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Effect of lactic acid bacteria and lactose on growth performance and intestinal microbial balance of artificially reared calves

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ABSTRACT

The aim of the present study was to evaluate the effect of lactose and a microbial inoculum integrated by three lactic acid bacteria (LAB) strains of bovine origin to improve the growth performance and the intestinal microbial balance in young calves. The experimental group of calves was administered with an inoculum consisting of three microorganisms (*Lactobacillus casei* DSPV 318 T, *Lactobacillus salivarius* DSPV 315 T and *Pediococcus acidilactici* DSPV 006 T) suspended in a solution of NaCl 0.15 M, at a daily dose of 10^9 CFU/kg for 35 days, whereas the control group was administered only NaCl solution as placebo. A factorial design with repeated measures in complete blocks at random was used. Four blocks with six calves each were built and the six proposed treatments randomly distributed. The experiment was performed in 35 days. We found no differences in the growth performance parameters for the probiotic and lactose factors. The probiotic and lactose factors showed significant differences in the values of the *Lactobacillus* spp. counts in feces. The animals supplemented with the probiotic showed the highest LAB counts, which remained constant during the experiment. The animals which consumed the intermediate level of lactose showed the highest LAB counts. Although the probiotic treatment did not reduce the fecal count of coliforms, it generated differences ($P<0.05$) in the *Lactobacillus*/coliforms ratio. The strategy achieved with lactose in this study allowed generating a controlled imbalance in the gastrointestinal tract of calves. This model may be useful to evaluate the beneficial effect of the microbial inoculum with a probiotic potential, especially when adequate sanitary and environmental conditions hinder viewing.

1. Introduction

The indigenous intestinal microbiota of the calf is a complex microbial community that plays an important role in nutrition and health. This ecosystem is under the influence of the healthy status of the host, and this, in turn, is influenced by its environment. The gastrointestinal health may thus be defined as the ability to maintain a balance within the constantly changing ecosystem of the gastrointestinal tract (Melin et al., 1997). In intensive rearing, especially in early-weaned calves, the possibility of acquiring a natural autochthonous microbiota is strongly diminished, and, as a result, pathogenic microorganisms are much more likely to colonize the intestine (Rosmini et al., 2004). If level of protection of the

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intestinal microbiota is diminished, the number of coliforms in the intestine can increase. The health care of meat-producing animals, as well as the maintenance of the food and feed safety throughout the production chain, is a key issue for both the production system and public health. Since lactic acid bacteria (LAB) are known to constitute a barrier against pathogens the use of LAB, as a probiotic supplement, agrees with a worldwide trend that promotes preventive and natural feeding practices, as well as residue-free, healthy and highly nutritious diets.

Although some LAB strains have shown beneficial effects when administered therapeutically, the supply of microorganisms together with feed by way of prophylaxis from birth allows incorporating and establishing the strains selected for the inoculum together with the microbiota of calves. This early colonization of LAB in the intestinal ecosystem allows the action of the inoculum in physiological situations and allows the calf to be in an advantageous position for a possible invasion by a pathogen.

Lactose can be digested by calves (Cruywagen et al., 1996) and other newborn mammals. The good use of any disaccharide or polysaccharide other than lactose is highly limited in young calves because, except for lactase, all the other enzymes that hydrolyze carbohydrates present a relatively low activity in the newborn digestive tract of calves (Davis and Drackley, 2001). The bacterial inoculum tested in this research showed a successful growth capacity in milk medium containing lactose as the only source of carbohydrate. Indeed, this capacity makes the use of this disaccharide interesting to facilitate the permanence of microorganisms at intestinal level. On the other hand, the presence of increased lactose levels in feed may imbalance the diet and thus lead to high incidences of the osmotic type of diarrhea (Fischer and Sutton, 1949). The addition of 100 g/d and 200 g/d of lactose in the diet of artificially raised young calves may cause a nutritional stress that may facilitate the observation of the beneficial effects caused by potentially probiotic LAB.

We have previously observed that some LAB are able to colonize the intestinal tract of mice and remain in it without affecting feed intake, and protecting animals against *Salmonella dublin* DSPV 595 T (Frizzo et al., 2005; Frizzo et al., 2006). In addition, LAB can colonize the gastrointestinal tract of calves without translocating to the internal organs (Frizzo et al., 2010).

