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Intestinal populations of *Lactobacilli* and coliforms after *in vivo* *Salmonella dublin* challenge and their relationship with microbial translocation in calves supplemented with lactic acid bacteria and lactose

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ABSTRACT

The aim of the present study was to evaluate the relationship between intestinal microbial populations and bacterial translocation in young calves supplemented with lactic acid bacteria (LAB) and lactose and experimentally infected with *Salmonella dublin* DSPV 595T. Fifteen calves were divided into three groups (a control group, a group inoculated with LAB, and a group inoculated with LAB and lactose), with 5, 6 and 4 animals each, were used. The LAB inoculum, composed of *Lactobacillus casei* DSPV 318T, *L. salivarius* DSPV 315T and *Pediococcus acidilactici* DSPV 006T was administered together with the milk replacer. The groups inoculated with LAB and LAB and lactose received a daily dose of 10^9 CFU/kg body weight of each strain throughout the experiment. Lactose was provided in doses of 100 g/d. The administration of the pathogen was performed on all animals on day 11 of the experiment with 2×10^{10} CFU. The levels of probiotic inoculum found in the large intestine of the animals from the two groups inoculated with the probiotic [0] were of 5 $\log_{10}$ CFU/g, whereas those in the small intestine were of 5 $\log_{10}$ CFU/g in the animals inoculated with LAB and lactose, and approximately 1 $\log_{10}$ CFU/g less in the group inoculated with LAB. The levels of *Salmonella* in both, the small and large intestines, were between 3 $\log_{10}$ CFU/g and 4 $\log_{10}$ CFU/g. High microbial loads were found in the internal organs. *Lactobacillus* spp. were found only in the lymph nodes of the two groups inoculated with the probiotic, in very low amounts (<1 $\log_{10}$ CFU/g). Despite the high concentration of *Salmonella* administered to calves, the LAB inoculum of bovine origin was not capable of translocating to the internal organs in the extreme situations of intestinal imbalance generated by the pathogen.

Abbreviations: BBA, brucella blood agar; BW, body weight; CFU, colony forming unit; IleoLN, ileocaecal lymph node; LAB, lactic acid bacteria; LAMVAB, *Lactobacillus* anaerobic MRS with vancomycin and bromocresol green; MesLN, mesenteric lymph node; TDN, total digestible nutrients; VRBL, violet red bile agar with lactose; XLD, xylose lysine deoxycholate.

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1. Introduction

Healthy animals have in their gastrointestinal tracts a typical microbiota that, after the colonization during the first days of life, reaches a symbiotic state. *Lactobacilli* are common components in the normal intestinal microbiota, both in human beings and domestic animals (Rosmini et al., 2004). Competitive colonization by beneficial microorganisms like *Lactobacillus* sp. and *Streptococcus* sp. happens at an early age (Ziemer and Gibson, 1998), in order to protect animals when exposed to pathogens like *Salmonella* spp. and *Escherichia coli*.

In previous studies, the levels of lactose used were able to generate an increase in the number of microorganisms of the genus *Lactobacillus* (Frizzo et al., 2010b). The explanation for the favourable influence of lactose on the intestinal lactic acid bacteria (LAB) to be found is the fact that when lactose is present, even in small quantities, the best environmental conditions are created for these organisms in particular, without a positive change for the other bacteria normally present. The calves inoculated with probiotics may take a better advantage of lactose and thus have a better growth performance. It is thus desirable to make further experiments to evaluate whether the inoculum is able to act against the primary pathogens that cause diarrhea and assess their behavior in this extreme condition (Frizzo et al., 2010b).

Bacterial translocation is the passage of viable indigenous bacteria from the gastrointestinal tract to extra-intestinal places (Berg, 1995). The study of intestinal microbial populations and their relationship with bacterial translocation is very important in safety experiments of potentially probiotic strains (Frizzo et al., 2010a). Experimental models of intestinal disease could be used to evaluate the ability of different components of the intestinal microbiota to enter the internal organs in a situation of total disruption of the barrier effect generated by an intestinal pathogen. Thus, it could appreciate the affinity or otherwise of some populations to translocate in an extreme situation of intestinal imbalance.

The aim of the present study was to evaluate the relationship between intestinal microbial populations and bacterial translocation in young calves supplemented with LAB and lactose and experimentally infected with *Salmonella dublin*.

2. Materials and methods

2.1. Animals and facilities

The study was carried out in an area designed for Artificial Calf Rearing that belongs to the Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina, and involved 15 male calves (*Bos taurus*), aged 5 days old in average. Colostrum intake was verified by the supplier according to their routine procedures. The calves were kept separated from each other in single cages to avoid re-infections. Intensive rearing was carried out on dirt floor covered with natural grass. Every week, each animal was moved to a new area 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 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 was offered to calves *ad libitum*. The experiment lasted 15 days and was conducted according to the Guide for the Use and Care of Agricultural Animals in Agricultural Research and Teaching (FASS, 1998). The protocol used was previously approved by an Advisory Committee on Ethics and Security of the Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral.

