecium</i>	3.55×10 ³	CFU/g
<i>Aspergillus oryzae</i>	1.183×10 ³	CFU/g
<i>Bacillus coagulans</i>	85.207×10 ³	CFU/g
Total	1 × 10 ⁶	CFU/g
Vitamins		
Retinol Palmitate (Vitamin A)	10,000.00	IU
Cholecalciferol (Vitamin D3)	2500.00	IU
DL-Alfa Tocopherol Acetate (Vitamin E)	5.00	mg
Menadione (as Menadione sodium bisulfite (Vitamin K3)	4.50	mg
Thiamine (as Thiamine hydrochloride) (Vitamin B1)	1.10	mg
Riboflavin (as Riboflavin 5-phosphate sodium dihydrate) (Vitamin B2)	1.50	mg
Nicotinamide (Vitamin B3)	10.00	mg
Calcium Pantothenate (Vitamin B5)	5.50	mg
Pyridoxine (as Pyridoxine hydrochloride) (Vitamin B6)	0.75	mg
Biotin (Vitamin B7)	0.40	mg
Folid Acid (Vitamin B9)	0.20	mg
Cyanocobalamin (Vitamin B12)	0.01	mg
Sodium Pangamate (Vitamin B15)	0.20	mg
Inositol (Vitamin BH)	0.50	mg
Ascorbic Acid (as Sodium Ascorbate) (Vitamin C)	1.00	mg
Amino Acids		
Aspartic Acid (L-Aspartic Acid)	1.91	mg
Glutamic Acid (as Monosodium Glutamate)	3.30	mg
Alanine (L-Alanine)	0.84	mg
Arginine (as L-Arginine Hydrochloride)	0.47	mg
Cysteine (as L-Cysteine Hydrochloride Monohydrate)	0.43	mg
Glycine	4.13	mg
Histidine (as L-Histidine Hydrochloride Monohydrate)	0.42	mg
Isoleucine (L-Isoleucine)	1.12	mg
Leucine (L-Leucine)	1.78	mg
Lysine (L-Lysine hydrochloride)	15.00	mg
Methionine (DL-Methionine)	10.00	mg
Proline (L-Proline)	1.01	mg
Serine (L-Serine)	3.30	mg
Tyrosine (L-Tyrosine)	0.40	mg
Threonine (L-Threonine)	1.26	mg
Tryptophan (L- Tryptophan)	0.32	mg
Valine (L-Valine)	1.07	mg
Minerals and Electrolytes		
Anhydrous Citric Acid	50.00	mg
Sodium Chloride	50.00	mg
Potassium Chloride	87.50	mg
Cobalt (as Cobalt Gluconate)	0.33	mg
Copper (as Copper-Edetate)	1.88	mg
Chromium (as Chromium-Methionine)	0.00	mg
Ferrun (as Ferrum-Edetate)	0.65	mg
Manganese (as Manganese-Edetate)	1.63	mg
Sodium Selenite (as Sodium Selenite Pentahydrate)	0.50	mg
Magnesium Sulfate Anhydrous	12.50	mg

Table 1 (continued)

Components	Quantity	Unit
Potassium Iodide	2.50	mg
Zinc (as Zinc-Edetate)	1.88	mg
Other		
Inulin	5.00	mg

¹ Composition of the Product commercial Probiolyte® WS of Agrovot Market Animal Health, Peru. ProBiolyte is a nutritional supplement that includes 15 vitamins, 18 amino acids, chelated organic minerals, and a blend of probiotics. Registration SENASA Peru A.16.07.N.015. CFU= colony-forming units; IU= international units.

Table 2
Composition per 1 g of PMC Polimeve Solúve®¹.

Components	Concentration	Unit
Bacterial strains		CFU/g
<i>Bacillus cereus</i> var. <i>Toyo</i>	4 × 10 ⁸	
<i>Bacillus subtilis</i>	4 × 10 ⁸	CFU/g
<i>Bifidobacterium bifidum</i>	3.5 × 10 ⁸	CFU/g
<i>Enterococcus faecium</i>	3.5 × 10 ⁸	CFU/g
<i>Lactobacillus acidophilus</i>	3.5 × 10 ⁸	CFU/g
Folic acid (mg)	1.00	mg
Calcium pantothenate (min)	10.00	mg
Niacin (min)	19.00	mg
Methionine	0.21	g
Lysine (min)	0.10	g
Chloride (min)	0.81	mg
Potassium (min)	0.80	mg
Sodium (min)	0.06	mg
Magnesium (min)	0.07	mg
Vitamin B1 (min)	0.01	mg
Vitamin B2 (min)	0.02	mg
Vitamin B6 (min)	0.02	mg
Vitamin B12 (min)	0.09	µg
Vitamin C (min)	0.01	mg
BHA	0.001	mg

¹ Composition of the Product commercial Polimeve Solúve® of Imeve Laboratory, Brazil. Polimeve Solúve® is a supplement with probiotics, vitamins, amino acids, and electrolytes. CFU= colony-forming units.

Table 3
Description of zoometric variables based on anatomical foundations in alpacas.

Zoometric variables	Anatomical points	References
Live Weight (LW)	Live weight of the animal in kg	(Ablondi et al., 2023)
Withers Height (WH)	Distance in cm from the ground to the top of the withers	(Ablondi et al., 2023)
Rump Height (RH)	Distance in cm from the ground to the top of the rump	(Ablondi et al., 2023)
Body Length (BL)	Distance in cm from the scapulohumeral joint to the tip of the rump	(Ablondi et al., 2023)
Thoracic Girth (TG)	Measurement in cm of the thoracic circumference, taken around the sternum and the seventh thoracic vertebra.	(Ablondi et al., 2023)

2.6. Statistical analysis

For the analysis of the information, descriptive and inferential statistics were used. Normal distribution and homogeneity of variances were verified. The least squares means (MLmeans) and standard error (SE) of body weight, body size, immune response, and intestinal function were calculated. The effect of the treatments was analyzed via analysis of variance (ANOVA) with a univariate linear model. Mean comparisons were performed using Tukey's multiple range test. Data that did not meet normality or homogeneity assumptions were analyzed using the non-parametric Kruskal-Wallis test, and means were compared with the Wilcoxon rank-sum test. Morbidity and mortality rates were

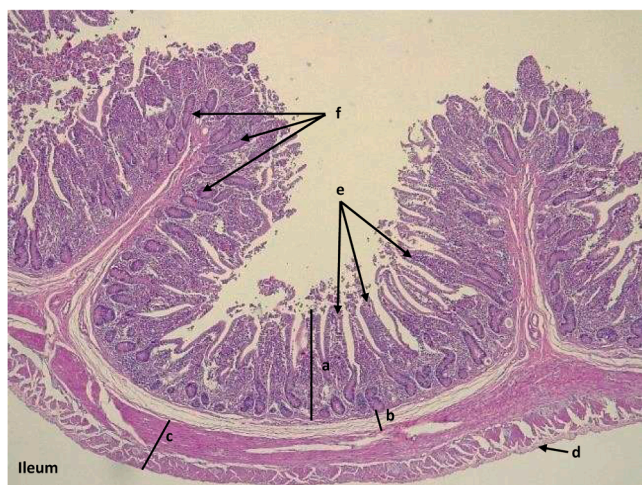


Fig. 2. Morphometric evaluation of intestinal structure. The intestinal mucosal layer (a), submucosal layer (b), muscular layer (c), serosal layer (d), intestinal villi (e), and intestinal crypts (f) are shown. Villi length was measured only in the ileum, as it was the only section that preserved its histological structure for proper evaluation. H-E staining, 4 × magnification.

determined using frequency tables, and associations with treatments were tested using the Chi-square test. Statistical significance was set at $P < 0.05$ or $P < 0.001$. Data were processed using R-statistical software version 4.4.0. The statistical model used was:

$$Y_{ijk} = \mu + \tau_i + \varepsilon_{ijk}$$

Where: Y_{ijk} = k observations in treatment i at age j , μ = mean, τ_i = effect of treatment or type of probiotic i ($i = 1, 2, 3$), ε_{ijk} = residual.

