Colegio de Postgraduados

Reduction in the incidence and recurrence of diarrhea in multibreed kids supplemented with *Bacillus subtilis* PB6 in Northern Mexico

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ABSTRACT
Objective: To evaluate the effect of probiotic supplementation (*Bacillus subtilis* PB6) on weight gain, as well as the incidence and recurrence of diarrhea and pneumonia in multibreed kids in northern Mexico.
Design/Methodology/Approach: The study was conducted in Ejido Corona, Municipality of Matamoros, Coahuila (northern Mexico). The experiment lasted 25 days in October 2022. Ninety-nine kids were randomly assigned to two homogeneous groups based on body weight (BW; 3.7±0.2 kg). The control group (CG; n=50) received 5 mL of milk per kid per day. The treated group (TG; n=49) received 5 mL of milk per kid per day supplemented with 1 g/kg BW of *Bacillus subtilis* PB6. Administration was performed daily at 12:00 p.m. The probiotic dose was adjusted every five days, coinciding with body weight measurements.
Results: No significant difference was observed in final BW ($p>.05$; 7.7±0.1 kg *vs.* 7.4±0.2 kg for TG and CG, respectively). A significant difference was found in diarrhea incidence ($p<.05$; 28% *vs.* 50% for TG and CG, respectively), as well as in diarrhea recurrence ($p<.05$; 29% *vs.* 48% for TG and CG, respectively). These findings indicate that supplementation with *Bacillus subtilis* PB6 reduced the incidence and recurrence of diarrhea and may have contributed to the prevention of pneumonia.
Limitations/Implications: A longer supplementation period may be required to observe significant effects on body weight in kids.
Findings/Conclusions: Probiotic supplementation with *Bacillus subtilis* PB6 improved health outcomes by reducing diarrheal incidence and recurrence in multibreed kids raised under semi-arid conditions in northern Mexico.

Keywords: goats; intestinal health; probiotics; weight gain; diarrhea

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INTRODUCTION

Semi-arid environments play a decisive role in the establishment of vegetation cover and the availability of forage resources, as they are characterized by low precipitation, high thermal variability, and vegetation dominated by natural grasslands and

xerophytic shrubs (Tarango, 2005; Sánchez *et al.*, 2023). Under these agroecological conditions, goat production has become established as a viable livestock activity due to the physiological adaptability of goats, their hardiness, and their ability to utilize low-quality forage.

In Mexico, goat production is concentrated primarily in the arid and semi-arid regions of the northern and central parts of the country, where annual precipitation ranges between 350 and 600 mm (FAO, 2011; Tarango, 2005). In these areas, extensive production systems predominate, based on daytime grazing and nighttime confinement, which helps reduce feeding costs by utilizing natural rangelands. Beyond its ecological suitability, goat production also plays a significant socioeconomic role, as it provides meat and milk that contribute to food security and income generation for small-scale rural producers (Echavarría *et al.*, 2006; Maldonado-Jáquez *et al.*, 2014).

Despite its productive importance, goat production in semi-arid regions faces significant constraints, particularly during the neonatal stage. Kid mortality represents one of the main factors affecting system profitability, resulting in considerable economic losses. Neonates possess an immature immune system, which increases their susceptibility to gastrointestinal and respiratory diseases (Cuéllar *et al.*, 2012). Among the primary causes of morbidity and mortality are infectious diarrheal diseases, whose etiological agents include *Escherichia coli*, *Salmonella* spp., *Clostridium perfringens*, coccidia of the genus *Eimeria*, and *Cryptosporidium* spp. (Alarcón-Aburto, 2005; Suárez *et al.*, 2007). Additionally, environmental factors such as low temperatures may increase the incidence of respiratory diseases, particularly pneumonia (Windeyer *et al.*, 2014; Cheng *et al.*, 2021).

Traditionally, antibiotics have been used for the prevention and treatment of these diseases; however, their indiscriminate use has raised concerns due to the development of antimicrobial resistance and the presence of residues in animal-derived products intended for human consumption. This scenario has prompted the search for sustainable alternatives that improve animal health without posing risks to public health. In this context, probiotics defined as live microorganisms that, when administered in adequate amounts, confer health benefits on the host (FAO/WHO, 2002) have emerged as a promising strategy in animal production. In ruminants, probiotic supplementation has been associated with improvements in intestinal health, feed efficiency, weight gain, and reduced incidence of diarrhea.