2.2. Feed composition

Feeds used were not supplemented with antibiotics. The milk replacer contained 230 g/kg crude protein, 150 g/kg fat, 10 g/kg crude fiber, 10 g/kg Ca and 8 g/kg P (AF-80™, ACA). The lactose contained 990 g/kg lactose (Milkaut, S.A.). 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 feed supplier.

2.3. Microorganisms

Three bacterial strains of bovine origin – *Lactobacillus casei* DSPV 318T, *Lactobacillus salivarius* DSPV 315T and *Pediococcus acidilactici* DSPV 006T – showing probiotic properties (Frizzo et al., 2010a,b, 2011) were used. Their Genbank accession numbers of 16S rDNA gene sequences are FJ787305, FJ787306 and FJ787307, respectively. The *Salmonella dublin* DSPV 595T strain of bovine origin was isolated from organs of a necropsy conducted at the Animal Health Hospital of the Facultad de Ciencias Veterinarias. Its Genbank accession number of 16S rDNA gen sequence is FJ997268.

2.4. Selection of mutants resistant to antibiotics

The inoculum strains were made resistant to rifampicin so as to be able to trace down the inoculum during the *in vivo* study. Resistance of the inoculum strains to the antibiotic was obtained from serial cultures in MRS medium, from low levels up to a concentration of 100 μg/ml rifampicin (Demecková et al., 2002). Rifampicin was prepared in a stock solution

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(10 mg/ml), and used at 100 μg/ml of final concentration. An overnight microorganism culture was spread over MRS agar plates supplemented with rifampicin (MRS_{rif}) and, afterwards, incubated for 48 h at 37 °C. Finally, a colony was obtained by using the isolation method. The isolated strains resistant to rifampicin were cultured in MRS broth (for 24 h at 37 °C). The physiological and biochemical parameters of both the original strains and the ones resistant to rifampicin were compared in order to guarantee that resistance was the only difference between them. Inoculum strains resistant to rifampicin were kept at −80 °C (MRS broth with 35% of glycerol), and later administered to calves. A similar procedure was used with the *Salmonella dublin* DSPV 595T strain using XLD medium and novobiocin (50 mg/ml) and nalidixic acid antibiotics (10 mg/ml) ($XLD_{nov\ nal}$). Physiological, biochemical and genotypic parameters of both the original and resistant strains were compared in order to guarantee that resistance was the only difference between them. The verification was performed with biochemical tests and amplification by PCR of the specific *Inv*A gene of *Salmonella* sp.

2.5. Experimental design

Animals were randomly divided into three experimental groups: the Control Group (C-G), the LAB-Inoculated Group (LAB-G) and the LAB-Inoculated Group supplemented with lactose (L-LAB-G), each with 5, 6 and 4 animals, respectively. The probiotic inoculum was administered to each calf of the LAB-G and L-LAB-G, once daily, together with the milk replacer supplied in the afternoon, during the 15 days of the experiment. The calves of the C-G were inoculated in the same way but with 40 ml of 0.15 M NaCl, which served as placebo. The lactose was administered to each calf in the L-LAB-G at a concentration of 100 g/d. Body weight (BW) was recorded on days 0 and 10. The faecal samples were taken on days 1, 5, 10, 11, 12, 13, 14 and 15. Counts of total *Lactobacillus*, inoculum members and coliforms were carried out in the first three samplings. The determination of the presence/absence of *Salmonella* was conducted both before and after experimental infection. Scheduled necropsies of one calf from each experimental group from day 11 of the experiment until completing all experimental units were performed daily.

2.6. Preparation and administration of the LAB inoculum

Bacteria were cultured in a MRS broth for 18–20 h at 37 °C. The optical density of the cultures was determined at 560 nm, and bacterial concentration was calculated using a calibration curve. Cultures were centrifuged at $3000 \times g$ for 10 min at 18 °C, and suspended in a NaCl solution. Afterwards, the three strains were mixed and completed to reach 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 and administered daily via the milk replacer to the calves of the LAB-G and L-LAB-G. Control calves were inoculated with 40 ml 0.15 M NaCl solution as placebo using the same practice.