The aim of the present study was to evaluate the effect of lactose and a microbial inoculum integrated by three LAB strains of bovine origin on the growth performance and development of fecal *Lactobacillus* and coliforms in young calves.

2. Materials and methods

2.1. Animals and facilities

The study was carried out at the Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Esperanza (Santa Fe province, Argentina), in an area specially designed for artificial calf rearing, and involved 24 male calves (*Bos taurus*), aged 10 days old on average. Intensive rearing was carried out in an area with natural pasture. Each animal was confined to its individual feeder by a 3-m long tether, with a limited space to move. Every week, each animal was moved to a new space with the same soil characteristics and free of droppings. Throughout the experiment, all the animals were fed with milk replacer and drinking water. The milk replacer (Booster®, Alimental, Provimi) was reconstituted at 11% of dry matter (DM), and administered to calves at 06:00 A.M. (2 l/animal) and 06:00 P.M. (2 l/animal), at approximately 38 °C. A commercial concentrate pellet (starter) (Concentrados Garay S.A.) was offered to calves *ad libitum*. The experiment lasted 35 days and was conducted according to the Guide for the Use and Care of Agricultural Animals in Agricultural Research and Teaching (FASS, Federation of Animal Science Societies, 1998).

2.2. Lactic acid bacteria

Three bacterial strains of bovine origin – *Lactobacillus casei* DSPV 318 T, *Lactobacillus salivarius* DSPV 315 T and *Pediococcus acidilactici* DSPV 006 T – showing probiotic properties (Frizzo et al., 2005, 2006, 2010) were used. The strains were isolated from healthy dairy calves artificially reared by a work team from the "Departamento de Salud Pública Veterinaria", Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Esperanza (Santa Fe, Argentina), and then kept at −80 °C in MRS medium with glycerol (35% v/v) and identified using molecular techniques (Schneider et al., 2004). Their 16 S rDNA gene GenBank accession numbers are: FJ787305, FJ787306 and FJ787307, respectively.

2.3. Preparation and administration of the inoculum

Bacteria were multiplied in MRS broth for 18–20 h at 37 °C. The optical density of the cultures was determined at 560 nm and the bacterial concentration was calculated using a calibration curve (Frizzo et al., 2006). Cultures were centrifuged at 3000 x*g* for 10 min, and suspended in a NaCl solution. The three strains were then mixed and completed until they reached final volume. The probiotic inoculum consisted of a 40-ml dose of the three microorganisms, in a 0.15 M NaCl solution. The inoculum was dosed at 10^9 CFU/kg BW daily via the milk replacer. In the groups where the probiotic factor was at level 0 (P_0), calves were inoculated only with 40 ml of 0.15 M NaCl, which served as placebo.

2.4. Feed composition

The feeds used were not supplemented with antibiotics. The milk replacer contained 210 g/kg crude protein, 400 g/kg lactose and 160 g/kg ether extract. The lactose contained 990 g/kg lactose. The starter was formulated with the following ingredients: ground corn grain, soy bean pellet, wheat bran, dicalcic phosphate, sodium chloride and a vitamin–mineral supplement. The starter also contained 900 g/kg DM, 180 g/kg crude protein, 2.9 Mcal ME/kg DM, 800 g/kg total digestible nutrients, 50 g/kg crude fiber, 12 g/kg Ca, 8 g/kg P and 50 g/kg ether extract. All feed composition data were indicated in the label provided by the feed supplier.

