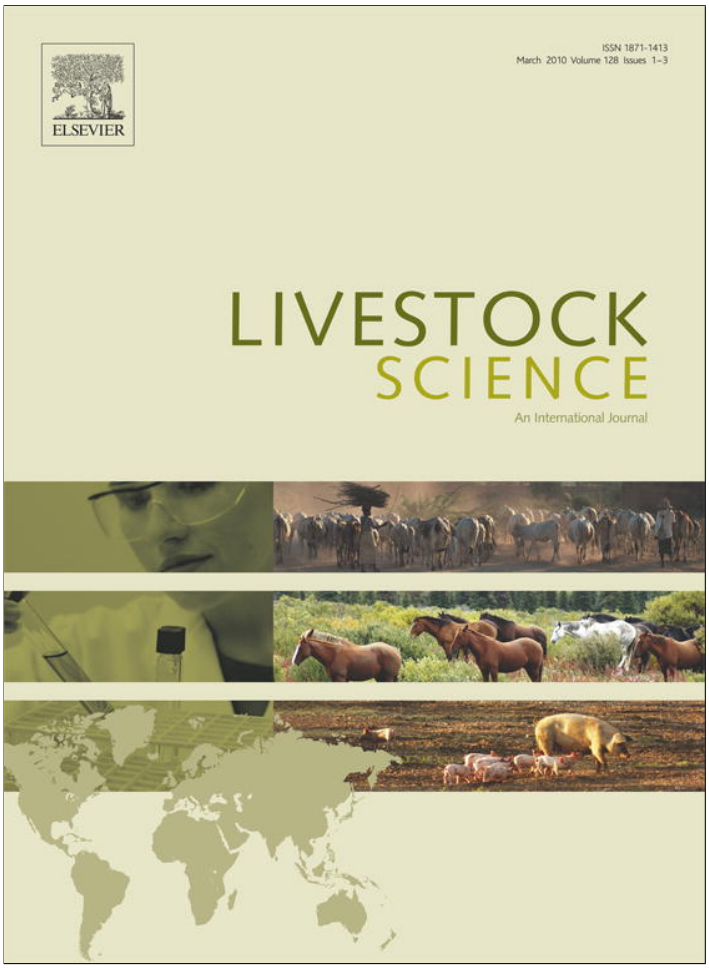

Livestock Science 128 (2010) 28–35

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Studies on translocation, acute oral toxicity and intestinal colonization of potentially probiotic lactic acid bacteria administered during calf rearing

L.S. Frizzo [a], E. Bertozzi [a], L.P. Soto [a], G.J. Sequeira [a], R. Rodriguez Armesto [b], M.R. Rosmini [a,*]

[a] Departamento de Salud Pública Veterinaria (DSPV), Facultad de Ciencias Veterinarias (FCV), R.P. Kreder 2805 (S3080HOF) Esperanza (Santa Fe), Universidad Nacional del Litoral (UNL), Argentina
[b] Departamento de Clínicas, Hospital de Salud Animal, Facultad de Ciencias Veterinarias (FCV), R.P. Kreder 2805 (S3080HOF) Esperanza (Santa Fe), Universidad Nacional del Litoral (UNL), Argentina

ARTICLE INFO

Article history:
Received 19 January 2008
Received in revised form 10 October 2009
Accepted 12 October 2009

Keywords:
Intestinal tract
Lactobacillus
Probiotic
Translocation
Young calves

ABSTRACT

In order to test the harmlessness of potentially beneficial bacterial strains when raising young calves, their safety level should be verified before they are included into a probiotic formulation. In the present study, an inoculum composed of three lactic acid bacteria of bovine origin, *Lactobacillus casei* DSPV 318T, *Lactobacillus salivarius* DSPV 315T and *Pediococcus acidilactici* DSPV 006T, was evaluated to define its acute oral toxicity and capacity to colonize, remain in the gastrointestinal tract and translocate to the organs in the internal medium. The inoculum was orally administered to a group of experimental calves in doses of 10^9 CFU/kg/day suspended in a NaCl 0.15 M solution. A control group received only a NaCl solution as placebo. The results showed that the bacteria of the used probiotic inoculum did not translocate to the internal medium and that there were no adverse effects on the general health state, weight gain and feed consumption in the animals treated with the inoculum. This situation suggests that the strains used are not pathogenic and will be probably safe if used as a food additive in calf diets.