2.7. Pathogen inoculation

Salmonella dublin DSPV 595T, developed in BHI broth for 18 h at 37 °C, was administered by the milk replacer to all calves from the three experimental groups on day 11 of the experiment. To establish the strain concentration, tenfold dilutions were made from a culture. In this culture and its dilutions, absorbance was determined at 560 nm and, simultaneously, counts on plates were performed. Regression analysis was performed with both parameters. Subsequently, to quantify the amount provided we used the equation $y = 0.4735\ln(x) + 8.2162$, where y corresponds to $\log_{10}$ CFU/ml and the variable x measures the absorbance of the culture. The inoculum was administered at a dose of 2×10^{10} CFU/calf. The infective dose was chosen based on the bibliographic information available (Masalski et al., 1987; Steinbach et al., 1996; Deignan et al., 2000) and by experimental verification carried out in a previous study in two calves.

2.8. Detection of Salmonella dublin in faeces

The faecal samples (approx. 5 g) were collected on days 1, 5, 10, 11, 12, 13, 14 and 15 of the experiment from the rectum of all calves by rectal massage. That is, −240, −144, −24, 0, 24, 48, 72 and 96 h of infection. Faeces were cultured either in selenite cystine broth for 12 h at 42 °C and in Rappaport Vassiliadis broth for 18 h at 42 °C (1 g and 0.1 g, respectively). After incubation, $XLD_{nov\ nal}$ agar plates (XLD on days 1, 5 and 10) were cultured and incubated for 24 h at 37 °C. The finding of typical colonies with positive agglutination with a polyclonal antibody (OS-A and OS-B, A.N.L.I.S Dr. Carlos G. Malbrán) was considered positive for *Salmonella dublin*.

2.9. Necropsies

From day 11 of experiment, programmed necropsies were performed in one animal from each experimental group per day. The necropsies were performed 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 conventional necropsy techniques were followed. Liver, spleen, mesenteric lymph node (MesLN), ileocaecal

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lymph node (IleoLN), small intestine (jejunum and ileum) and large intestine (cecum and colon) tissues were collected using sterile instruments, minimizing possible bacterial contamination among the samples (Lee et al., 2000).

2.10. Recovery of LAB and coliforms from faeces of calves

The faecal samples of all calves (approx. 5 g) were obtained by rectal massage on days 1, 5 and 10 of the experiment and then weighed in sterility conditions and diluted 1/100 in Ringer ¼ solution. All samples were collected and analysed individually for each calf and sampling occasion. Later, samples were 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), $LAMVAB_{rif}$ to retrieve only the inoculum used (Frizzo et al., 2010a), and VRBL for coliforms (Demecková et al., 2002). The Petri dishes with LAMVAB and $LAMVAB_{rif}$ media were incubated at 37 °C for 48 h under anaerobic conditions, whereas those with VRBL medium were incubated at the same temperature for 24 h under aerobic conditions. Subsequently, characteristic colonies were counted and CFU/g faeces were calculated for each bacterial group.

2.11. Inoculum and Salmonella dublin recovery from different segments of the intestinal tract of calves

The small intestine (jejunum and ileum) and large intestine (cecum and colon) from calves were aseptically obtained. Decimal dilutions from mucosal scraping were carried out in a Ringer ¼ solution to facilitate the total *Lactobacilli* counts and those that were part of the inoculum. To determine the intestinal tract colonization by means of the inoculum, the number of viable colonies (CFU) recovered from mucosal scrapings was determined. The presence of a bacterial inoculum in the intestinal tract was interpreted as colonization by those bacteria (Lee et al., 2000). Each sample was diluted in series; LAMVAB agar plates were spread in triplicate to count total lactic microbiota and $LAMVAB_{rif}$ agar plates to recover only the inoculum that had been used. Petri dishes were incubated at 37 °C for 48 h in anaerobic conditions and the characteristic colonies were counted. The recovery of the pathogen was carried out on the $XLD_{nov\ nal}$ agar plates incubated for 24 h at 37 °C. The finding of typical colonies with positive agglutination with a polyclonal antibody (OS-A and OS-B, A.N.L.I.S Dr. Carlos G. Malbrán) was considered positive for *Salmonella dublin* and the characteristic colonies were counted.

2.12. Translocation test

Samples of liver, spleen and complete mesenteric and ileocaecal lymph nodes were obtained in aseptic conditions and homogenized with a Stomacher Seward biomaster in buffered peptone water. To measure translocation in these internal organs, homogenized samples were spread in the following medium: $LAMVAB_{rif}$ (administered inoculum), LAMVAB (*Lactobacilli*), VRBL (coliforms), Slanetz Bartley (enterococci), BBA supplemented with Vit K_1 and hemine (total aerobic bacteria) and $XLD_{nov\ nal}$ (*Salmonella*). Subsequently, characteristic colonies were counted and CFU/g organ were calculated for each bacterial group. The finding of typical colonies with positive agglutination with a polyclonal antibody (OS-A and OS-B, A.N.L.I.S Dr. Carlos G. Malbrán) was considered positive for *Salmonella dublin* and the characteristic colonies were counted.