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2.5. Experimental design

A factorial design of randomized blocks was used. Four blocks with six calves each were built and the six proposed treatments (without probiotic and lactose-free, P_0L_0; without probiotic + lactose at level 1, P_0L_1; without probiotic + lactose at level 2, P_0L_2; with probiotic and lactose-free, P_1L_0; with probiotic + lactose at level 1, P_1L_1; with probiotic + lactose at level 2, P_1L_2) randomly distributed. The probiotic inoculum was administered to each calf in the experimental group where the probiotic factor was level 1 (P_1), once daily, together with the milk replacer supplied in the afternoon, during the 35 days of experiment. In the groups where the probiotic factor was at level 0 (P_0), calves were inoculated with placebo. Lactose was administered to each calf in the experimental group where the lactose factor was at level 1 ($L_1 = 100$ g/d) and at level 2 ($L_2 = 200$ g/d). For the preparation of feed for each calf, the milk replacer was weighed (11% = 220 g/calf) and lactose was added (0 g for L_0, 50 g for L_1 and 100 g for L_2) and finally all were dissolved in 2 liters of water. The solid was mixed strongly with the liquid to achieve a homogeneous mixture. The frequency of diarrhea was measured daily during macroscopic analyses of the feces and assigning values from 1 to 4 according to their particularities. The evaluation of such characteristics was performed following the parameters of fecal consistency and according to the scores proposed by Meyer et al. (2001): 1-Normal: solid feces, but not hard; its original shape is slightly modified when falling; 2-soft, looser than normal, it has no shape it is set down in heaps and is slightly scattered; 3-fluid: is rapidly scattered forming 6-mm-thick sheets; 4-aqueous: liquid consistency, diarrheal feces. An animal scoring 4 was considered "with diarrhea". The animals with diarrhea remained fasting for 24 h and were then given 1 l hydrating dextrose saline solution (06.23 g/kg glucose, 10.65 g/kg sodium chloride, 2.685 g/kg sodium carbonate, 1.935 g/kg potassium chloride) orally, twice daily. The milk replacer diet was started again when the fecal consistency index (FCI) was 3 or less. No antibiotic treatments were administered. The FCI was used as an indicator of the intensity and duration of the depositions. The higher this index, the more intense and lasting the softening of feces is:

$$\mathrm{FCI} = \frac{[(\mathrm{dE1} \times 1) + (\mathrm{dE2} \times 2) + (\mathrm{dE3} \times 3) + (\mathrm{dE4} \times 4)]}{Td \times 4} \times 100$$

where dE1, dE2, dE3 and dE4 represent the number of days with fecal consistency scoring 1, 2, 3 and 4 respectively and Td represents the total number of days involved in the experiment (Td = 35). The weekly FCI was also calculated (Td = 7).

Body weight (BW), wither height (WH) and heart girth (HG) were recorded weekly. Feed and water consumption (WC) were measured daily. Total feed intake (TFI) was calculated according to the consumption of milk replacer and starter (SI). Live weight gain (LWG) was obtained by means of the difference between weights in the corresponding time period. Feed efficiency (FE) was calculated by relating TFI (kg) and LWG (kg). The fecal counts of *Lactobacilli* and coliforms were carried out weekly. *Lactobacillus* counts in the jejunal mucosa were carried out at the end of the experiment.

2.6. Necropsies

Programmed necropsies were performed at the end of the experiment, in two animals from each experimental group, 22 h after the last administration of the probiotic inoculum. The animals were desensitized by means of a euthanasic drug (Euthanyle®, Brouwer S.A.) administered in aseptic conditions.

Later, animals were bled and then conventional necropsy techniques were followed (Rodríguez Armesto, 2004). The spleen and jejunum were collected using sterile instruments, minimizing the possible bacterial contamination between samples (Lee et al., 2000). Spleen weight was determined and, together with BW, spleen weight index (SWI) was calculated as follows:

$$\mathrm{SWI} = \frac{\text{Spleen weight (g)}}{\text{Body weight (kg)}}.$$

2.7. Recovery of LAB and coliforms from feces

The fecal samples obtained weekly from three calves from each experimental group were weighed in sterile conditions and diluted 1/100 in ¼ Ringer solution. All the samples were collected and analyzed individually for each calf and sampling occasion and then homogenized with a magnetic stirrer. To determine the microbial load, serial dilutions of the samples were spread in selective media: LAMVAB for *Lactobacillus* spp. (Timmerman et al., 2005) and VRBL for coliforms (Deneckova et al., 2002). The petri dishes with LAMVAB medium were incubated at 37 °C for 48 h under anaerobic conditions and those with VRBL medium were incubated at the same temperature for 24 h under aerobic conditions. Subsequently, characteristic colonies were counted and CFU/g stool were calculated for each bacterial group.

2.8. Recovery of LAB from the jejunum

The jejunum was aseptically obtained from all calves. The mucosa of the jejunum was scraped, and decimal dilutions were carried out in ¼ Ringer solution to facilitate the total *Lactobacillus* spp. counts (Frizzo et al., 2010). Each sample was diluted in series and LAMVAB agar plates were spread in triplicate to count total lactic microbiota. Petri dishes were incubated at 37 °C for 48 h in anaerobic conditions and the characteristic colonies were counted.