Nevertheless, information regarding the use of specific probiotic strains in kids raised under production systems in semi-arid regions remains limited. The strain *Bacillus subtilis* PB6 has demonstrated potential to maintain intestinal microbiota balance and improve productive performance in farm animals (Kritas & Morrison, 2005; Kritas *et al.*, 2006); however, its effects on the health and growth of neonatal kids have not been sufficiently documented. Therefore, the objective of the present study was to evaluate the effect of dietary supplementation with *Bacillus subtilis* PB6 on weight gain, as well as on the incidence and recurrence of diarrhea and pneumonia in multibreed kids raised under semi-arid conditions in northeastern Mexico.

MATERIALS AND METHODS

All methods and management of the experimental groups were conducted in accordance with international (ASAS, 2020) and national (NAM, 2002) guidelines for the care, ethical use, and welfare of research animals, with institutional approval (UAAAN-UL: 3811-425502002-2792).

Description of location and environmental conditions

The study was conducted in the municipality of Matamoros, Coahuila, a semi-arid region in northeastern Mexico (103°15′38” W, 25° 35′03” N; 1,111 m) (INEGI, 2020). The mean annual precipitation is 230 mm, with an average annual temperature of 22 °C, a maximum of 38 °C, and a minimum of 8 °C (CONAGUA, 2018).

Animals and treatments

Ninety-nine kids were randomly selected and assigned to two homogeneous groups based on birth weight: Treatment Group (TG; n=49; 3.8 kg body weight [BW]) and Control Group (CG; n=50; 3.7 kg BW). Colostrum intake was ensured through direct suckling.

During part of the lactation period (25 days), from October 7 to November 1, 2022, both groups received 5 mL of milk per kid administered orally using a disposable syringe. The milk provided to the TG was supplemented with 1 g of a commercial product containing 2×10^8 colony-forming units (CFU) of *Bacillus subtilis* PB6 per kilogram of body weight. Administration was performed daily at 12:00 p.m. The probiotic dose was adjusted according to BW every five days, coinciding with the weighing of the kids.

Evaluated variables

Body weight

Body weight was recorded using a digital hanging scale (WH-100) at the beginning of the study and every five days over a 25-day period.

Incidence and recurrence of diarrhea

Each kid was monitored daily through observation of feces in the perianal area. When diarrhea was detected, a small sample was collected and transported to the pathology laboratory at UAAAN, where fecal color (dark green, white, or orange) was recorded. Diagnosis was performed using coprological examinations and Ziehl-Neelsen staining to determine the etiological agent of the diarrhea. Additionally, each kid was examined daily for clinical signs of pneumonia. Disease incidence was determined by recording the number of confirmed cases, and recurrence was defined as the number of cases that resolved and subsequently reoccurred within the 25-day study period.

Statistical analysis

To determine differences in weight gain between groups was performed using the Student’s t test. Differences in the proportions of kids with incidence and recurrence of diarrhea and pneumonia were evaluated using the chi-square test. A significance level of $p<.05$ was established to determine statistical differences. Statistical analyses were conducted using SYSTAT version 12. Incidence was defined as the number of new cases occurring within a specified period divided by the population at risk (I=amount of cases/population at risk). Recurrence was defined as the number of existing cases within a population at a given time, without distinguishing between new and previous cases (WHO & PAHO, 2018).

RESULTS AND DISCUSSION

For the final body weight variable (25 days of age), no significant difference was observed ($p>.05$; 7.7±0.1 kg *vs.* 7.4±0.2 kg) between the TG and CG, respectively (Table 1).

Incidence of diarrhea

Laboratory diagnosis identified Cryptosporidium parvum as the etiological agent responsible for the diarrheal cases observed, infecting the intestinal tract of the kids. The incidence of diarrhea was higher in the control group than in the treated group ($p<.05$; 50% *vs.* 28%). Likewise, a significant difference was observed in the recurrence of diarrhea (48% *vs.* 29%), favoring the TG (Table 2).

Regarding pneumonia, only one kid death was recorded with an initial suspicion of this condition (control group). However, postmortem examination revealed that the cause of death was drowning due to animal overcrowding during the night.

In the present study, greater weight gain (kg) was hypothesized in the TG, along with lower incidence and recurrence of diarrhea and pneumonia. The results showed no statistically significant difference in body weight between groups. This finding is consistent with Souza *et al.* (2021), who administered *Bacillus subtilis* PB6 in poultry, cows, and deer and reported no significant weight gain. Similarly, studies evaluating weight gain in kids have reported significant differences only after 30 days of supplementation (Sivadasan, 2020). However, in our study, a significant difference was observed in diarrhea incidence.

Table 1. Body weight (kg) of two groups of multibreed kids from northeastern Mexico supplemented with *Bacillus subtilis* PB6 for 25 days after birth.

Body weight (kg)	TG (n = 49)	CG (n = 50)
Initial	$3.8±0.1^{a}$	$3.7±0.2^{a}$
Final	$7.7±0.1^{a}$	$7.4±0.2^{a}$

[a, b] Means within rows do not differ significantly ($p>.05$).