2.13. Statistical analysis

The variables (BW and microbial counts – in faeces, mucosa and internal organs –) were analysed with ANOVA by the general linear model with SPSS 11.0 for Windows software. Differences between treatment means were tested for significance ($P<0.05$) by Tukey's test. Results were expressed as the arithmetic mean and SEM.

3. Results

3.1. Effect of treatment on body weight of calves before infection with the pathogen

The animals from the L-LAB-G were the only ones that gained weight during this stage (37.1 in 0 d vs 37.6 in 10 d), while those from the LAB-G and C-G decreased their weight slightly (37.8 in 0 d vs 36.4 in 10 d and 38.3 in 0 d vs 37.6 in 10 d, respectively). No differences between experimental groups were found.

3.2. Effect of treatment on the faecal microbial populations of coliforms and Lactobacillus in young calves before infection with Salmonella dublin DSPV 595T

Fig. 1 shows the counts of the two microbial populations studied and the *Lactobacillus*/coliforms ratio in calves from the three experimental groups. In the first 10 d of probiotic treatment, the number of *Lactobacillus* spp. in the faeces from the L-LAB-G and L-LAB-G was higher than in the C-G ($P<0.05$) (Fig. 1A and B). On day 10 of the experiment, the values of *Lactobacillus* spp. reached 9 $\log_{10}$ CFU/g faeces in both groups inoculated with LAB, exceeding 1.5 $\log_{10}$ CFU/g levels attained by the members of the inoculum. The levels of *Lactobacillus* in the faeces of calves of the C-G were similar to the experimental inoculum charge in both inoculated groups. The probiotic treatment did not reduce faecal coliform counts but generated differences ($P=0.02$) in the *Lactobacillus*/coliforms ratio during day 10 of the experiment (Fig. 1E). This was because a steady

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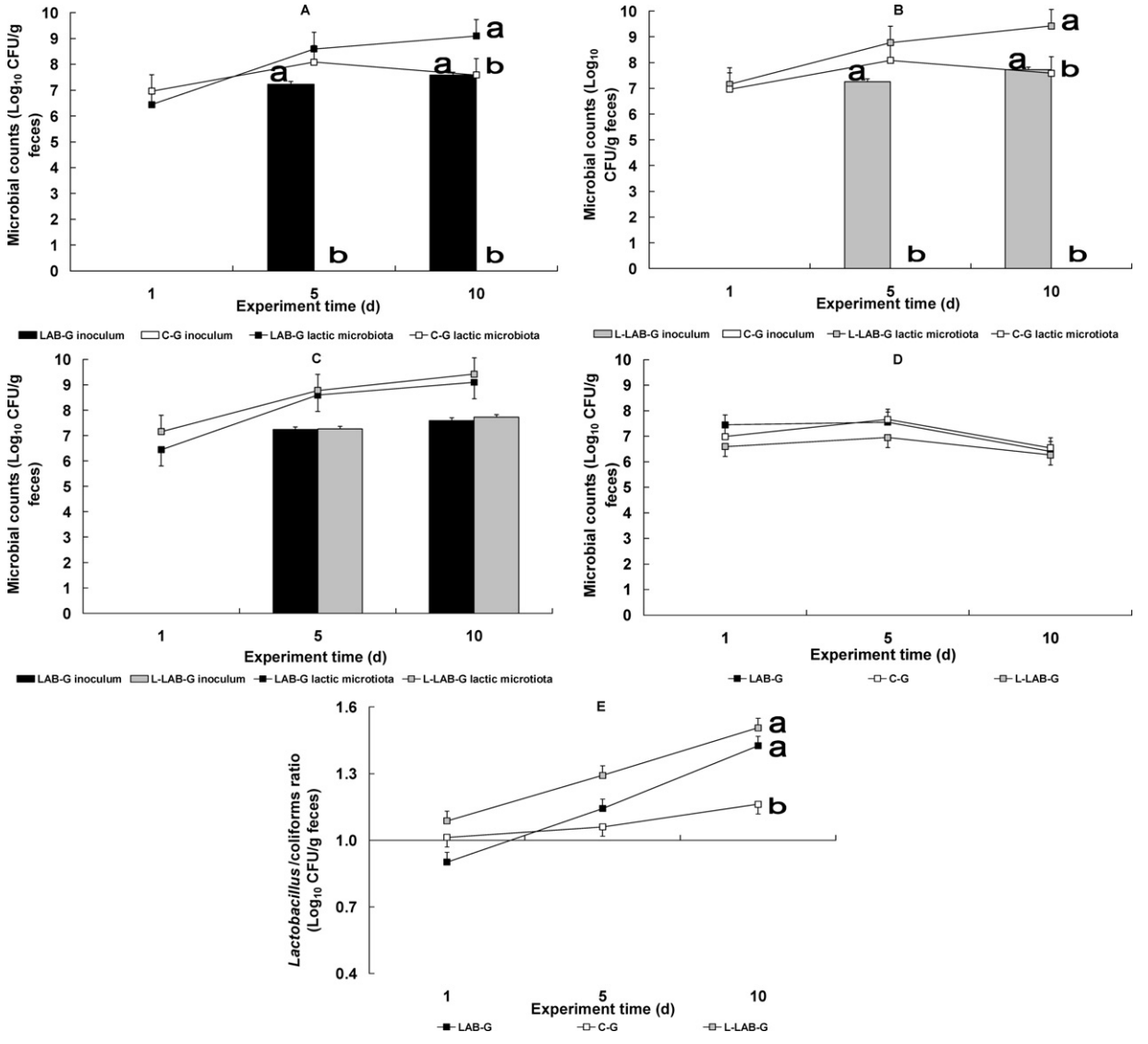