3. Results

3.1. Growth performance

PMC supplementation had no significant effect on weight gain ($p > 0.05$). The initial live weight of neonatal alpaca was 7.90 ± 1.03 , 7.69 ± 0.69 , and 8.11 ± 1.01 kg for the control, Probiolyte, and Polimeve groups, respectively (Table 4). The Polimeve-treated group tended to increase live weight (LW) and average daily gain (ADG) at 60 and 90 days compared to the other groups; similarly, they showed improvements in percentage weight gain (PWG) and specific growth rate (SGR), although these differences were not statistically significant ($p > 0.05$). The PMC effect on body size is shown in Table 5 and Fig. 3. A positive response was observed in the Polimeve-supplemented groups, which

Table 4
Effect of PMC supplementation on body weight gain in alpaca neonatal up to 90 days of age.

Variable	Control	Probiolyte	Polimeve	SE	p-value
LW (kg)					
1 d	7.90	7.69	8.11	0.09	0.190
15 d	10.39	9.84	10.34	0.14	0.193
30 d	11.84	11.70	11.99	0.17	0.800
60 d	15.89	15.66	16.10	0.24	0.766
90 d	19.15	18.73	19.80	0.29	0.151
ADG (kg/day)					
1–15 d	0.17	0.14	0.15	0.01	0.211
16–30 d	0.13	0.13	0.13	0.00	0.801
31–60 d	0.13	0.13	0.14	0.00	0.736
61–90 d	0.10	0.10	0.13	0.00	0.056
PWG (%)	1.38	1.44	1.49	0.03	0.374
SGR	0.12	0.12	0.13	0.00	0.196

LW: Live weight, ADG: Average daily gain, PWG: Percentage of weight gain, SGR: Specific growth rate, SE: Standard error of the mean.

Table 5
Effect of PMC supplementation on growth variables of alpaca neonatal up to 90 days of age.

Variable	Control	Probiolyte	Polimeve	SE	p-value
TG (cm)					
1 d	45.21	44.30	44.53	0.31	0.449
15 d	48.82 ^{ab}	48.30 ^b	50.00 ^a	0.28	0.010 *
30 d	51.97	51.09	52.38	0.30	0.200
60 d	59.16 ^{ab}	57.41 ^b	59.54 ^a	0.32	0.014 *
90 d	61.75 ^b	61.55 ^b	63.96 ^a	0.41	0.036 *
WH (cm)					
1 d	53.36	53.15	53.20	0.24	0.932
15 d	56.88 ^a	55.85 ^b	57.67 ^a	0.24	0.016 *
30 d	58.64	58.06	59.24	0.26	0.192
60 d	62.91	61.55	63.00	0.32	0.114
90 d	64.81	64.79	66.30	0.31	0.081
RH (cm)					
1 d	55.18	55.48	55.10	0.27	0.833
15 d	58.97 ^a	57.88 ^b	59.47 ^a	0.25	0.029 *
30 d	60.82	60.00	61.10	0.22	0.114
60 d	64.72	64.08	64.75	0.27	0.525
90 d	66.81	66.98	68.30	0.30	0.099
BL (cm)					
1 d	39.21	38.30	38.53	0.22	0.183
15 d	43.00	42.48	43.47	0.22	0.202
30 d	45.42	45.27	45.83	0.22	0.576
60 d	49.97	50.25	51.14	0.36	0.227
90 d	51.48	51.68	52.56	0.27	0.162

TG: Thoracic girth, WH: Withers height, RH: Rump height, BL: Body length, SE: Standard error of the mean. Superscript letters (^{a,b}) in rows indicate significant differences at $p \leq 0.05$ (*).

showed higher body size values. Polimeve-treated groups showed a significantly greater increase in thoracic girth after 15 days of age, as well as withers and rump height at 15 days, compared to the other groups ($p < 0.05$). However, body length did not differ significantly between groups ($p > 0.05$).

3.2. Immune response

At 90 days of age, the Polimeve group (9.00%) had a higher proportion of basophils (BA) compared to the other groups, while the Probiolyte (9.00%) and control (9.00%) groups had higher proportions of monocytes (MO) relative to Polimeve ($p \leq 0.05$). PMC supplementation had no significant effect on the proportion of neutrophils, eosinophils, or lymphocytes ($p > 0.05$). At 30 days of age, all three groups had a higher proportion of band neutrophils (BNE) than segmented neutrophils (SNE), and this ratio reversed at 90 days. IgG concentrations at both 30 and 90 days of age showed no significant differences among groups ($p > 0.05$) (Table 6).

3.3. Intestinal functional status

Groups supplemented with PMC had shorter ileal villi compared to the control group (Table 7; $p < 0.05$). PMC supplementation significantly affected crypt depth in the jejunum and cecum ($p < 0.05$). Histological signs of inflammatory processes were observed across all treatment groups, with the intestinal mucosa of neonates showing structural damage, including a discontinuous epithelial layer and autolytic changes (Fig. 4). Especially in the ileum portion of the neonates showed mild to moderate enteritis to severe acute diffuse catarrhal enteritis associated with *Coccidia* (Fig. 5 and Fig. 6).

3.4. Morbidity and mortality indices

No neonatal mortality was recorded during the experiment, and morbidity did not show a significant association among groups ($p > 0.05$), although the Polimeve and Probiolyte groups had a proportional increase of diarrhea cases (Table 8). It is worth noting that the diarrheas

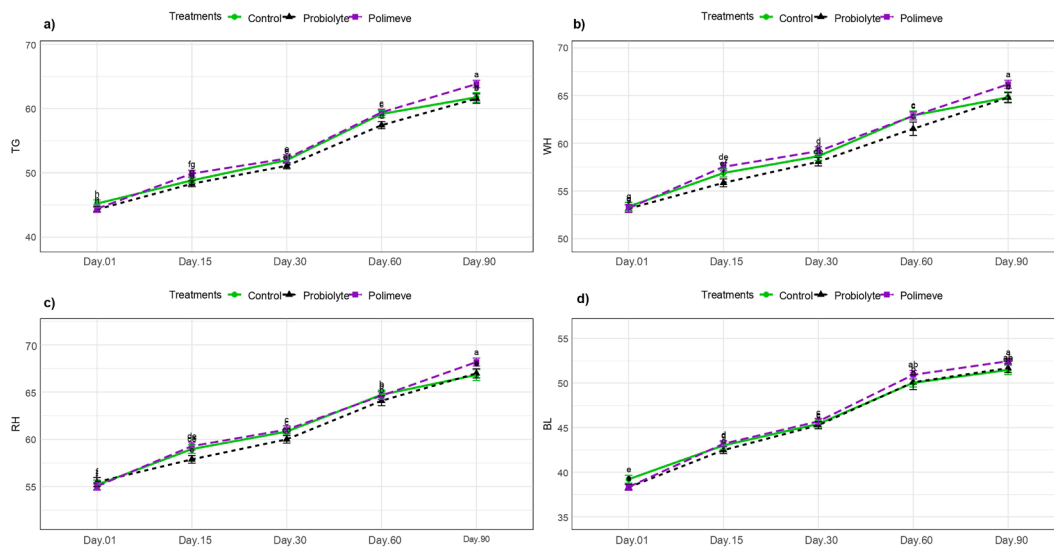


Fig. 3. Effect of PMC supplementation on body size performance up to 90 days of age. a) Thoracic Girth (TG), b) Withers Height (WH), c) Rump Height (RH), d) Body length (BL).

Table 6
Effect of PMC supplementation on immune response in alpaca neonatal up to 90 days of age.

Variable	Control	Probiolyte	Polimeve	SE	p-value
BNE (%)					
30 d	0.16	0.14	0.16	0.01	0.628
90 d	0.09	0.12	0.10	0.01	0.472
SNE (%)					
30 d	0.20	0.17	0.14	0.01	0.712
90 d	0.33	0.34	0.32	0.01	0.765
EO (%)					
30 d	0.06	0.10	0.08	0.01	0.149
90 d	0.03	0.03	0.05	0.00	0.223
BA (%)					
30 d	0.15	0.14	0.16	0.01	0.063
90 d	0.06 ^b	0.06 ^b	0.09 ^a	0.01	0.050*
LI (%)					
30 d	0.35	0.37	0.39	0.01	0.600
90 d	0.39	0.36	0.37	0.01	0.659
MO (%)					
30 d	0.08	0.08	0.08	0.01	0.459
90 d	0.09 ^a	0.09 ^a	0.06 ^b	0.01	0.023*
IgG (mg/dL)					
30 d	883.83	1162.10	689.04	124.68	0.313
90 d	2066.63	1479.78	1609.04	123.56	0.125

BNE: Band neutrophils, SNE: Segmented neutrophils, EO: Eosinophils, BA: Basophils, LI: Lymphocytes, MO: Monocytes, IgG: Immunoglobulin G, SE: Standard error of the mean. Superscript letters ^(a,b) in rows indicate significant differences at $p \leq 0.05$ (*).

observed in neonates were physiological in nature, resulting from probiotic use an expected response due to microbial shifts in the gastrointestinal tract. Additionally, this is a pilot study that demonstrates an alternative supplement to improve survival and resilience in neonates.