Table 2. Incidence and recurrence of diarrhea and pneumonia in multibreed kids from northeastern Mexico supplemented with *Bacillus subtilis* PB6 for 25 days after birth.

Diarrhea	GT (n=49)	GC (n=50)
Diarrhea incidence, n (%)	$14/49\ (28)^{a}$	$25/50\ (50)^{a}$
Diarrhea recurrence, n (%)	$4/14\ (29)^{a}$	$12/25\ (48)^{a}$
Pneumonia, n (%)	$0/49\ (0)^{a}$	$1/50\ (2)^{a}$

[a, b] Means within rows do not differ significantly ($p>.05$).

The control group presented 25 cases (50%) of diarrhea, compared with only 14 cases (28%) in the probiotic-treated group. Likewise, recurrence of this condition was lower in the TG than in the CG (29% *vs.* 48%). These findings are consistent with studies evaluating probiotic supplementation in goats, which reported a reduction in diarrhea cases (Galina & Puga, 2009).

Microbial populations in the digestive system of animals are highly specialized and composed of diverse microorganisms, including bacteria, fungi, and protozoa (Figueroa & Vilades, 2008). These microorganisms are responsible for essential digestive and fermentative functions, such as vitamin synthesis, maintenance of intestinal mucosal integrity, and protection against pathogenic agents (Relling & Mattioli, 2003). Based on this premise, the findings of the present study may be interpreted in light of the lower incidence of diarrhea observed in the treated group compared with the control group. This reduction may be attributed to the inhibitory effects of probiotics on pathogenic agents, thereby decreasing the occurrence of the disease.

Studies evaluating probiotics in animals have demonstrated that their beneficial effects are associated with specific mechanisms of action. Probiotic strains can positively influence digestive system balance by adhering to the intestinal epithelium and exerting competitive exclusion, bacterial antagonism, and immunomodulatory effects. These mechanisms are reflected in reduced populations of pathogenic bacteria and improved nutrient utilization (Markowiak & Śliżewska, 2018).

Probiotic supplementation in goats has been shown to improve intestinal health and support disease prevention by reducing pathogenic bacterial strains that affect the gastrointestinal tract, thereby decreasing disease incidence. Studies in sheep supplemented with Bacillus subtilis have reported improvements in intestinal health, productivity, metabolic profile, and reduced disease occurrence (Devyatkin, Mishurov, & Kolodina, 2021; Sheikh *et al.*, 2022).

The favorable results obtained in the present study regarding intestinal health may be attributed to the ability of probiotics to produce bacteriocins, adhere to the intestinal epithelium, and modify pH levels. These mechanisms create unfavorable conditions for pathogenic microorganisms, thereby limiting their survival and contributing to a reduction in disease occurrence (Corr *et al.*, 2007; Shim *et al.*, 2012; Jordan *et al.*, 2014).

Previous research has reported a reduction in pathogenic strains (*Escherichia coli*, *Clostridium* spp., and *Salmonella* spp.) in various animal species, including poultry and piglets, following probiotic supplementation. These studies have also documented an increase in populations of *Lactobacillus* and *Bifidobacterium*, resulting in improvements in gut microbiota composition, enhanced digestive health, reduced incidence of diarrhea, and improved nutritional status (Shim *et al.*, 2012; Liu *et al.*, 2014; Mookiah *et al.*, 2014; Zhang & Kim, 2014; Zhang *et al.*, 2016). Other studies have reported that supplementation with lactic acid bacteria and *Bacillus* spp. modifies microbial populations within the gastrointestinal tract (Abdelqader, Irshaid, & Al-Fataftah, 2013). Additionally, probiotic supplementation has been shown to improve intestinal health, enhance milk quality, support disease prevention, and promote weight gain after 30 days of supplementation (Abd El-Tawab *et al.*, 2016).

Although no cases of pneumonia were recorded in the present study, this absence may be attributed to the environmental conditions of the study area, as well as to the lack of coccidiosis cases, which have been associated with increased pneumonia incidence (Donkin & Boyazoglu, 2004; Kattimani *et al.*, 2020). Given the limited number of studies evaluating weight gain in kids supplemented with probiotics, it is possible that supplementation periods longer than 30 days are required to observe measurable effects on body weight.

CONCLUSIONS

Supplementation with *Bacillus subtilis* PB6 improved the health status of the kids and reduced both the incidence and recurrence of diarrhea. Additionally, it may have contributed to the prevention of pneumonia. A longer supplementation period with *Bacillus subtilis* PB6 may enhance body weight in multibreed kids raised in northern Mexico.

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