Colegio de Postgraduados

Conjugated probiotics dispensed from birth to weaning for the survival of goat kids

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ABSTRACT

Objective: To prevent morbidities, mortalities and increase weight gain and growth of kids by administering oral probiotic conjugate (PC).

Design/Methodology/Approach: A randomized design comparing treatments (supplemented dose), percentage of morbidity and percentage of mortality. Goats were administered weekly from birth to 56 days of age. PC of *Bifidobacterium bifidum essensis*, *Lactococcus lactis*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were dosed weekly. Treatments, TC: Control, T2: 2.0 mL PC/kg body weight (BW). T3: 3 mL PC/Kg BW. T4: 4 mL PC/Kg BW.

Results: Diarrhea was present in: TC=16%, T2=1%, T3 and T4=0% (TC *vs*. T2, T3, T4, $P<0.05$). Mortality percentages: TC=17%, T2, T3 and T4=0% (TC *vs*. T2, T3, T4, $P<0.05$). T2 and T3 had an increase ($P<0.05$) of 1.9 kg weight gain (WG) *vs*. TC. T3 was the best treatment at 56 d ($P<0.05$).

Study Limitations/Implications: The doses used were defined based on other studies and experimental doses were used; the results consider that the facilities and management are optimal and in accordance with animal welfare standards.

Findings/Conclusions: The most adequate dose was 1014 CFU/kg BW since it improved WG and reduced mortality. It is suggested to compare oral doses of probiotics to reduce death due to enteric diseases.

Keywords: Goat kids, diarrhea, probiotic, mortality.

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INTRODUCTION

Intestinal probiotics adequately maintain the digestive health of animals and humans; its benefits depend on several factors, such as strains chosen, concentration, the viability of colony-forming units, and frequency of consumption (Salazar & Montoya, 2003). The excellent choice of a probiotic allows to maintain the health of the animals and improve the growth rate; there are reports were lactobacilli, and bifidobacteria have improved body weight and decreased mortality in piglets (Giraldo-Carmona *et al*., 2015), lambs (Castro & Rodriguez, 2005) and kids (Markowiak-Kopeć & Ślizewska, 2019). The main reason for

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probiotic use is to avoid colonization of intestinal pathogens that adhere to the intestinal mucosa and to obtain a better immune response in the digestive tract (Delia *et al.*, 2012). Antibiotics are commonly used to treat digestive disorders and control intestinal pathogens, although, its continuous use causes resistance to microorganisms and may end as residual compounds in food (Abd El-Tawab *et al.*, 2016). In neonatal kids, the mortality rate is higher than 15% due to deficiency, respiratory and digestive diseases. The etiologies of the deficiencies are the lack of energy, protein, and selenium (Diaz-Sánchez *et al.*, 2018). In the case of infectious diseases, protozoa, viruses, and bacteria participate (Salazar & Montoya, 2003). Probiotics in neonates, have generally been used to prevent digestive diseases and have been extensively studied in the calves, piglets, and lambs. However, the information published in kids is limited, some works describe the effect of probiotics in short times, but there are no studies that evaluate the use of probiotics in kids throughout the neonatal lactation cycle, which includes from birth to weaning. Therefore, the objective of the present study was to evaluate the oral use of a probiotic conjugate, administered weekly in kids from birth to 56 days of age.

MATERIAL AND METHODS

Study Site

The study was carried out at the "San José" Tepoxtla ranch, Yauquemehcan, Tlaxcala, Mexico. It is located between parallels 19° 21' and 19° 26' north latitude: meridians 98° 08' and 98° 13' west longitude. The altitude of 2,420 meters above sea level and temperature from 12 to 16 °C.

Herd Characteristics

The production unit has 300 French Alpine goats, with ages ranging from two to four years. The zootechnical purpose is the production of milk and kids for breeding and market supply. The animals are housed, and the essential rations are prepared based on the herd's physiological stages, formulated with oats, alfalfa, rootlets, barley, corn, and minerals. Initially, a total of 100 pregnant goats were selected, with breeding records, the females calved from December 2021 to February 2022. During deliveries, kids were identified, the weight and sex were recorded, and each animal was assigned to an experimental group.

Characteristics of the Probiotic Conjugate

The probiotic conjugate (PC) is a beverage; it was prepared in a cheese whey medium, including *Bifidobacterium bifidum essensis*, *Lactococcus lactis*, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus*. The probiotic conjugate consists of a certified guarantee of viable microorganisms of 106 colony forming units (CFU) per mL. The determination of the CFUs was performed with a colony counter (Kert-Lab. CM-1) described by Schell and Beermann (2014), in the Veterinary School of the Autonomous University of Tlaxcala's biochemical lab. During the kids' dosing, the probiotic complex was thawed and diluted in whey at 30 °C for 10 min. It was then cooled to room temperature and refrigerated during the oral administration process to the kids.