4. Discussion

4.1. Growth performance

In recent years, the use of PMC-based additives have significantly raised expectations among livestock producers' (Cholewińska et al., 2020). It is widely used to promote the development of a protective microflora at the intestinal barrier level, especially in neonates (Wang et al., 2022b). Studies have shown that PMCs can improve milk quality, increase weight gain, and feed efficiency (Adjei-Fremah et al., 2018;

Table 7
Effect of PMC supplementation on intestinal morphometry in alpaca neonatal up to 90 days of age.

Variable	Control	Probiolyte	Polimeve	SE	p-value
VL (µm)					
Ileum	367.70 ^a	311.50 ^{ab}	268.65 ^b	14.34	0.013 *
CD (µm)					
Duodenum	211.70	194.55	184.90	5.56	0.136
Jejunum	139.85 ^a	208.15 ^b	163.10 ^a	7.66	<0.001***
Ileum	219.00	196.20	194.45	6.01	0.180
Cecum	253.35 ^b	358.60 ^a	347.45 ^a	11.15	<0.001***
CM (µm)					
Duodenum	237.00	145.00	182.00	23.83	0.350
Jejunum	224.50	170.50	287.00	30.38	0.363
Ileum	144.00	187.00	171.00	20.74	0.789
Cecum	151.00	314.50	277.50	34.34	0.069
LM (µm)					
Duodenum	100.50	92.50	80.50	4.74	0.252
Jejunum	109.50	110.50	133.00	10.13	0.676
Ileum	107.00	132.50	79.00	16.43	0.519
Cecum	41.00	137.00	80.50	19.11	0.058

VL: villus length, CD: crypt depth, CM: circular muscle, LM: longitudinal muscle, SE: Standard error of the mean. Superscript letters ^(a,b) in rows indicate significant differences at $p \leq 0.001$ (***), $p \leq 0.05$ (*).

Hasunuma et al., 2011; Liu et al., 2021). In the early stages of life, animals go through critical periods for their survival, as their immune systems are not yet fully developed, making them entirely dependent on the maternal immunity they receive through colostrum. These physiological factors, combined with environmental ones, can seriously impair the normal growth of newborns (Liu et al., 2014).

In this study, PMC supplementation had no significant effect on daily weight gain (DWG) or final body weight in neonatal alpacas. No previous research was found on the effect of PMC supplementation on the growth of neonatal alpaca. In other species, such as guinea pigs (Valdizán et al., 2019), lambs (Esfokhi et al., 2024), and calves (Wang et al., 2022a), no significant effect on weight gain was reported. In contrast, favorable effects have been observed in suckling piglets (Konieczka et al., 2023). Differences between studies may be related to factors such as animal species, physiological and immunological status, age, probiotic concentration and dose, rearing conditions, diet composition, and route of administration (Hoseinifar et al., 2018; Lambo et al., 2021). Esfokhi et al. (2024) confirm that the viability and efficacy of probiotics are related to the specific strains used, the composition of the

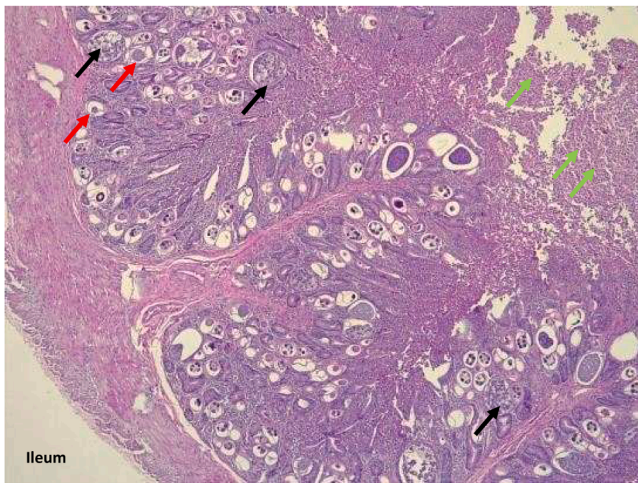


Fig. 4. Micrograph of the ileum of a young alpaca showing histological signs of acute inflammation. Catarrh and mucosal desquamation (green arrow), immature microgamete (black arrow), and mature schizont (red arrow). H-E stain, 4 × magnification.

gut microbiota, and the host's diet.

The use of PMC in this study had no significant effect on growth. No comparable studies were found in alpacas; however, Wang et al. (2022a) reported a negative effect on withers and hip height in neonatal calves, whereas Noori et al. (2016) found a positive response in piglets. Probiotics are capable of synthesizing amylase, cellulase, protease, improving digestive function and support growth in newborn calves (Cangiano et al., 2020). The bioavailability of minerals such as calcium, phosphorus, and magnesium contained in the supplement would also be influencing growth (Noori et al., 2016). In other studies, it was demonstrated that probiotics based on *Bacillus subtilis* and *Bacillus*

amyloliquefaciens can enhance the production of GH/IGF-1 because these bacteria stimulate the gene expression responsible for that hormone, which has a direct relationship with body growth (Du et al., 2018; Salehizadeh et al., 2019). Furthermore, variations in the animals' physical development are a result of the environmental conditions in which they are raised and the production model adopted (Heinrichs et al., 2007)

4.2. Immune response in neonates

The immune system in neonatal animals is underdeveloped. Studies on the use of PMC as a supplement in ruminants (Ali et al., 2024; Konieczka et al., 2023; Varada et al., 2022; Wang et al., 2022a) and pseudo-ruminants (Hancock, 2018) have shown improved innate immunity and enhanced preparation of adaptive (both humoral and cellular) immune responses (Ali et al., 2024; Varada et al., 2022). Immunity can be subdivided into innate and adaptive components. Innate immunity comprises neutrophils, monocytes, basophils, eosinophils, and natural killer cells (NKCs), and acts as the first line of defense against pathogenic microbial colonization (Rasmussen et al., 2009). Adaptive immunity is represented by T and B lymphocytes (Schenten and Medzhitov, 2011), with B cells being responsible for producing IgG, which function as antigen receptors (Ramos-Medina et al., 2012).

In this study, no significant effects were observed on the mean proportions of neutrophils, eosinophils, and lymphocytes, but there were effects on basophils and monocytes. Hancock (2018) reported that probiotics had no effect on neutrophils, but positively influenced monocytes (20.14%) and negatively affected lymphocytes (21.33%). This positive effect on monocytes would explain the content of the bacterial strains in the supplement, as Hancock (2018) used *Lactobacillus bulgaricus* and *Streptococcus thermophilus* compared to this study.

In apparently healthy 90-day-old neonatal alpaca, Velásquez (2023) reported neutrophil proportions ranging from 50.83 to 60.80%, lymphocytes from 38.00 to 43.50%, monocytes from 3.20 to 5.00%,