Fig. 1. Counts of coliforms populations and *Lactobacillus* and *Lactobacillus*/coliforms ratio of calves before infection with *Salmonella dublin* DSPV 595T. Values represent the $\log_{10}$ CFU/g faeces and are expressed as the mean ± SEM. (A) Total lactic microbiota and inoculum charge in the C-G and LAB-G. (B) Total lactic microbiota and inoculum charge in the C-G and L-LAB-G. (C) Total lactic microbiota and inoculum charge in the LAB-G and L-LAB-G. (D) Coliforms. (E) *Lactobacillus*/coliforms ratio.

number of *Lactobacillus* spp. due to the inoculum, together with a decrease in the number of coliforms over time, was found along the experiment (Fig. 1D). The number of *Lactobacillus* spp. exceeded the number of coliforms in about 3 $\log_{10}$ CFU/g on day 10 of the experiment.

3.3. Detection of Salmonella dublin DSPV 595T in faeces

Salmonella dublin was detected in faeces from calves 24 h after infection and during the rest of the experiment. Only one animal, belonging to the LAB-G, was negative to the isolation of the pathogen 96 h after infection. Nevertheless, the presence of the pathogen in faeces was not related to the administration of the probiotic inoculum.

3.4. Effect of treatment on microbial populations of coliforms, Lactobacillus, Salmonella and experimental inoculum in different sectors of the intestinal tract of young calves infected with Salmonella dublin DSPV 595T

Figs. 2 and 3 show the populations of coliforms, *Lactobacillus*, *Salmonella* and experimental inoculum and the *Lactobacillus*/coliforms ratio in different sectors of the intestinal tract of calves infected with *Salmonella dublin* DSPV 595T. The levels

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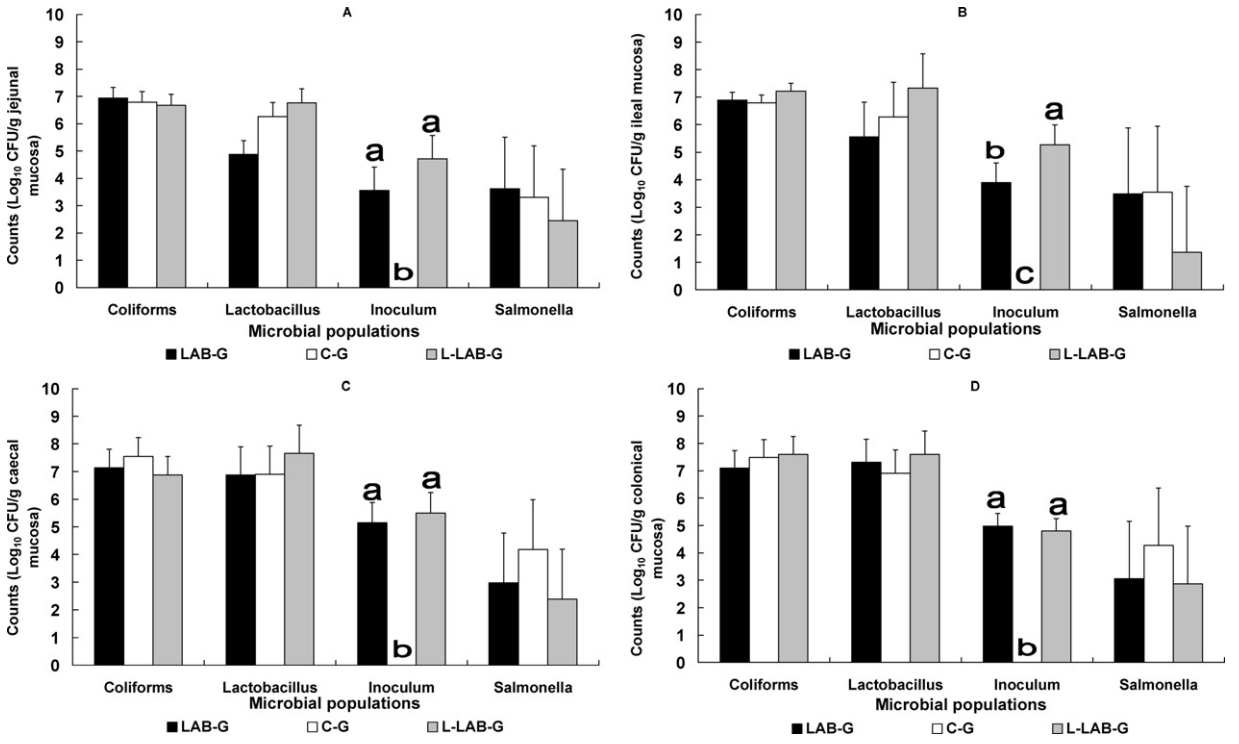