2.9. Statistical analysis

The variables BW, WH, HG, TFI, SI, LWG, FCI and microbial counts in the feces were analyzed as a randomized complete block design in a factorial arrangement with repeated measures. The variables SWI, FE and microbial counts in the jejunal mucosa were analyzed as a randomized complete design in a factorial arrangement. Statistical analysis was conducted with SPSS for Windows software. Differences between treatment means were tested for significance ($P < 0.05$) with the Duncan's test.

The incidence density of diarrhea in each experimental group was obtained as the proportion of animals which suffered diarrhea during a specific period of time (daily) over

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the total time during which the animals of the population were at risk (35 d). The association between the probiotic and lactose variables and incidence of diarrhea was compared by the χ^2-test. The null hypothesis was that there were no differences between groups and alpha was 5%. A logistic regression was performed to identify the significant differences across the levels of lactose used in the experiment. The estimation method was maximum likelihood with a convergence criterion of 0.01.

3. Results

3.1. Effects of the treatment on animal performance

The initial values of BW, WH and HG were similar in all the groups studied ($P>0.05$). BW, WH, HG, TFI, LWG and SI showed an increase along the experiment, regardless of the treatment (Table 1). There were no differences in BW, WH, HG, TFI, SI, and FE for the probiotic and lactose factors.

3.2. Effect of the treatment on diarrhea frequency, fecal consistency index and mortality rate

Although some animals suffered diarrhea, the general health status was good (without signs of other diseases). The lactose factor showed differences in the values of the FCI ($P=0.001$; Table 2). The animals which consumed the maximum level of lactose (regardless of the probiotic treatment) showed the highest FCI. A decrease in FCI was observed along the experiment, and the lowest level was reached at week 5 (Fig. 1). The animals that did not consume lactose or were exposed to its intermediate level (L_1) showed a decrease in the FCI during the first week and remained constant until the end of the experiment. On the other hand, the animals that consumed the highest level of lactose (L_2) showed an increase in the FCI values, which reached their maximum level at week 3 and then decreased sharply until week 5 (Fig. 1). Also, the animals that consumed higher amounts of lactose (L_2) presented the highest incidence density of diarrhea (Table 2). The relative risk for the L_2 group, calculated by logistic regression, indicated that this group had a 19.85 times (95% CI 4.72–83.41, $P<0.001$) increased risk of diarrhea than those animals which had not consumed lactose. It was not possible to determine any effect of the inoculum on the frequency of diarrhea or the FCI. No deaths were recorded during the experiment.

3.3. Effect of the treatment on the intestinal microbial balance

The probiotic and lactose factors showed no significant differences in the values of the coliform counts (Table 3). This microbial population presented a decrease during the experiment, without considering the treatment. The animals which consumed the intermediate level of lactose (L_1) and probiotic showed a significant decrease in the number of coliforms, while the animals which had not received the probiotic presented an increase in the coliform counts. On the other hand, animals that were supplemented with the probiotic presented an increase in the coliforms when ingesting the maximum level of lactose (L_2).

The probiotic and lactose factors showed significant differences in the values of the *Lactobacillus* spp. counts (Table 3). The animals that were supplemented with the probiotic showed the highest LAB counts, which remained constant during the experiment. The animals that consumed the intermediate level of lactose showed the highest LAB counts. This microbial population presented a decrease during the experiment, without considering the treatment. While the LAB counts in the animals supplemented with the probiotic remained constant during the experiment, a progressive decrease was observed in the animals not supplemented with the probiotic.

The probiotic factor showed significant differences in the values of the *Lactobacillus*:coliforms ratio (Table 3). The animals that were supplemented with the probiotic showed the highest ratio, which remained constant during the experiment. While the ratio in the animals supplemented with the probiotic increased during the experiment, a progressive decrease was observed in the animals not supplemented with the probiotic (Fig. 2). The animals which consumed lactose did not show differences.

3.4. Effect of the treatment on the total lactic microbiota of the jejunum

The recovery of the total lactic microbiota of the jejunum in the animals killed at the end of the experiment showed significant differences ($P<0.05$) for the probiotic factor, being the values higher in the P_1 group (3.5 logCFU/g) than in the P_0 group (2 logCFU/g). No effect of lactose was observed on the bacterial counts in the jejunum.