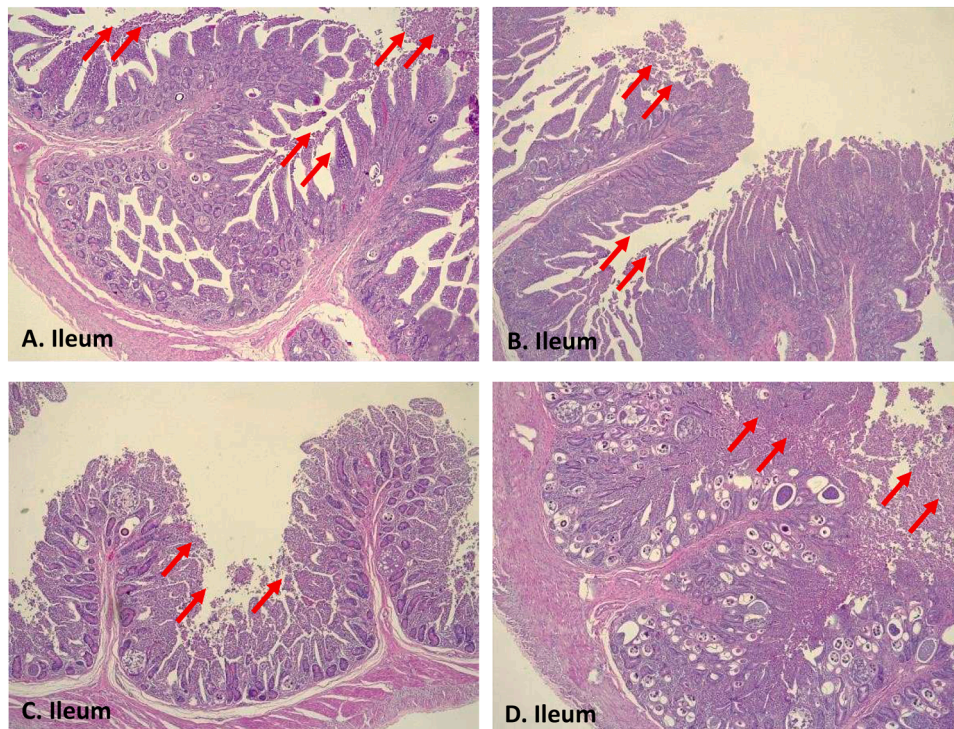


Fig. 5. Micrographs of the ileum in alpaca neonatal showing histological signs of mild, moderate, and severe inflammation according to treatment group. A) Ileum with moderate to severe catarrhal enteritis; B) Ileum with mild to moderate catarrhal enteritis; C) Ileum with moderate to severe catarrhal enteritis; D) Ileum with severe acute catarrhal enteritis.

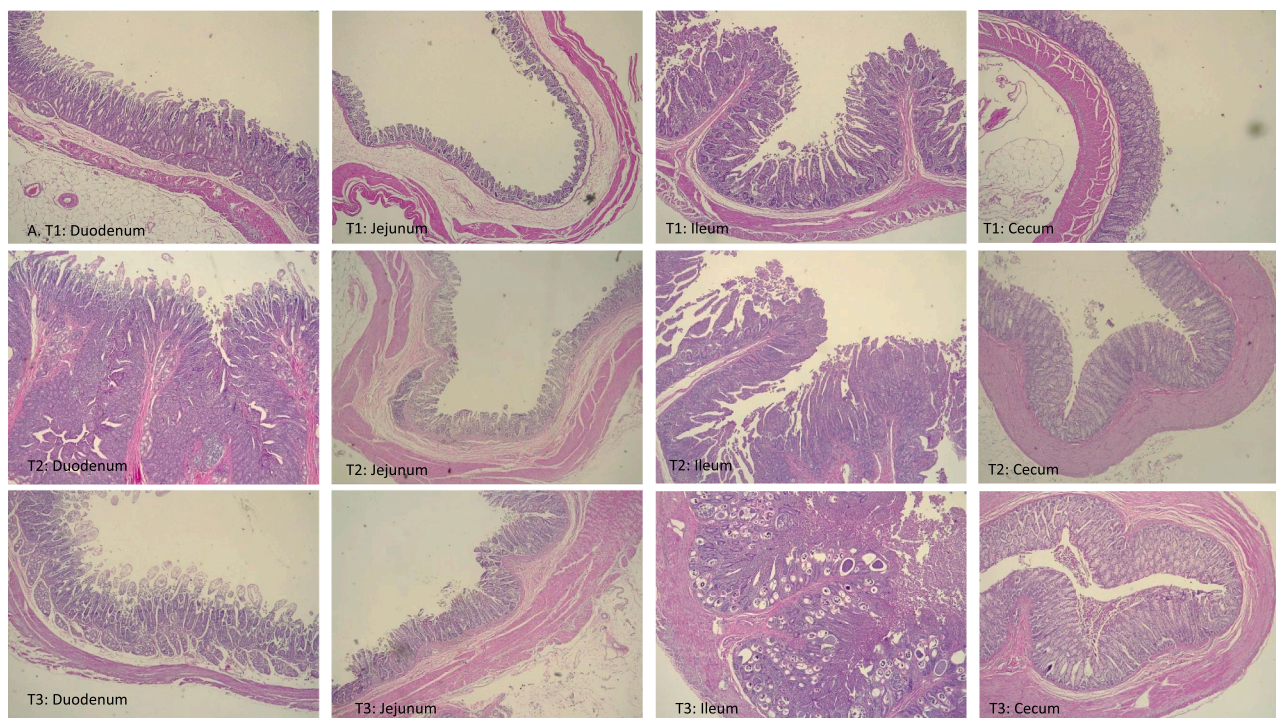


Fig. 6. Micrographs of intestinal health status in alpaca neonatal supplemented with PMC. The top panel shows images of alpacas from the control group. The middle panel shows images of alpacas supplemented with the probiotic Probiolyte. The bottom panel shows images of alpacas supplemented with the probiotic Polimeve. H&E staining, 4 × magnification.

Table 8

Effect of PMC supplementation on survival in alpaca neonatal up to 90 days of age.

Variables	Treatments	%	X ²	p-value
Morbidity	Control	3.03 (1/32)	1.047	0.592
	Probiolyte	9.09 (3/32)		
	Polimeve	6.67 (2/30)		
Mortality	Control	-	-	-
	Probiolyte	-		
	Polimeve	-		

eosinophils from 1.50 to 2.00%, and basophils from 0.17 to 0.33%. These values were higher than the proportion of neutrophils and lower than the proportion of monocytes, eosinophils, and basophils observed in the control group of this study. Sierra et al. (2010) found that *Lactobacillus salivarius*-based probiotics increased monocyte percentages, although this strain was not included in any of the PMC used in this study. In another study, Gutiérrez-Castro and Corredor-Matus (2017) found no effect of PMC on leukocyte parameters.

The effects of PMC supplementation on leukocyte concentrations in alpacas have not been well studied. Leukocytes are understood to be the first line of defense against pathogenic microorganisms (Rasmussen et al., 2009); however, they are influenced by environmental conditions, age, sex, nutritional status, and the animal's health status (Foster et al., 2009; Udeh et al., 2021). Lymphocyte levels tend to be higher in neonatal alpacas than in adult alpacas whereas the opposite trend is observed for eosinophils and neutrophils (Hengrave et al., 2005; Wagener et al., 2024). Similar patterns have been reported in calves (Mohri et al., 2007). Likewise, elevated lymphocyte and neutrophil levels during the first month of life may be attributed to increased cortisol concentrations, which peak during late gestation and parturition (Martín-Barrasa et al., 2023).

PMC supplementation had no significant effect on IgG concentration; however, at 30 days of age, the Probiolyte group showed a numerically

higher IgG level (1162.099 mg/dL) compared to the other groups. By contrast, at 90 days, the control group exhibited a higher IgG concentration (2066.63 mg/dL), followed by the Polimeve group (1609.04 mg/dL). Hanco (2018) reported lower IgG values (646.40 mg/dL and 664.70 mg/dL) using different probiotic doses. The higher IgG concentrations observed in the present study may be attributed to the specific doses used (Wang et al., 2022a), strain concentrations, strain diversity, and the presence of micronutrients and amino acids in the PMC formulations. Nevertheless, Velásquez (2023) demonstrated that amino acid supplementation had no effect on IgG levels in alpaca crias.

Other studies have shown that probiotics containing *Enterococcus*, *Lactobacillus*, and *Bifidobacterium* can activate the production of IgA, IgE, and IgG (Ali et al., 2024; Letnická et al., 2017; Sierra et al., 2010). To activate IgA, probiotics first activate dendritic cells, through interaction with Toll-like receptors, this process differentiate promotes the differentiation of B lymphocytes into IgA+B cells, thereby increase IgA production (Ali et al., 2024; Dogi et al., 2016). Parada et al. (2019) mentioned that probiotics allow the increase in the gene expression of proteins responsible for the synthesis of immune cells.

In alpacas, due to the epitheliochorial type of placentation, immunoglobulins cannot cross the placenta; for this reason, the immunity of newborns depends on the absorption of immunoglobulins and lymphocytes from the mother's colostrum (Pachari, 2008; Weaver et al., 2000). It has been demonstrated that by day 79, neonatal alpaca begins to independently produce adequate levels of IgG (Quispe, 2019), suggesting that before this age, their immunity depends entirely on maternal immunoglobulin transfer. Though, Maximiliano et al. (2018) found no association between serum IgG concentrations and mortality in calves due to enterotoxemia.