Experimental Distribution of Kids

The distribution of the kids at birth was done randomly in four treatments, considering the sex of the kid as a weighted variable, so that the groups would be homogeneous. The groups were distributed as follows:

TC: Control treatment, saline solution: 2.0 mL kg/body weight (BW), 12 goats and 12 kids.
T2: Dose of 2.0 mL PC/kg BW: 13 kids and 12 kids.
T3: 3.0 mL dose PC/Kg BW, 12 kids and 13 kids.
T4: 4.0 mL dose PC/Kg BW: 12 kids and 12 kids.

The first dosage of probiotics was given 24h after birth, and these were repeated every eight days for seven consecutive times, adjusting the dose of each treatment based on the weight of the registered kid. All the kids remained with their mothers, and the zootechnical management was similar in the four treatments. The zootechnical routine in the production unit is to disinfect the umbilical cord with a solution of iodine; it is ensured that the animal ingested colostrum during the first 24h of birth, the kids were identified with a plastic earring in the right ear, they were injected with two doses of selenium (25mg Se/kg BW) at three and 25 days after birth. The pens were cleaned daily, avoiding puddles or areas with humidity to elude the presence of coccidia. Milking of the goats was done two times a day, and a remnant of milk was left in the udder for the young's suckling. Additionally, there was a pen that allowed only the walk-through of the kids (Creep-feeding) to learn to ingest solid food that is high in protein and energy. Weaning was done at around 60 days of age.

The response variables measured during the 56 days of study were:

a) Presence of diarrhea: (number of kids with diarrhea/number of kids per treatment) ×100.
b) Percentage of total mortality: dead kids/weaned kids.
c) Findings in the necropsy.
d) the body weight gains (BWG).

This last variable was measured at days: 1, 8, 16, 24, 32, 40, 48, and 56 from birth.

Statistical Analysis

The presence of diarrhea and percentage of mortality were analyzed with the Kruskal-Wallis non-parametric test. Necropsy findings were interpreted visually. The BWG was analyzed with a 4×8 factorial arrangement considering the four treatments and the eight weight sequences registered and identified as BWG.

Mean comparisons between treatments and measurements recorded over time were analyzed with the PROC MIXED SAS Statistical Analysis Program version 14.1 (SAS/STAT, 2015). Statistical significance was performed at the value of $P<0.05$ using the following statistical model:

$$Y_{ij} = \mu + T_i + M_j + TM_{ij} + \varepsilon_{ij}$$

Where: μ = mean; T_i = Effect of the i-th treatment ($i = 1, 2,..., T$); M_j = Effect of the j-th sampling time ($j = 1, 2,..., M$); TM_{ij} = Effect of the interaction of the i-th treatment for the umpteenth time; ε_{ij} = Random error for treatment i and time j.

RESULTS

Presence of Diarrhea and Mortality

The diarrhea presences were TC=16%, T2=1%, T3, and T4=0% (TC *vs.* T2, T3, T4, P<0.05).

Mortality percentages were TC=17%, T2, T3, and T4=0% (TC *vs.* T2, T3, T4, P<0.05).

All the diarrheas presented in the kids were semi-liquid, the perianal surface was dirty, with no foul odor, the excretion color was yellow-brown, indicative of colibacillosis. No diarrhea was melena type, ruling out the clinical presence of coccidiosis. The kids that presented the highest incidence of diarrhea were the TC, and all died. During the necropsy, clinical signs of dehydration, intestinal gasses, little presence of perirenal fat, and weight loss were observed.

Body Weight Gain

Table 1 shows the BWGs of the kids. There was no interaction effect between treatments and sampling times (P>0.05). Between the period times from 16 to 24 days, T2 and T3 increased (P<0.05) 1.9 kg in the BWG, unlike the TC. Then, during the period from 32 to 56 days, all the treatments supplemented with probiotics were better than the TC (~9.81 *vs.* 6.62 kg, P<0.05). Specifically, T3 was the best treatment, unlike T2 and T4, at 56 days (P<0.05).

Table 1. Body weight gains in kids Alpine-French supplemented with conjugate of probiotics*.