1. Introduction

The composition of the intestinal microbiota varies because of many environmental and host factors (Nousiainen and Setälä, 1998). When animals are reared either in a grazing-based system (extensive systems) or in the wild form, the spontaneous colonization of the digestive tract by the microbiota of the surrounding environment takes place (Fuller, 1997). Segments from different parts of the intestine excised from healthy animals can be colonized by a typical microbiota, which adapts and develops in a beneficial symbiosis with the host (Kurzak et al., 1998). In contrast, in livestock intensive rearing, particularly when weaned calves are placed in intensive breeding systems without their mothers, the natural acquisition of autochthonous microbiota is drastically reduced, thus altering the intestinal environment and making it easier for pathogens to settle in (Rosmini et al., 2004). In such handling practice, farm animals are more susceptible to an imbalance of the enteric bacteria, thus generating an inefficient digestion and absorption of nutrients and, consequently, a growth delay in early weaned young calves (Nousiainen and Setälä, 1998).

Probiotics are live microorganisms that provide beneficial effects to the hosts when administered in adequate amounts (FAO/WHO, 2001). The use of autochthonous microorganisms with probiotic capacity provides an efficient alternative for the treatment and prevention of some animal illnesses (Rosmini et al., 2004).

From the animal production and public health standpoint, a key issue is to ensure animal health and food safety along the complete food chain, bearing in mind that they will be part of the human diet. Consequently, probiotics, as preventive supplements in feed, constitute an answer to a worldwide tendency of promoting natural, waste-free healthy food with improved nutritional quality.

* Corresponding author. Tel.: +54 3496 420639 int. 128; fax: +54 3496 426304.
E-mail address: mrosmini@unl.edu.ar (M.R. Rosmini).

1871-1413/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.livsci.2009.10.009

Bacterial translocation is the passage of viable indigenous bacteria from the gastrointestinal tract to extra-intestinal places such as the mesenteric lymph nodes, the liver, the spleen and peripheral blood. Three main mechanisms permit bacterial translocation: overgrowth of intestinal bacteria, deficiencies in the host's immune defenses and an increase in the intestinal permeability or the physical damage in the intestinal mucosal barrier (Berg, 1995). The gastrointestinal tract of a young animal at birth is physiologically immature when compared with that of an adult animal (Lee et al., 2000). Young ruminants might be more vulnerable to the translocation of microorganisms, because their monogastric digestive systems undergo a physiological transition to become a polygastric one. Consequently, the capacity of a probiotic candidate to translocate could be used as a parameter to evaluate its safety level (Trevisi et al., 2007). Translocation is, indeed, an undesirable characteristic when a live microbial inoculum is administered.

In previous studies, lactic acid bacteria (LAB) from young healthy calves fed with a milk replacer were isolated and identified (Schneider et al., 2004). Later, their *in vitro* probiotic properties and their capacity to protect lab animals when facing a *Salmonella dublin* experimental challenge were studied (Frizzo et al., 2005, 2006). The present work gives continuity to these two previous studies and intends to develop a probiotic inoculum of bovine origin. The objective was to study the acute oral toxicity of a LAB inoculum and evaluate its capacity to both colonize and remain in the gastrointestinal tract of calves, and then translocate to the organs in the internal medium.

2. Materials and methods

2.1. Animals

Thirty-six Holstein calves (*Bos taurus*) 10 ± 3 (SD) days of age and weighing 41.1 ± 3.5 (SD) kg, were used in the trial. Artificial rearing was carried out on a dirt floor covered with natural grass. 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 *ad libitum* with a commercial pelleted starter and twice daily with a milk replacer (4 l/day) and water, directly rationed in the individual feeder. The milk replacer was reconstituted at 11% of dry matter (DM) and administered to calves at 06:00 A.M. and 06:00 P.M., at approximately 38 °C. The animals were handled following the Guide for the Use and Care of Agricultural Animals in Agricultural Research and Teaching (FASS, 1998).