4.3. Functional status of the intestine

Epithelial cells play a crucial role in maintaining the intestinal ecosystem and integrity to preserve intestinal health (Ding et al., 2021). They also function as a selectively permeable barrier with the capacity

to absorb nutrients and provide protection against potential pathogens and allergens (Chen et al., 2015; Groschwitz and Hogan, 2009; Prudencio, 2005). Probiotics have been shown to exert a trophic effect on the barrier function of epithelial cells by increasing Peyer's patches (Raabis et al., 2019; Rhayat et al., 2019; Shini et al., 2020).

In this study, neonates supplemented with PMC showed shorter ileal villi compared to the control group. No similar reports were found in alpaca neonates; however, in guinea pigs (Puente et al., 2019), pigs (Konieczka et al., 2023), and chickens (Sefcová et al., 2023), probiotics have been shown to increase ileal villus height. The ileum is primarily involved in the reabsorption of electrolytes and water (Ross and Pawlina, 2013). Nutrient absorption is more efficient with longer villi due to increased surface area, whereas shorter villi are associated with poor intestinal health (Awad et al., 2009). The efficacy of probiotics on villus length is influenced by factors such as animal species, product dosage, animal age, probiotic strains, and their viability, persistence in the intestinal tract (Serrano, 2015), and external factors such as the prevalence of protozoa (Centro Experimental Canaán, INIA), since Sharma et al. (2015) have confirmed that infection with *Eimeria* spp. it causes atrophy and fusion of the villi.

In the Probiolyte and Polimeve groups, the crypts of the jejunum and cecum were deeper than in the control group. Similar studies in guinea pigs (Puente et al., 2019) and pigs (Konieczka et al., 2023) reported no effect of probiotics on crypt depth. It is suggested that shallower crypts are more efficient in nutrient absorption, whereas deeper crypts contain mostly immature cells due to active regeneration of the villi, thus reducing absorption efficiency (Wang et al., 2019a; Bogucka et al., 2019). The results could be attributed to environmental conditions (Torres-Rodríguez et al., 2007), since this study revealed the initial stage of *Eimeria* spp. infection, which would explain the active regeneration of the crypts. It is also important to note that these results may be influenced by the number of animals evaluated, since at the end of the study, only two animals per group underwent histological analysis.

Histological evaluation of the newborns treated with PMC revealed structures consistent with coccidia. The histopathological diagnosis of the ileum revealed acute catarrhal enteritis associated with coccidia, with few lymphocytes and epithelial cell desquamation. These results suggest that these probiotics may be effective only against bacterial pathogens, not protozoal ones (Maria, 2021). Furthermore, the occurrence of coccidiosis in alpaca newborns may be related to the duration of PMC supplementation, because in this study, the newborns received probiotics only on five occasions during the first month of life, which reduces the probiotic's efficacy, since daily administration of probiotics is recommended in the animal industry (Wang et al., 2022a; Konieczka et al., 2023).

Likewise, there seems to be a high prevalence of *Eimeria* spp. in breeding areas, which may be compromising intestinal health status, where the protozoan would take advantage of the susceptibility of the neonates. Damage to the intestinal mucosa may impair the mode of action of probiotics, as they primarily interact with epithelial cells, dendritic cells, macrophages, and intraepithelial lymphocytes (Kalita et al., 2021; Raabis et al., 2019).

4.4. Morbidity and mortality rates in neonates

In this study, PMC supplementation had no significant effect on morbidity or mortality rates in alpaca neonates. A higher morbidity rate was observed in PMC-treated groups (9.09 to 9.38%) compared to the control group (3.03%). The diarrhea observed in the treated neonates may be due significant changes in the gastrointestinal microbiota resulting from supplementation, which may involve anaerobic shifts and an increase in lactic acid bacteria, leading to dysbiosis in the intestinal tract (Gomez et al., 2022). Llamas and alpacas between 3 and 7 days of age are affected by diseases such as coccidiosis, clostridiosis, and colibacillosis in approximately 23% of cases, which is often associated with septicemia and ultimately leads to high mortality among newborns

(Foster et al., 2008; Rojas et al., 2016).

No mortality in neonates was reported in this study, while Hanco (2018), working in Puno, reported a mortality rate of 6.67% in neonates supplemented with probiotics. High mortality rates due to enterotoxemia (*Clostridium perfringens*) have been reported in alpaca neonates, particularly between 8 and 35 days of age (Wiedner, 2021). In this region, *Escherichia coli* has been identified as a primary cause of neonatal mortality (Centro Experimental Canaán, INIA). It is crucial that neonates receive colostrum within the first hours after birth and that it is effectively absorbed in the intestinal barrier. The absence of maternal antibodies can lead to death from colibacillosis (*E. coli*) by 3 or 4 days of age (Tsur et al., 1996).

As final considerations, the implementation of probiotics as a supplement in neonatal alpacas with the aim of improving growth and gastrointestinal health in alpaca neonates is an emerging strategy that warrants greater scientific attention, particularly given the promising results reported in other ruminants such as buffaloes (Varada et al., 2022), cattle (Wang et al., 2022a), and goats (Taboada et al., 2022).

More research is needed to better understand the relationship between probiotic bacterial strains and the functional integrity of the intestinal mucosa in alpacas. Several reports indicate that probiotics release various metabolites (bacteriocins, organic acids, amines, etc.), with bacteriocins being the main metabolite because they act at the level of the bacterial cytoplasmic membrane and alter the driving force of the bacteria (Kumar et al., 2013; Umu et al., 2017). Probiotics also act as a competitive exclusion factor, as they compete for receptor sites in the intestinal tract, leaving no space for pathogenic bacteria (Bermudez-Brito et al., 2012).

5. Conclusion

The use of probiotics in alpaca neonates could serve as an alternative supplementation and therapeutic strategy against digestive disorders. The administration of Polimeve can improve neonatal vigor, promoting faster weight gain and body growth. At 90 days of age, Polimeve was found to improve basophil concentration. No significant effect was observed on the IgG concentration; however, an increase in the average IgG levels was noted during the first 30 days with the use of Probiolyte. The supplemented animals showed shorter ileal villi compared to the control group, as well as greater crypt depth in the jejunum and cecum. Likewise, epithelial cell loss from the villi was observed in all three groups, indicating structural damage to the intestinal mucosa, likely associated with enteritis caused by *Coccidia*. This gastrointestinal issue in neonates may be related to the grazing area, which appears to be contaminated with protozoa of the *Coccidia* genus. The use of PMC showed no effect on neonatal survival. Among the limitations are the limited availability of samples and the lack of specialized laboratories nearby to process samples. It is worth noting that further studies are required on the use of probiotics in alpaca neonates, including the identification of potential probiotic microorganisms and the relationship between digestive physiology and the intestinal microbiota.

Funding

This research was funded by the project "Mejoramiento de los Servicios de Investigación y Transferencia de Tecnología en Ganadería Altoandina en 33 Distritos de los Departamentos de Apurímac, Arequipa, Ayacucho, Cusco, Huancavelica, Junín, Moquegua, Pasco, Puno y Tacna", CUI N° 2491159, of the Instituto Nacional de Innovación Agraria.

CRedit authorship contribution statement

Walter Palomino-Guerrera: Conceptualization, Methodology, Software, Data curation, Writing – original draft, Visualization, Writing – review & editing. **Wenfil Daisi Mariño Huaraca:** Conceptualization,

Methodology. **Mijail Contreras Huamani:** Conceptualization, Visualization, Supervision, Validation, Writing – review & editing. **Pedro Coila:** Validation, Writing – review & editing. **Natalia Verónica Taboada:** Visualization, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the producers from the various communities of the Chuschi district for their willingness to collaborate with the experimental units. We also extend our gratitude to the technical team of the PROGAN Project at the Canaán Experimental Station of INIA – Ayacucho.

Data availability

The raw data supporting the conclusions of this article will be made available by the authors on request.