Supplement Days	TC 0 mL/kg BW	T2 2 mL/kg BW	T3 3 mL/kg BW	T4 4 mL/kg BW	SEM
1	3.02a	3.25a	3.67a	3.45[a]	0.48
8	3.99a	4.98a	5.02a	4.47[a]	0.48
16	5.40b	6.47a	6.87a	5.82ab	0.52
24	5.31c	7.42ab	8.28a	6.66bc	0.64
32	5.81c	8.21a	9.35b	7.58 a	0.48
40	6.25c	8.92ab	9.78a	8.01b	0.48
48	6.37c	10.21a	11.35b	9.16[a]	0.52
56	8.07c	11.5a	13.07b	10.58[a]	0.64

*Lactic acid bacteria *Bifidobacterium bifidum essensis*, *Lactococcus lactis*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* with viable microorganisms of 10^6 colony forming units per mL. SEM=Standard error of the mean. Different letters between the same line show significant difference (P<0.05).

Table 2 shows the gains in body weight between males and females, regardless of the type of treatment, there were no significant differences by type of sex (P>0.05). It is worth mentioning that kids with signs of diarrhea, were not recorded their weight to affect the gains in body weight.

There was no mortality in the treatments supplemented with probiotics, while in the TC group was 12%, the causes of death are attributed to the onset of diarrhea, which in some way complicated the health of the animals with the presence of dehydration and body condition losses. The results indicate that the minimum dose of 1012 CFUs prevented colonization of pathogenic bacteria in kids. The objective of the study was to colonize the intestine with probiotic bacteria, prevent the invasion of pathogens, and prevent diarrhea from the first days after birth. Studies suggest the use of probiotics on the occurrence of diarrhea, decreased their presence in 37% (Görgülü *et al.*, 2003), recovering kids is usually after three days (Anandan *et al.*, 1999) as digestibility improves production and balance of the microbiota in the rumen; better BWGs were reflected in the kids (Galina *et al.*, 2009).

Other studies have administered single 2.3mL doses of CFU (*Lactobacillus* and *Lactococcus* spp.) per kg BW and no response was observed in the BWG (Gómez, 2020). Changes in microbial composition and population in the digestive tract led to a healthier condition. Probiotics are defined as dietary supplements containing potentially beneficial bacteria and yeasts and generally provide health benefits to the host. However, it also depends on the probiotic strain. The study by Santos *et al.* (2003) demonstrated that *L. acidophilus* CYC 10051, and *L. kefiranofaciens* CYC 10058 were more viable in intestinal health, although benefits were not always obtained in weight gains in kids (Anandan *et al.*, 1999; Ayişiği *et al.*, 2005; Görgülü *et al.*, 2003). Considering the digestive health benefits there are more advantages when using probiotics.

Other studies have evaluated food consumption, however, in this study, the consumption of milk and the sporadic ingestion of solid food was not measured, it is known that a good vitality of the kid helps it be more active to increase consumption. Baldwin *et al.* (2004) noted that kids fed only dairy diets, had limited rumen development and less capacity

Table 2. Body weight gains in three female and male Alpine-French kids without considering the assigned treatments

Number	Males*	Females*	SE
1	3.0833	3.4889	0.09
8	4.9167	4.7306	0.23
16	6.3083	6.2322	0.28
24	7.0833	7.1528	0.41
32	8.1667	7.9306	0.46
40	8.5833	8.4722	0.50
48	9.75	9.6111	0.39
56	11.00	11.3039	0.21

* Without significant statistical differences (P>0.05). SE=standard error.

than kids exposed to solid diets as a supplement. The efficacy of probiotic use can have coincidences or contradictions, Yoon, and Stern (1995) reviewed several studies with probiotics and concluded that at least 40% of the studies had positive responses to microbial supplements. Although, the answer depends on the environmental conditions (Donovan *et al*., 2002, Krehbiel *et al*., 2002) the comfort or overcrowding environment, immune status, hygiene conditions and the care given to the young (Cruywagen *et al*., 1996). Concerning the differences between the sexes of the offspring; the study by Castillo-Rodríguez *et al*. (2013) reported higher weight in male goats than in neonatal females supplemented with probiotics, in the case of our study, there were no differences.

The continuous application of probiotics per week increased the management of the kids; perhaps it is assumed that there is a more significant economic expense. However, the result of not presenting any dead animal in the groups that were given the probiotics, justifies its continuous application and, it is considered an excellent strategy to maintain the survival of the kids. The effect is due to the continuous presence of probiotics in the digestive tract that causes dominance over pathogens and the dominance of probiotic conjugated microorganisms. Krehbiel *et al*. (2002) suggested that the efficacy of probiotics should be evaluated more in terms of their health benefits, as higher growth presented.

CONCLUSION

The seven-fold application from 10^{12} to 10^{16} CFU/kg BW is a conjugate of probiotics *Bifidobacterium bifidum Essensis*, *Lactococcus lactis*, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus*, improved health in kids and did not show any death. The dose of 10^{14} CFU/kg BW was the most suitable, improving the BWG up to 56 days. The dosage of these probiotics is suggested from the first day of birth to weaning of the kids, to reduce the causes of death from enteric diseases.

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