Fig. 2. Counts of the populations of coliforms, *Lactobacillus*, *Salmonella* and members of the experimental inoculum in different sectors of the intestinal tract of calves infected with *Salmonella dublin* DSPV 595T. Values represent the $\log_{10}$ CFU/g intestinal contents and are expressed as the mean ± SEM. (A) Jejunum, (B) ileum, (C) cecum and (D) colon.

of coliforms in the three groups were similar in both the small and large intestines (approximately 7 $\log_{10}$ CFU/g). A high degree of recovery of lactic microbiota was observed in different parts of the intestine independently the treatment. *Lactobacillus* spp. reached in the large intestine larger amounts than in the small intestine, although no differences between groups were found. The levels of probiotic inoculum in the large intestine of the animals from both groups inoculated with the probiotic were of 5 $\log_{10}$ CFU/g. In the small intestine probiotic inoculum counts were of 5 $\log_{10}$ CFU/g in the animals from the L-LAB-G, and of approximately 1 $\log_{10}$ CFU/g less in the LAB-G. Only in the ileum differences were found between the three experimental groups ($P<0.05$; Fig. 2b). The levels of *Salmonella* in both the small and large intestines were between 3 $\log_{10}$ CFU/g and 4 $\log_{10}$ CFU/g (Fig. 2). Although the animals from the L-LAB-G showed lower levels of *Salmonella* than those of the C-G, no differences between groups were found. During infection with *Salmonella,* only the calves from the L-LAB-G had higher levels of *Lactobacillus* than of coliforms in all the sectors of the digestive tract studied (Fig. 3) but no differences between groups were found.

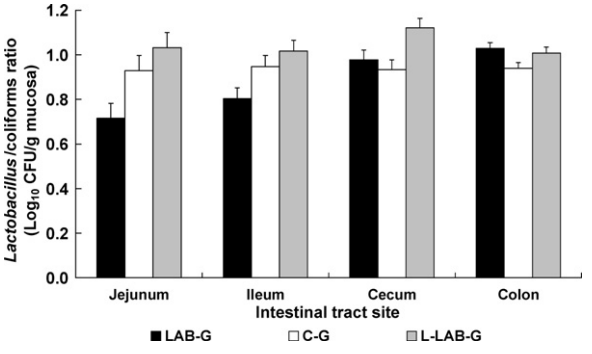

Fig. 3. *Lactobacillus*/coliforms ratio in different sectors of the intestinal tract of calves infected with *Salmonella dublin* DSPV 595T. Values represent the $\log_{10}$ CFU/g intestinal contents and are expressed as the mean ± SEM. No differences between experimental groups were found.