References

Ablondi, M., et al. (2023). Body measures, growth curves and body weight prevision of alpacas (*Vicugna pacos*) reared in Italy. *Italian Journal of Animal Science*, 22(1), 1196–1204. <https://doi.org/10.1080/1828051X.2023.2273982>

Adjei-Fremah, S., et al. (2018). Effect of probiotic supplementation on growth and global gene expression in dairy cows. *Journal of Applied Animal Research*, 46(1), 257–263. <https://doi.org/10.1080/09712119.2017.1292913>

Alawneh, J. I., et al. (2020). Systematic review of an intervention: The use of probiotics to improve health and productivity of calves. *Preventive Veterinary Medicine*, 183, Article 105147. <https://doi.org/10.1016/j.prevetmed.2020.105147>

Ali, M., et al. (2024). Interrelations between probiotics, gut microbiota, intestinal barrier, and immune response; focusing on diarrhea in dairy calves. *Journal of Integrative Agriculture*, (1) <https://doi.org/10.1016/j.jia.2024.05.022>

Awad, W. A., et al. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science*, 88(1), 49–55. <https://doi.org/10.3382/ps.2008-00244>

Barba-Vidal, E., Martín-Ortúe, S. M., & Castillejos, L. (2019). Practical aspects of the use of probiotics in pig production: A review. *Livestock Science*, 223, 84–96. <https://doi.org/10.1016/j.livsci.2019.02.017>

Bermudez-Brito, M., et al. (2012). Probiotic mechanisms of action. *Annals of Nutrition and Metabolism*, 61(2), 160–174. <https://doi.org/10.1159/000342079>

Bogucka, J., et al. (2019). Microstructure of the small intestine in broiler chickens fed a diet with probiotic or synbiotic supplementation. *Journal of Animal Physiology and Animal Nutrition*, 103(6), 1785–1791. <https://doi.org/10.1111/jpn.13182>

Caman Salazar, J. E. (2018). *Causas de mortalidad en alpacas y su impacto económico, en la SAIS Tupac Amaru Ltda-N 1*, 2010–2016. Thesis Medico Veterinario y Zootecnista.

Cangiano, L. R., et al. (2020). Invited review: Strategic use of microbial-based probiotics and prebiotics in dairy calf rearing. *Applied Animal Science*, 36(5), 630–651. <https://doi.org/10.15232/aas.2020-02049>

Chen, J., et al. (2015). Identification of potential biomarkers for gut barrier failure in broiler chickens. *Frontiers in Veterinary Science*, 2, 14. <https://doi.org/10.3389/fvets.2015.00014>

Cholewińska, P., et al. (2020). The microbiome of the digestive system of ruminants—a review. *Animal Health Research Reviews*, 21(1), 3–14. <https://doi.org/10.1017/S1466252319000069>

Ding, S., et al. (2021). The impact of probiotics on gut health via alternation of immune status of monogastric animals. *Animal Nutrition*, 7(1), 24–30. <https://doi.org/10.1016/j.aninu.2020.11.004>

Dogi, C., et al. (2016). *Lactobacillus rhamnosus* RC007 intended for feed additive: Immune-stimulatory properties and ameliorating effects on TNBS-induced colitis. *Beneficial Microbes*, 7(4), 539–548. <https://doi.org/10.3920/BM2015.0147>

Du, R., et al. (2018). Probiotic *Bacillus amyloliquefaciens* C-1 improves growth performance, stimulates GH/IGF-1, and regulates the gut microbiota of growth-retarded beef calves. *Frontiers in Microbiology*, 9, 2006. <https://doi.org/10.3389/fmicb.2018.02006>

Esfiokhi, S. M., et al. (2024). Effects of probiotic-supplemented milk replacer on growth, blood biochemistry, fermentation, digestibility, and carcass traits in lambs. *Veterinary and Animal Science*, 25. <https://doi.org/10.1016/j.vas.2024.100368>

Espinoza, J. (2018). *Determinación de las características morfológicas de llamas (Lama glama L.), alpacas (Vicugna pacos L.) e híbrido en zonas del municipio de Catacora provincia José Manuel Pando del departamento de la Paz*. Universidad Mayor de San Marcos.

Foster, A., et al. (2009). Haematology and biochemistry in alpacas and llamas. *In Practice*, 31(6), 276–281. <https://doi.org/10.1136/inpract.31.6.276>

Foster, A. P., et al. (2008). Naturally occurring intestinal lesions in three alpacas (*Vicugna pacos*) caused by attaching and effacing *Escherichia coli*. *Veterinary Record*, 162(10), 318–320. <https://doi.org/10.1136/vr.162.10.318>

Gomez, D. E., et al. (2022). Calf diarrhea is associated with a shift from obligated to facultative anaerobes and expansion of lactate-producing bacteria. *Frontiers in Veterinary Science*, 9, Article 846383. <https://doi.org/10.3389/fvets.2022.846383>

Gómez-Quipe, O. E., et al. (2022). Analysis of alpaca (*Vicugna pacos*) cria survival under extensive management conditions in the high elevations of the Andes mountains of Peru. *Small Ruminant Research*, 217, 106–839. <https://doi.org/10.1016/j.smallrumres.2022.106839>

Groschwitz, K. R., & Hogan, S. P. (2009). Intestinal barrier function: Molecular regulation and disease pathogenesis. *Journal of Allergy and Clinical Immunology*, 124(1), 3–20. <https://doi.org/10.1016/j.jaci.2009.05.038>

Guo, H., et al. (2024). Compound probiotics starter: A solution for aflatoxin B1 reduction and meat quality improvement in fermented chicken jerky. *Food Control*, 65, Article 110601. <https://doi.org/10.1016/j.foodcont.2024.110601>

Guo, Y., et al. (2022). Effects of a multi-strain probiotic on growth, health, and fecal bacterial flora of neonatal dairy calves. *Animal Bioscience*, 35(2), 204–216. <https://doi.org/10.5713/ab.21.0084>

Gutiérrez-Castro, L. L., & Corredor-Matus, J. (2017). Parámetros sanguíneos y respuesta inmune en pollos. *Revista Veterinaria y Zootecnia*, 11(2), 81–92. <https://doi.org/10.17151/vetzo.2017.11.2.7>

Hanco, E. (2018). *Efecto benéfico de los probióticos, en el complejo entérico neonatal, nivel de Ig séricas y recuento de leucocitos, en crías de alpacas (Vicugna pacos) del INIA -Puno*. Universidad Nacional del Antiplano.

Hasunuma, T., et al. (2011). Effect of cellooligosaccharide or synbiotic feeding on growth performance, fecal condition and hormone concentrations in Holstein calves. *Animal Science Journal*, 82(4), 543–548. <https://doi.org/10.1111/j.1740-0929.2010.00861.x>

Heinrichs, A. J., et al. (2007). Variability in Holstein heifer heart-girth measurements and comparison of prediction equations for live weight. *Preventive Veterinary Medicine*, 78(3–4), 333–338. <https://doi.org/10.1016/j.prevetmed.2006.11.002>

Hengrave, I., et al. (2005). Neuweltkameliden in der Schweiz. II. Referenzwerte für hämatologische und blutchemische parameter. *Schweizer Archiv Für Tierheilkunde*, 147(8), 335–343. <https://doi.org/10.1024/0036-7281.147.8.335>

Hoseinifair, S. H., et al. (2018). Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. *Frontiers in Microbiology*, 9. <https://doi.org/10.3389/fmicb.2018.02429>

Jorge Maximiliano, G., et al. (2018). Igg serum concentrations in alpacas neonates killed by enterotoxemia. *Revista de Investigaciones Veterinarias Del Peru*, 29(2), 635–642. <https://doi.org/10.15381/rivp.v29i2.14479>

Kalita, A., et al. (2021). Small intestinal mucosal cells in piglets fed with probiotic and zinc: A qualitative and quantitative microanatomical study. *Folia Morphologica*, 80(3), 605–617. <https://doi.org/10.5603/FM.a2020.0091>

Kelsey, A. J., & Colpoys, D. J. (2018). Effects of dietary probiotics on beef cattle performance and stress. *Journal of Veterinary Behavior*, 27, 8–14. <https://doi.org/10.1016/j.jvbeh.2018.05.010>

Konieczka, P., et al. (2023). Feeding Bacillus-based probiotics to gestating and lactating sows is an efficient method for improving immunity, gut functional status and biofilm formation by probiotic bacteria in piglets at weaning. *Animal Nutrition*, 13, 361–372. <https://doi.org/10.1016/j.aninu.2023.03.003>

Konieczka, P., et al. (2022). Bacillus-based probiotics affect gut barrier integrity in different ways in chickens subjected to optimal or challenge conditions. *Veterinary Microbiology*, 265, Article 109323. <https://doi.org/10.1016/j.vetmic.2021.109323>

Kumar, S., et al. (2025). Prospects of probiotics in improvement of health and productivity of dairy animals and reducing antibiotic load on the environment. *Biotechnology for the Environment*, 2(1), 13. <https://doi.org/10.1186/s44314-025-00025-6>