2.2. Experimental design

The animals were randomly divided into two experimental groups: the control group (C-G) and the inoculated group (LAB-G), each with 18 animals comparable in weight. Body weight (BW) was weekly recorded. Feed consumption was daily determined and calculated on the basis of the consumption of both the milk replacer and the commercial concentrated pelleted feed. The health state of the individuals and the frequency of diarrheas were recorded daily by means of a macroscopic analysis of feces (Meyer et al., 2001). Blood samples were taken weekly to evaluate the blood biochemical profile and the general immunological state. Fecal samples were taken to do a coproparasitological analysis. For statistical analysis only the results of the 12 animals per group that completed the study were considered. Programmed necropsies were carried out once a week during the entire test (35 days).

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., 2005, 2006, 2007) were used. The strains were isolated from healthy calves reared artificially by a work team at the "Departamento de Salud Pública Veterinaria" (DSPV) of the School of Veterinary Sciences, University of Litoral, Argentina. The isolated bacterial strains were kept at −80 °C in MRS medium (Biokar, France) with glycerol (35% v/v), and identified using molecular techniques (Schneider et al., 2004). Their Genbank accession numbers are: FJ787305, FJ787306 and FJ787307, respectively.

2.4. Selection of mutants resistant to rifampicine

The inoculum strains were made resistant to rifampicine in order to be able to trace down the inoculum during the *in vivo* study. The resistance of the inoculum strains to the antibiotic effect was obtained from serial cultures in MRS medium, from low levels up to a concentration of 100 µg/ml rifampicine (Kurzak, 2000; Demecková et al., 2002). Rifampicine was prepared in a stock solution (10 mg/ml), and used at a 100 µg/ml final concentration. An overnight microorganism culture was spread over MRS agar plates supplemented with rifampicine (MRS_{rif}) and, afterwards, incubated for 48 h at 37 °C. Finally, a colony was obtained by using the isolation method. Isolated strains resistant to rifampicine were cultured in MRS broth (24 h at 37 °C). The physiological and biochemical parameters of both the original strains and the ones resistant to rifampicine were compared in order to guarantee that resistance was the only difference between them. Inoculum strains resistant to rifampicine were kept at −80 °C (MRS broth with 35% glycerol), and later administered to calves.

2.5. Inoculum preparation and administration

Bacteria were multiplied in MRS broth for 18–20 h at 37 °C. Optical density was determined at 560 nm in order to calculate a calibration curve, and then used to calculate the bacterial concentration (Frizzo et al., 2006). Cultures were centrifuged at $3000 \times g$ for 10 min and suspended in a NaCl solution. The three strains were then mixed and leveled until the final volume was reached. The probiotic inoculum consisted of a 40 ml dose of a suspension of three rifampicine-resistant microorganisms into a 0.15 M NaCl solution and then dosed with at least 10^9 CFU/kg BW (1 daily dose). This inoculum was administered to LAB-G calves by incorporating it to the milk replacer during the 35-day experimental period. The C-G group was inoculated in the same way but with 40 ml of the 0.15 M NaCl solution as placebo.

2.6. Coproparasitological analysis of fecal samples

The possible influence of the parasitological load on the health of the animals was evaluated by coproparasitological analysis on a weekly basis. Fecal samples were collected directly from the rectum of each calf, and the number of parasite eggs, related to the verminous gastroenteritis complex, per gram of feces was determined microscopically by means of the McMaster technique, modified by Roberts and O'Sullivan (1950).

2.7. Blood biochemical profile and leukogram

Blood samples (15 ml) were taken every 7 days from the jugular vein of each animal using syringes with hypodermic needles. Sodium heparin was added as anticoagulant to 10 ml of the samples (Matsuzaki et al., 1997). Some of these blood samples were used for the leukogram in a Neubauer's chamber and the differential count (leukocyte formula) was performed using a Zeiss microscope from Giemsa colored smears. Other samples were centrifuged to obtain the plasma, which was stored at −80 °C until measuring uremia, cholesterolemia and glycemia. The remaining 5 ml of the blood samples was processed without anticoagulant, with the same frequency, to measure total serum protein. Once the coagulum was formed, a similar procedure was used in order to obtain and store blood serum. Serum proteins and urea were measured using a spectrophotometer and Wiener Lab's reagents by means of the biuret (540 nm) and urease (570 nm) techniques, respectively. Total cholesterol was determined by means of an enzymatic method (cholesterol-oxidase/peroxidase 505 nm) using Wiener Lab's reagents. Glucose was determined using Wiener Lab's reagents, by means of an enzymatic method (505 nm).