Table 1
Growth performance of lactose-supplemented calves at three levels (L_0, L_1 and L_2) and probiotic-supplemented calves at two levels (P_0 and P_1). The probiotic supplement was administered at a daily dose of 10^9 CFU/kg BW for 35 d only in P_1. The lactose supplement was administered at a daily dose of 100 g and 200 g in L_1 and L_2 respectively.

Parameters	Treatment						SEM	P-value [a]						
	P_0L_0	P_0L_1	P_0L_2	P_1L_0	P_1L_1	P_1L_2		P	L	P*L	T	T*P	T*L	T*P*L
Calves (#)	4	4	4	4	4	4	–	–	–	–	–	–	–	–
Body weight (kg)	59.7	62.2	63.5	65.5	65.4	62.6	17.73	NS	NS	NS	<0.001	NS	NS	NS
Live weight gain (kg/wk)	2.8	3.2	3.6	3.9	3.9	3.3	2.34	NS	NS	NS	<0.001	NS	NS	NS
Starter intake (kg DM/calf)	16.9	15.2	19.3	20.9	19.1	15.1	1.49	NS	NS	NS	<0.001	NS	NS	NS
Total feed intake (kg DM/calf)	32.2	33.7	38.8	36.2	37.3	34.9	1.53	NS	NS	NS	<0.001	NS	NS	NS
Feed efficiency (kg DMI/kg gain)	2.4	2.1	2.3	1.9	2.0	2.1	0.12	NS	NS	NS	–	–	–	–
Heart girth (cm)	90.8	93.8	94.8	93.8	95.8	93.3	8.13	NS	NS	NS	<0.001	NS	NS	NS
Wither height (cm)	87.3	85.3	85.3	85.5	84.5	84.8	3.13	NS	NS	NS	<0.001	NS	NS	NS

[a] Not significant, defined as $P>0.05$, is denoted by NS.

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Table 2
Fecal consistency index and diarrhea frequency of lactose-supplemented calves at three levels (L_0, L_1 and L_2) and probiotic-supplemented calves at two levels (P_0 and P_1). The probiotic supplement was administered at a daily dose of 10^9 CFU/kg BW for 35 d only in P_1. The lactose supplement was administered at a daily dose of 100 g and 200 g in L_1 and L_2 respectively.

Parameters	Treatment						SEM	P-value [a]						
	P_0L_0	P_0L_1	P_0L_2	P_1L_0	P_1L_1	P_1L_2		P	L	P*L	T	T*P	T*L	T*P*L
Calves (#)	4	4	4	4	4	4	–	–	–	–	–	–	–	–
Fecal consistency index (%)	37.9	44.5	60.4	39.3	45.7	58.4	13.22	NS	0.001	NS	<0.001	NS	NS	NS
Incidence density of diarrhea (%)	0.7	2.1	13.6	0.7	3.6	11.4	–	NS	<0.001	–	–	–	–	–

[a] Not significant, defined as P>0.05, is denoted by NS.

3.5. Effect of the treatment on SWI

The values of the SWI of calves were between 1.8 g/kg (SD = 0.4 g/kg) and 2.7 g/kg (SD = 1.6 g/kg). There were no significant differences (P>0.05) for both the probiotic and lactose factors.

4. Discussion

In the present study, we hypothesized that the addition of lactose in the diet of young calves may cause a nutritional stress that may facilitate the observation of the beneficial effects of the administration of a mix of three potentially probiotic LAB. Thus, the multispecies inoculum used in our experiment could be beneficial in making a more efficient use of lactose and in preventing the gastrointestinal problems caused by that nutritional stress.

The high levels of lactose (200 g/d) fed to the animals in our work caused a significant imbalance in the intestines of the calves, a fact that was clearly reflected in the high frequency of diarrhea observed in these animals. This is because any luminal or serosal factor affecting the transport systems will also affect electrolyte and water movements. Besides the specific mechanisms involved in water movement in the gut, osmotic diarrhea can also be induced when a non-absorbable compound reaches the intestinal lumen and maintains an osmotic gradient between the intestinal lumen and the blood. A typical case of osmotic diarrhea is that induced by lactose malabsorption in the case of lactase deficiency (Heyman and Ménard, 2002). In our work, both the fact that the FCI and the diarrhea frequency were significantly higher in the animals fed lactose and the fact that lactose was responsible for generating it are unquestionable. Calves are particularly vulnerable to digestive disorders during the first weeks of life and nutritional imbalances and gastroenteric diseases are common in intensive rearing systems. Under such conditions of management, the probiotic inoculum during rearing could be used to prevent diarrheal diseases of nutritional origin. However, further experiments should be carried out to assess whether the inoculum is able to act against the primary pathogens of diarrhea and to evaluate their behavior in this extreme condition.