Kumar, M., et al. (2022). Microencapsulated and lyophilized *Lactobacillus acidophilus* improved gut health and immune status of preruminant calves. *Probiotics and Antimicrobial Proteins*, 14(3), 523–534. <https://doi.org/10.1007/s12602-021-09821-4>

Kumar, M., et al. (2013). Probiotic metabolites as epigenetic targets in the prevention of colon cancer. *Nutrition Reviews*, 71(1), 23–34. <https://doi.org/10.1111/j.1753-4887.2012.00542.x>

Lambo, M. T., Chang, X., & Liu, D. (2021). The recent trend in the use of multistrain probiotics in livestock production: An overview. *Animals*, 11(10). <https://doi.org/10.3390/ani11102805>

Lee, J. S., et al. (2021). Effects of microencapsulated organic acids on growth performance, nutrient digestibility, fecal microbial counts, and blood profiles in weaning pigs. *Journal of Animal Science and Technology*, 63(1), 104–113. <https://doi.org/10.5187/JAST.2021.E16>

Lee, S. J., et al. (2024). Dietary supplementation with host-associated low-temperature potential probiotics improves the growth, immunity, digestive enzyme activity, and intestinal microbial population of olive flounder (*Paralichthys olivaceus*). *Aquaculture Reports*, 36, Article 102128. <https://doi.org/10.1016/j.aqrep.2024.102128>

Lele, V., et al. (2019). Milk production and ruminal parameters of dairy cows fed diets containing *Lactobacillus sakei* KTU05-6 and *Pediococcus pentosaceus* BaltBio02. *Polish Journal of Veterinary Sciences*, 22(2). <https://doi.org/10.24425/pjvs.2019.129224>

Letnická, A., et al. (2017). Influence of oral application of *Enterococcus faecium* AL41 on TGF- β 4 and IL-17 expression and immunocompetent cell distribution in chickens challenged with *Campylobacter jejuni*. *Acta Veterinaria Hungarica*, 65(3), 317–326. <https://doi.org/10.1556/004.2017.031>