2.8. Necropsies

Programmed necropsies were performed every 7 days, in one animal from each experimental group. The necropsies were performed at 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). Liver, spleen, mesenteric lymph node, ileocecal lymph node, small intestine (duodenum, jejunum) and large intestine (cecum) tissues were collected using sterile instruments minimizing possible bacterial contamination among the samples (Lee et al., 2000). Spleen weight was determined and, together with BW, spleen weight index (SWI) was calculated as follows:

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

2.9. Inoculum recovery from different segments of the intestinal tract of calves

The small intestine (duodenum, jejunum) and large intestine (cecum) 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; MRS agar plates were spread in triplicate to count the total lactic flora, and MRS_{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.

2.10. Translocation test

Samples of liver, spleen and complete mesenteric and ileocaecal lymph node were obtained in aseptic conditions and homogenized with a Stomacher Seward biomaster in a Ringer ¼ solution. To measure translocation in the internal medium, homogenized samples were spread in the following medium: MRS_{rif} (administered inoculum), MRS (Lactobacilli), VRBL (coliforms), KF (enterococci) and BBA supplemented with Vit K_1 and hemine (total aerobic bacteria).

2.11. Statistical analysis

A repeated measures ANOVA test was carried out to determine whether there were significant differences between the C-G and the LAB-G studied (microbial counts, BW, food intake, SWI, leukogram, parasite egg count and glucose concentration, cholesterol, urea and total serum proteins). Results are expressed as the arithmetical mean and their standard deviations. Treatment differences with $P \leq 0.05$ were considered significant. Translocation was analyzed by means of the *chi*-square test. Statistical tests were performed with the Program SPSS 11.0 for windows.

3. Results

3.1. Lactic flora and inoculum recovery from the intestinal tract

A high degree of lactic flora recovery was observed in the different parts of the intestine (Fig. 1).

Before the inoculum was administered, the lactic flora from the three segments of intestine did not present significant differences between the groups, while the cecum was the segment with the most numerous flora ($P<0.05$) during all the experiment in both groups.

Once the inoculum was administered, the LAB values in the jejunum and cecum of the animals from the LAB-G were higher ($P<0.05$) than the values in similar intestinal segments taken from the C-G. Cecum colonization by the inoculum was significantly higher ($P<0.01$) than that in the duodenum and jejunum from the LAB-G animals. The microbial load of the lactic microbiota was similar in the duodenum from both groups. The inoculum loads were similar in the duodenum and jejunum for the LAB-G animals. The inoculum used reached a level lower than the lactic microbiota ($P<0.05$) in each intestinal segment from the LAB-G animals.