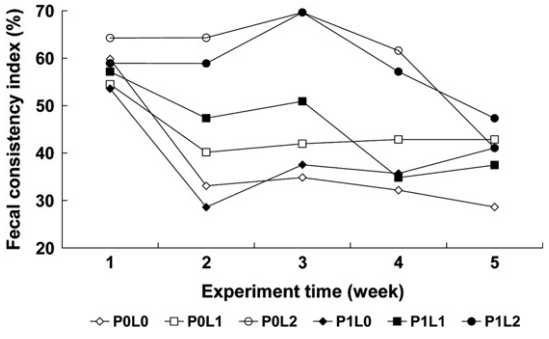

Fig. 1. Fecal consistency index of lactose-supplemented calves at three levels (L_0, L_1 and L_2) and probiotic-supplemented calves at two levels (P_0 and P_1). The probiotic supplement was administered at a daily dose of 10^9 CFU/kg BW for 35 d only in P_1. The lactose supplement was administered at a daily dose of 100 g and 200 g in L_1 and L_2 respectively.

Younger calves are more susceptible to diarrhea as a consequence of lactose addition, although, paradoxically, the activity of lactase decreases over the life of the young calf. During our experiment, the FCI decreased, showing that older animals are more tolerant to lactose. However, we observed that the level of tolerance is related to the concentration of lactose consumed by animals. The activity of intestinal microbiota may explain, at least in part, the fact that lactose is better used in older animals. Bacteria interfere with the intestinal physiology, but, conversely, the intestinal environment is important in conditioning bacteria. The gut environment is able to modify the ingested bacteria and thus affect their viability (Heyman and Ménard, 2002). The highest level of lactose used in our experiment backfired both for the parameters of growth performance, health and intestinal microbial balance. However, the intermediate level of lactose used in our work was able to generate an increase in the number of microorganisms of the genus *Lactobacillus* at the end of the experiment. Moreover, a very strong increase was observed in the animals supplemented with the inoculum. This could be related to an adequate housing in the gut by the microorganisms that make up the inoculum. The explanation for the favorable influence of lactose on intestinal lactic acid bacteria is that when lactose is present, even in small quantities, the best cultural and environmental conditions are created for these organisms in particular, without a positive change for other bacteria normally present. Thus, a diet high in lactose has a marked influence on the intestinal flora (Atkinson et al., 1957) and the persistence of the ingested lactic acid bacteria in the gastrointestinal tract, at least for a few days, is necessary for their beneficial effect (Heyman and Ménard, 2002). Although the number of coliforms did not decrease in the calves supplemented with the inoculum, no increases due to the increased dietary content of lactose (a carbohydrate easily used by this microbial population) were observed. However, the interaction of the factors showed a favorable effect on the intestinal microbial balance. Although lactose is easily used by many intestinal microorganisms, it

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Table 3
Average weekly count of coliforms and Lactobacillus spp. and Lactobacillus:coliforms ratio in feces of lactose-supplemented calves at three levels (L_0, L_1 and L_2) and probiotic-supplemented calves at two levels (P_0 and P_1). The probiotic supplement was administered at a daily dose of 10^9 CFU/kg BW for 35 d only in P_1. The lactose supplement was administered at a daily dose of 100 g and 200 g in L_1 and L_2 respectively.

Parameters	Treatment						SEM	P-value [a]						
	P_0L_0	P_0L_1	P_0L_2	P_1L_0	P_1L_1	P_1L_2		P	L	P*L	T	T*P	T*L	T*P*L
Calves (#)	3	3	3	3	3	3	–	–	–	–	–	–	–	–
Coliforms (CFU/g feces)	7.41	7.86	7.40	7.86	7.30	7.71	0.858	NS	NS	0.049	<0.001	NS	NS	NS
Lactobacillus sp (CFU/g feces)	6.87	7.50	7.09	8.61	8.61	8.25	0.617	<0.001	0.026	NS	<0.001	<0.001	NS	NS
Lactobacillus:coliforms ratio	0.93	0.97	0.96	1.11	1.21	1.08	0.024	<0.001	NS	NS	NS	0.002	NS	NS