- Liu, H., et al. (2014). Oral administration of *Lactobacillus fermentum* 15007 favors intestinal development and alters the intestinal microbiota in formula-fed piglets. *Journal of Agricultural and Food Chemistry*, 62(4), 860–866. <https://doi.org/10.1021/jf403288r>
- Liu, K., et al. (2021). Ruminal microbiota–host interaction and its effect on nutrient metabolism. *Animal Nutrition*, 7(1), 49–55. <https://doi.org/10.1016/j.aninu.2020.12.001>
- Lorenz, I., et al. (2011). Calf health from birth to weaning. I. General aspects of disease prevention. *Irish Veterinary Journal*, 64(1). <https://doi.org/10.1186/2046-0481-64-10>
- Mallma, Y., et al., 2021. Morphologic, morphostructural and faneroptic characterization of alpaca (*Vicugna pacos*) in the provinces of Antabamba and Aymaraes, Apurímac, Peru. <https://www.cabidigitallibrary.org/doi/full/10.5555/20219847707>
- Maria, L. P. (2021). Avaliação do efeito da suplementação de um probiótico associado a diferentes níveis dietéticos de proteína balanceada em frangos de corte desafiados por *Eimeria maxima*.
- Martín-Barrasa, J. L., et al. (2023). Haematological and biochemical blood reference values for Canary island camels (*Camelus dromedarius*), an endangered dromedary species. *Saudi Journal of Biological Sciences*, 30(6). <https://doi.org/10.1016/j.sjbs.2023.103677>
- Ministerio de Desarrollo Agrario y Riego (MIDAGRI), 2019. El Perú es la primera potencia mundial en producción de fibra de alpaca. Ministerio de Desarrollo Agrario y Riego. <https://www.gob.pe/institucion/midagri/noticias/49289-el-peru-es-la-primera-potencia-mundial>.
- Ministerio de Desarrollo Agrario y Riego (MIDAGRI). SIEA - BI, 2025. https://siea.midagri.gob.pe/portal/siea_bi/index.html.
- Mohri, M., Sharifi, K., & Eidi, S. (2007). Hematology and serum biochemistry of Holstein dairy calves: Age related changes and comparison with blood composition in adults. *Research in Veterinary Science*, 83(1), 30–39. <https://doi.org/10.1016/j.rvsc.2006.10.017>
- Noori, M., Alikhani, M., & Jahanian, R. (2016). Effect of partial substitution of milk with probiotic yogurt of different pH on performance, body conformation and blood biochemical parameters of Holstein calves. *Journal of Applied Animal Research*, 44(1), 221–229. <https://doi.org/10.1080/09712119.2015.1031772>
- Pachari, G. S. (2008). *Tiempo de absorción de inmunoglobulina G en crías de alpaca*. Universidad Nacional Jorge Basadre Grohman.
- Parada Venegas, D., et al. (2019). Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Frontiers in Immunology*, 10, 277. <https://doi.org/10.3389/fimmu.2019.00277>
- Parasuraman, S., & Christopher, P. V. (2022). Anestesia y eutanasia en animales de experimentación. En: *Introducción a los fundamentos de la farmacología y la toxicología: Volumen 3: Farmacología experimental: Metodología de investigación y bioestadística* (pp. 65–75). Singapur: Springer Nature Singapore. https://doi.org/10.1007/978-981-19-5343-9_5
- Páucar, J., Perales, R., & Tabacchi, L. (2017). Frecuencia de neoplasias en cavidad oral de caninos y felinos, diagnosticadas histopatológicamente en el laboratorio de patología veterinaria de la universidad nacional mayor de San Marcos (periodo 2007-2013). *Revista de Investigaciones Veterinarias del Perú*, 28(1), 201–206. <https://doi.org/10.15381/rivep.v28i1.12929>
- Piewngam, P., et al. (2018). Pathogen elimination by probiotic *Bacillus* via signalling interference. *Nature*, 562(7728), 532–537. <https://doi.org/10.1038/s41586-018-0616-y>
- Plaza-Díaz, J., et al. (2019). Mechanisms of action of probiotics. *Advances in Nutrition*, 10, S49–S66. <https://doi.org/10.1093/advances/nmy063>
- Plaza-Díaz, J., et al. (2018). Immune-mediated mechanisms of action of probiotics and synbiotics in treating pediatric intestinal diseases. *Nutrients*, 10(1), 42. <https://doi.org/10.3390/nu10010042>
- Prudencio, J. (2005). *Estudio descriptivo longitudinal de diversos parámetros morfométricos del intestino del pollo de engorda*. Universidad Nacional de Loja.
- Puente, V. J., et al. (2019). Effect of supplementation with increasing levels of probiotics on the histomorphometry of the small intestine of Guinea pig (*Cavia porcellus*). *Revista de Investigaciones Veterinarias Del Perú*, 30(2), 624–633. <https://doi.org/10.15381/rivep.v30i2.16086>
- Quispe, M. W. (2019). *Determinación del tiempo de producción de IgG en crías de alpaca (Vicugna pacos)*. Universidad Nacional Jorge Basadre Grohmann.
- Raabis, S., Li, W., & Cersosimo, L. (2019). Effects and immune responses of probiotic treatment in ruminants. *Veterinary Immunology and Immunopathology*, 208, 58–66. <https://doi.org/10.1016/j.vetimm.2018.12.006>
- Ramos-Medina, R., Corbí, A. L., & Sánchez-Ramón, S. (2012). Inmunoglobulinas intravenosas: Llave inmunomoduladora del sistema inmunológico. *Medicina Clínica*, 139(3), 112–117. <https://doi.org/10.1016/j.medcli.2011.11.022>
- Rasmussen, S. B., Reinert, L. S., & Paludan, S. R. (2009). Innate recognition of intracellular pathogens: Detection and activation of the first line of defense. *Journal of Pathology, Microbiology and Immunology*, 117(5–6), 323–337. <https://doi.org/10.1111/j.1600-0463.2009.02456.x>
- Rhayat, L., et al. (2019). Effect of *Bacillus subtilis* strains on intestinal barrier function and inflammatory response. *Frontiers in Immunology*, 10(MAR). <https://doi.org/10.3389/fimmu.2019.00564>
- Rojas, M., et al. (2016). Outbreak of diarrhea among preweaning alpacas (*Vicugna pacos*) in the southern Peruvian highland. *Journal of Infection in Developing Countries*, 10(3), 269–274. <https://doi.org/10.3855/jidc.7398>
- Ross, M. H., & Pawlina, W. (2013). Histología: Texto y atlas color con biología celular y molecular. Aparato urinario. In *Histología: Texto y atlas color con biología celular y molecular*, 129 pp. 320–327. https://www.academia.edu/44858547/Histolog/C3/ADa_Texto_y_atlas_color_con_biol/C3/ADa_texto_y_molecular_6ed_2012_Ross_y_Pawlina.
- Salehzadeh, M., et al. (2019). Effects of probiotic lactic acid bacteria on growth performance, carcass characteristics, hematological indices, humoral immunity, and IGF-I gene expression in broiler chicken. *Tropical Animal Health and Production*, 51(8), 2279–2286. <https://doi.org/10.1007/s11250-019-01935-w>
- Salgado-Schüler, P. A. (2017). *Cuenta de leucocitos en frotis sanguíneo como alternativa de campo al método del hemocitómetro en especímenes de trucha arcoiris (Oncorhynchus mykiss) clínicamente sanos*. Universidad de Chile.
- Sharma, S., et al. (2015). Pathomorphological alterations associated with chicken coccidiosis in Jammu division of India. *Journal of Parasitic Diseases*, 39(2), 147–151. <https://doi.org/10.1007/s12639-013-0302-9>
- Schenten, D., & Medzhitov, R. (2011). The control of adaptive immune responses by the innate immune system. *Advances in Immunology*, 109, 87–124. <https://doi.org/10.1016/B978-0-12-387664-5.00003-0>
- Šefcová, M. A., et al. (2023). Effects of *Lactobacillus fermentum* administration on intestinal morphometry and antibody serum levels in *Salmonella-infantis*-challenged chickens. *Microorganisms*, 11(2). <https://doi.org/10.3390/microorganisms11020256>
- Servicio Nacional de Meteorología e Hidrología del Perú (SENAMHI) - Perú. Ministerio del ambiente, 2018. <https://www.senamhi.gob.pe/?p=monitoreo-hidrologico>.
- Serrano Martínez, T. (2015). Evaluación de la inclusión de probióticos en el agua de bebida de pollos broiler sobre parámetros productivos y morfometría intestinal. In *Evaluación de la inclusión de probióticos en el agua de bebida de pollos broiler sobre parámetros productivos y morfometría intestinal*, 7 pp. 37–72.
- Shini, S., et al. (2020). Probiotic *Bacillus amyloliquefaciens* H57 ameliorates subclinical necrotic enteritis in broiler chicks by maintaining intestinal mucosal integrity and improving feed efficiency. *Poultry Science*, 99(9), 4278–4293. <https://doi.org/10.1016/j.psj.2020.05.034>
- Sierra, S., et al. (2010). Intestinal and immunological effects of daily oral administration of *Lactobacillus salivarius* CECT5713 to healthy adults. *Anaerobe*, 16(3), 195–200. <https://doi.org/10.1016/j.anaerobe.2010.02.001>
- Singh, M., et al. (2021). Synbiotic formulation of *Cichorium intybus* root powder with *Lactobacillus acidophilus* NCD15 and *Lactobacillus reuteri* BFE7 improves growth performance in Murrah buffalo calves via altering selective gut health indices. *Tropical Animal Health and Production*, 53(2). <https://doi.org/10.1007/s11250-021-02733-z>
- Taboada, N., et al. (2022). Administration of selected probiotic mixture improves body weight gain and meat fatty acid composition of creole goats. *Food Bioscience*, 49, Article 101836. <https://doi.org/10.1016/j.fbio.2022.101836>
- Torres-Rodríguez, A., et al. (2007). Performance and condemnation rate analysis of commercial turkey flocks treated with a *Lactobacillus* spp.-based probiotic. *Poultry Science*, 86(3), 444–446. <https://doi.org/10.1093/ps/86.3.444>
- Trillo, F. Z., et al. (2021). Influence of sex, age, year and additive and permanent maternal effects on characteristics of economic importance in Huacaya alpacas. *Revista de Investigaciones Veterinarias Del Perú*, 32(1), Article e18493. <https://doi.org/10.15381/RIVEP.V32I1.19493>
- Tsur, I., et al. (1996). Meningoencephalitis and brain abscessation due to *Escherichia coli* in a 2 week old alpaca cria. *Australian Veterinary Journal*, 74(6), 437–438. <https://doi.org/10.1111/j.1751-0813.1996.tb07563.x>
- Udeh, N., et al. (2021). Effect of season, age, and sex on haematology and serum biochemistry of sport horses. *Journal of Sustainable Veterinary and Allied Sciences*, 1, 140–144. <https://doi.org/10.54328/covm/josvas.2021.032>
- Umu, Ö. C., Rudi, K., & Diep, D. B. (2017). Modulation of the gut microbiota by prebiotic fibres and bacteriocins. *Microbial Ecology in Health and Disease*, 28(1), Article 1348886. <https://doi.org/10.1080/16512235.2017.1348886>
- Valdizán, G. C., et al. (2019). Effect of dietary probiotic, prebiotic and their combination on the productive performance of Guinea pigs (*Cavia porcellus*). *Revista de Investigaciones Veterinarias Del Perú*, 30(2), 590–597. <https://doi.org/10.15381/rivep.v30i2.16071>
- Varada, V. V., et al. (2022). Autochthonous *Limosilactobacillus reuteri* BFE7 and *Ligilactobacillus salivarius* BF17 probiotics consortium supplementation improves performance, immunity, and selected gut health indices in Murrah buffalo calves. *Veterinary Research Communications*, 46(3), 757–767. <https://doi.org/10.1007/s11259-022-09896-6>
- Velásquez, X. (2023). *Suplementación proteica y su relación con la ganancia de peso, concentraciones de inmunoglobulinas G y hemograma de Alpacas*. Universidad Nacional Agraria La Molina.
- Wagener, M. G., et al. (2024). Hematologic parameters in female alpacas during age progression: A retrospective study. *Scientific Reports*, 14(1). <https://doi.org/10.1038/s41598-023-50572-9>
- Wang, H., et al. (2022a). Effects of compound probiotics on growth performance, rumen fermentation, blood parameters, and health status of neonatal Holstein calves. *Journal of Dairy Science*, 105(3), 2190–2200. <https://doi.org/10.3168/jds.2021-20721>
- Wang, M., et al. (2022b). The relationship between villous height and growth performance, small intestinal mucosal enzymes activities and nutrient transporters expression in weaned piglets. *Journal of Animal Physiology and Animal Nutrition*, 104(2), 606–615. <https://doi.org/10.1111/jpn.13299>
- Wang, X., et al. (2021). Hematological and cytochemical characteristics of peripheral blood cells in the argus snakehead (*Ophiocephalus argus* Cantor). *PeerJ*, 9, Article e11234. <https://doi.org/10.7717/peerj.11234>
- Wang, Y., et al. (2019a). Oral administration of *Lactobacillus rhamnosus* GG to newborn piglets augments gut barrier function in pre-weaning piglets. *Journal of Zhejiang University: Science B*, 20(2), 180–192. <https://doi.org/10.1631/jzus.B1800022>
- Wang, Y., et al. (2019b). *Lactobacillus casei* DBN023 protects against jejunal mucosal injury in chicks infected with *Salmonella pullorum* CMCC-533. *Research in Veterinary Science*, 127, 33–41. <https://doi.org/10.1016/j.rvsc.2019.09.010>

- Weaver, D. M., et al. (2000). Passive transfer of colostral immunoglobulin G in neonatal llamas and alpacas. *American Journal of Veterinary Research*, 61(7), 738–741. <https://doi.org/10.2460/ajvr.2000.61.738>
- Wiedner, E. (2021). Diseases of llamas and alpacas - exotic and laboratory animals. *MSD Veterinary Manual*. MSD Manual. <https://www.msdsvetmanual.com/exotic-and-laboratory-animals/llamas-and-alpacas/diseases-of-llamas-and-alpacas>.
- Zapata, C., et al. (2024). Alterations in the gut microbial composition and diversity associated with diarrhea in neonatal Peruvian alpacas. *Small Ruminant Research*, 235, Article 107273. <https://doi.org/10.1016/j.smallrumres.2024.107273>
- Zarrin, M., et al. (2020). Camelids: New players in the international animal production context. *Tropical Animal Health and Production*, 52(3), 903–913. <https://doi.org/10.1007/s11250-019-02197-2>