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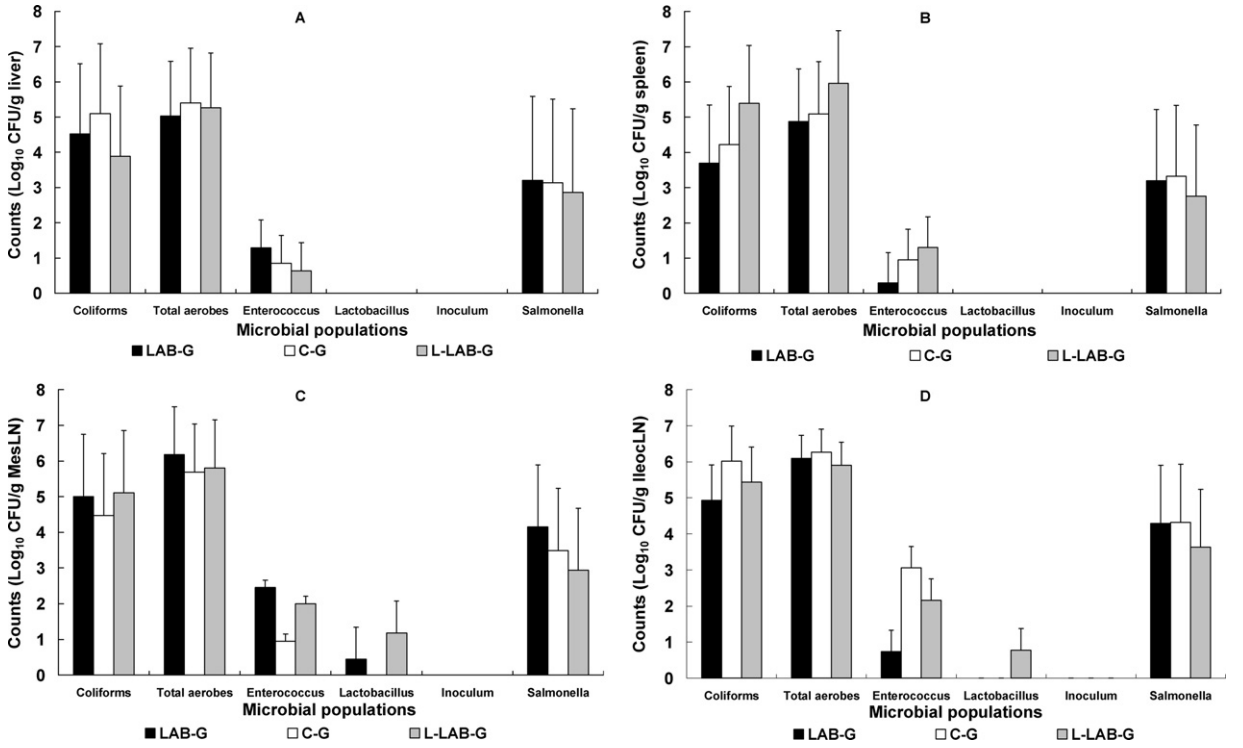

Fig. 4. Bacterial translocation in calves infected with *Salmonella dublin* DSPV 595T. Values represent the $\log_{10}$ CFU/g tissue and are expressed as the mean ± SEM. No differences between experimental groups were found. (A) Liver, (B) spleen, (C) mesenteric lymph node and (D) ileocaecal lymph node.

3.5. Translocation of microbial populations in young calves infected with Salmonella dublin DSPV 595T

High microbial loads were found in the internal organs (Fig. 4). The highest counts were those for total aerobic populations and were obtained in the lymph nodes (from 5 $\log_{10}$ CFU/g to 6 $\log_{10}$ CFU/g). Coliforms accounted for a high proportion of the total and showed levels of 1 $\log_{10}$ CFU/g lower than the aerobic populations. *Enterococcus* spp. reached levels of approximately 1 $\log_{10}$ CFU/g in the liver and spleen, and of about 2 $\log_{10}$ CFU/g in the mesenteric and ileocaecal lymph nodes. *Lactobacillus* spp. were found only in the lymph nodes of the animals inoculated with the probiotic, in very low amounts (<1 $\log_{10}$ CFU/g). None of the members of the inoculum probiotic was found in the internal organs (Fig. 4). The levels of *Salmonella* in all the internal organs were between 3 $\log_{10}$ CFU/g and 4 $\log_{10}$ CFU/g (Fig. 4). Although animals from the L-LAB-G showed lower values of *Salmonella* than the C-G, no differences between groups were found. Levels of this pathogen were higher in the internal organs than in the intestinal portions. The presence of microorganisms in the internal organs was not related to the administration of the probiotic inoculum.

4. Discussion

The ability of probiotic microorganisms to inhibit or at least counteract the negative effects of pathogens in live animals has been well studied in laboratory animals (Maia et al., 2001; Moura et al., 2001), but not sufficiently evaluated in farm animals. Young calves are particularly vulnerable to gastroenteric diseases during the first weeks of life. In the current study, we hypothesized that the behavior and ability of the potentially probiotic strains to either facilitate or hinder the translocation of other microbial populations could be evaluated in experimental models of intestinal disease, in order to obtain data on the safety of the strains in a situation of total disruption of the barrier effect generated by an intestinal pathogen.

The evolution in calves' weight showed the usual characteristics for this stage of animal growth and development before infection with *Salmonella dublin* in all the experimental groups. Calves are especially vulnerable to gastroenteric diseases during the first weeks of life. The maintenance of body weight during the first week could enhance the resistance against these diseases (Cruywagen et al., 1996).