[a] Not significant, defined as $P>0.05$, is denoted by NS.

appeared to have exerted, at the intermediate level used in our experiment, a considerable power of selection on the members of the intestinal microbiota and, together with the LAB of the inoculum, was responsible for generating a favorable microbial relationship. It is likely that these two factors improve the use of lactose in the intestine, and that this, in turn, might feedback the system by favoring the maintenance of beneficial bacterial populations that are better adapted to the conditions generated by the organic acid in the gut. In feces from healthy calves, *Lactobacillus* counts are usually greater than those of coliforms (*Lactobacillus*/coliforms ratio >1) but lower than those from calves suffering from diarrhea (Abu-Tarboush et al., 1996). After 5 weeks of probiotic treatment, the *Lactobacillus*/coliforms ratio was ≤1 in the animals not treated with the LAB inoculum and ≥1 in inoculated groups.

The bacteria given in the diet need to survive the passage through the stomach and remain active. It is thus important to use strains belonging to the indigenous population isolated from the same animal host species for which the inoculum is intended, because they are adapted to the environment of the gastrointestinal tract. The inoculum used in the present work contributed to the colonization of the jejunum of the animals inoculated with LAB (P_1) by microorganisms of the genus *Lactobacillus* because it allowed the establishment of these beneficial microorganisms at the level of the small intestine, where they found a suitable place for their development and lodging. The young animals used in the experiment significantly were able to modify the number of beneficial microorganisms present in the jejunal mucosa and this may partly explains some of the benefits detailed. However, additional research should be conducted.

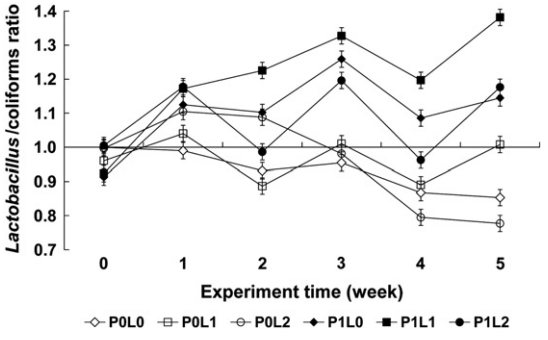

Fig. 2. *Lactobacillus*/coliforms ratio in feces from lactose-supplemented calves at three levels (L_0, L_1 and L_2) and probiotic-supplemented calves at two levels (P_0 and P_1). The probiotic supplement was administered at a daily dose of 10^9 CFU/kg BW for 35 d only in P_1. The lactose supplement was administered at a daily dose of 100 g and 200 g in L_1 and L_2 respectively.

Furthermore, because the consumption and weight gain were adequate and the health of the animals was good, it is unlikely that the treatment with the microbial inoculum generated an adverse effect on animal health, as observed in previous experiments (Frizzo et al., 2010). Moreover, the results obtained in the spleen weight index reinforce the assertion that the animals showed no signs of toxicity and remained in an adequate state of health.

The intake of milk replacer with a lactose concentration higher than the usual one (5% lactose in milk, 7.5% lactose in L_1 and 10.0% lactose in L_2) generated an unbalanced state, demonstrated by the high values of FCI and frequency of diarrhea found. This experimental model exposed calves to a higher vulnerability to suffer from digestive disorders. This situation was especially interesting to evaluate the microbial inoculum behavior due to the difficulties present when animals are in a very good health because of adequate sanitary and environmental conditions. Beyond the strategy achieved with lactose in this study, any other researcher aiming to generate a controlled imbalance in the gastrointestinal tract will find useful to evaluate the beneficial effect of microbial inocula with probiotic potential.

5. Conclusion

Very few studies have examined the effects of LAB as supplements in calves exposed to nutritional stress. The microbial inoculum used here was able to maintain a stable microbial load of *Lactobacillus* spp. at a critical stage of development of the animals and to improve the health status of calves. The strategy achieved with lactose in this study allowed generating a controlled imbalance in the gastrointestinal tract of calves. This model may be useful to evaluate the beneficial effect of the microbial inoculum with a probiotic potential, especially when adequate sanitary and environmental conditions hinder viewing.

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