Lactobacillus counts are usually greater than those of coliforms (*Lactobacillus*/coliforms ratio > 1) in healthy calves, but lower in calves suffering from diarrhea (Abu-Tarboush et al., 1996). The beneficial effect of viable LAB is possibly due to the transient proliferation of these bacteria in the digestive tract, *in vivo*, which represents a microbial barrier against the development of pathogenic bacteria (Heyman and Ménard, 2002). In the present work, within 10 days of treatment with the LAB inoculum, and just before the challenge with the pathogen, the *Lactobacillus*/coliforms ratio was greater than

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1 in all the experimental groups, but greater in calves supplemented with the LAB inoculum. Furthermore, the number of *Lactobacillus* spp. in the C-G was lower than in the groups supplemented with the probiotic. Under these ecological conditions of the intestinal tract, the pathogen was introduced to generate the infectious disease. In young calves, *Lactobacillus* is the dominant bacterial group, both in the digestive tract and in the faecal matter, with a fast colonization of the intestine, reaching populations of 10^7–10^8 CFU/g during the first week of life (Karney et al., 1986), when the intestinal bacterial community is highly unstable in the newborn animal (Lukás et al., 2007). Generally, the presence of viable inoculum members in the intestinal tract is the result of different factors: the number of inoculated microorganisms that are able to survive the biological barriers, their multiplication product, the saturation of lodging niches, and the evacuation due to adherence difficulty. In this study, the inoculated strains were able to survive a complex ecological niche like the gastrointestinal tract of calves. This characteristic is quite important for microorganisms with probiotic potential (Rogelj et al., 2002).

Bacterial pathogens have unique biological properties that allow them to colonize the mucous surfaces, penetrate them, grow in the host, avoid host defence and damage the host. The bacterial products responsible for these five properties are biological determinants of pathogenicity (virulence determinants) (Smith, 2000). In the present work, oral infection of animals led to the invasion and systemic dissemination of *Salmonella*. The pathogen was detected in faeces 24 h after infection. The average concentration of *Salmonella* in the internal organs was higher than that in the intestinal sections, where the pathogen count was similar in both the small and large intestines. The liver, spleen and mesenteric and ileocaecal lymph nodes (target organs) were the organs in which the pathogen was most frequently found, showing that both the lymphatic and the blood pathways were used for the entry and multiplication of *Salmonella*.

Bacterial translocation is a useful indicator to evaluate the safety level of a probiotic (Locascio et al., 2001), because it is the first step in the pathogenesis process of many opportunistic indigenous strains (Berg, 1995). Consequently, the ability to translocate is a good indicator of possible probiotic infectivity (Zhou et al., 2000). In our work, the bacterial strains of the LAB inoculum showed no capacity to translocate to extra-intestinal sites, or, in case they did, the host immune system eliminated them before they were detected. It is reasonable to think that the strains analysed do not have the ability to survive outside the animal's intestine. The results show that, despite the high concentration of *Salmonella* administered to calves, there was no translocation of the probiotic inoculum to the internal organs. This finding provides a solid basis to confirm the safety of the strains used. The translocation of other bacterial groups occurred in all experimental groups at an intensity higher than that found in calves that were not experimentally infected (Frizzo et al., 2010a). These results indicate that although the translocation was not inhibited by the probiotic treatment, it was not generated by it either, and that the probiotic inoculum was not responsible for the translocation of the microbial populations studied. It is also clear that the breakdown of the mucosal barrier generated by the pathogen facilitated the entrance of some intestinal populations, which in a situation of non-disease, becomes evident, showing only moderate levels of translocation to the internal organs. The translocation incidence and dissemination is an indicator of the intestinal mucosal barrier integrity (Lee et al., 2000), and, in young calves, the immaturity of the intestine and the presence of bacterial pathogens in the intestine could contribute to the transfer of microorganisms to extra-intestinal sites. No clear association was found between indigenous LAB counts in the intestine and those in the internal organs; no members of the inoculum were found in the latter.

The microbial loads of pathogens found in the different intestinal regions were lower than those found in the internal organs. However, when the rest of the microbial populations were analysed, higher levels in the intestine than in the internal organs were found. Some microbial populations showed some affinity to translocate in the extreme situation of intestinal imbalance generated by the pathogen. Thus, while coliforms showed high levels in the internal organs, the indigenous *Lactobacillus*, which was found at very low frequency (only in the groups treated with probiotics), expressed very little ability to enter the internal organs in a situation of total breakdown of the effect barrier generated in the intestinal mucosa. The *Enterococcus* occupied an intermediate position between the two populations mentioned. The members of the inoculum were not detected in the internal organs during *Salmonella dublin* infection. The use of an experimental model of salmonellosis was useful to test the safety of an experimental probiotic inoculum in a situation of extreme imbalance in the intestinal microbiota.

5. Conclusions

In the current study, although translocation was not inhibited by the probiotic treatment, it was not generated by it either, and the probiotic inoculum was not responsible for the translocation of the microbial populations studied. An important result of this study was that, despite the high concentration of *Salmonella* administered to calves, the LAB inoculum of bovine origin was not capable of translocating to the internal organs in the extreme situation of intestinal imbalance generated by the pathogen.

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