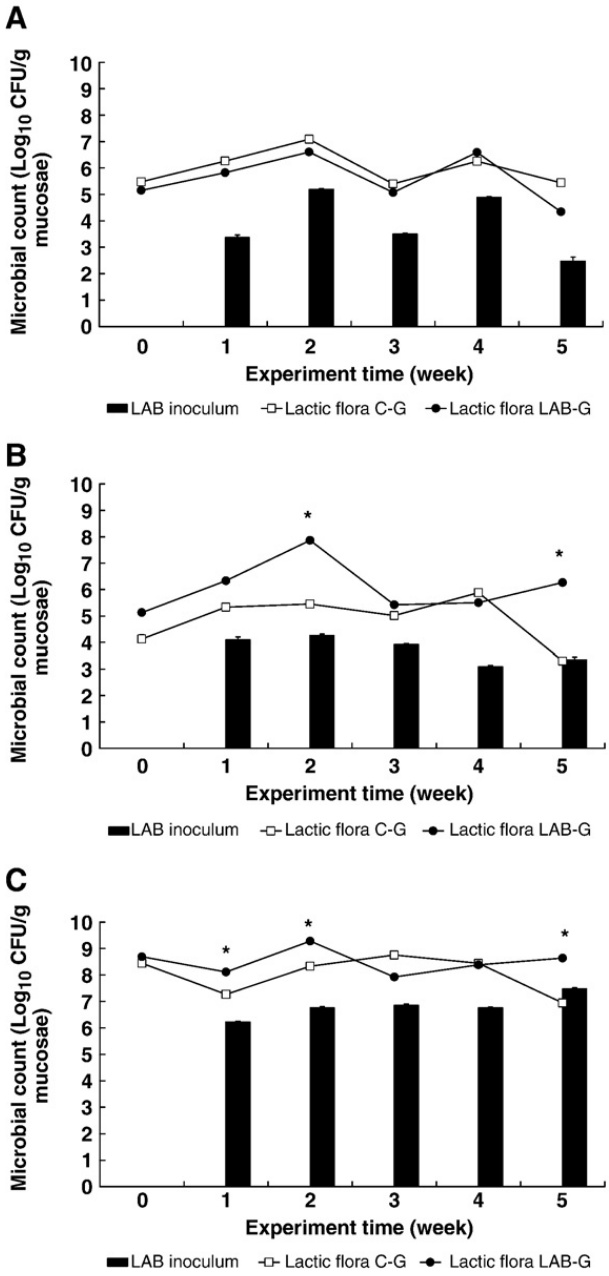

Fig. 1. Recovery of lactic microbiota from intestinal microbiota of calves either supplemented (LAB-G) or not supplemented (C-G) with an inoculum of lactic acid bacteria (LAB), at a dose of 10^9 CFU/kg BW for 35 days (*significant, $P<0.05$, compared with the C-G). A) Duodenum. B) Jejunum. C) Cecum. The indicated values are mean ± SD.

3.2. Translocation test

The incidence of bacterial translocation in the different organs is shown in Table 1. The administered inoculum was not present in the organs studied. The presence of microorganisms in the organs studied was not related to the probiotic inoculum supplementation. We did not find associations between intestinal LAB counts and those that translocated to the internal medium. The health of calves was not modified by the microbial populations found in the animals' internal medium. Independently of the experimental group, the spleen was the organ in which translocation was less frequent.

Table 1
Bacterial translocation to different tissues after the administration of a probiotic inoculum in the control group (C-G) and the inoculated group (LAB-G).

Microorganisms	C-G				LAB-G			
	Liver	Spleen	MLN	ILN	Liver	Spleen	MLN	ILN
Aerobes	3/5	1/5	3/5	2/5	4/5	2/5	4/5	4/5
Enterococii	2/5	0/5	0/5	0/5	1/5	1/5	1/5	1/5
Coliform	0/5	0/5	0/5	0/5	1/5	0/5	2/5	2/5
Lactic microbiota	2/5	0/5	1/5	3/5	0/5	0/5	4/5	4/5
Lactic microbiota $_{rif}$	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

MLN: mesenteric lymph node. ILN: ileocecal lymph node. Lactic microbiota$_{rif}$: lactic microbiota resistant to rifampicine (100 μg/ml).
Data presented are: number of positive cultures/number of tested tissues; *chi*-square test: $P>0.05$.

3.3. Morbidity and mortality

Health in all calves was excellent throughout the study. Neither illness signs or symptoms nor deaths were observed in the individuals from both experimental groups.

3.4. Inoculum effects on weight gain, feed consumption and diarrhea frequency in animals

There were no significant differences in live weight gain and feed consumption (Figs. 2 and 3). There were no cases of diarrhea while raising the animals; consequently, no significant differences were observed as regards the frequency of diarrhea episodes.

3.5. Blood biochemical profile, leukogram, coproparasitological analysis and spleen weight index

The blood biochemical parameters, the leukogram results, the coproparasitological analysis and the calves' spleen weight index (SWI) showed no significant differences between the individuals from the C-G and those from the LAB-G (Figs. 4–7, respectively).

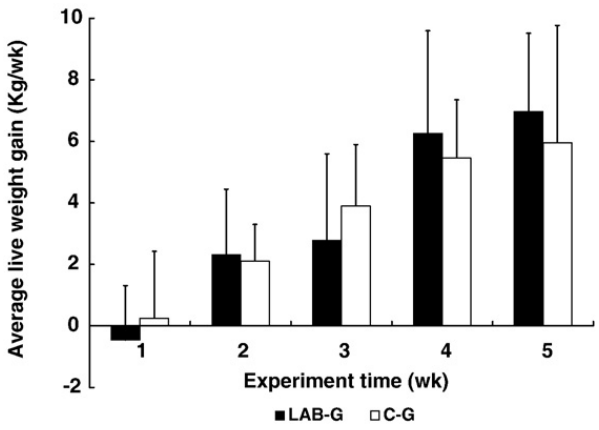

Fig. 2. Average live weight gain in calves either supplemented (LAB-G) or not supplemented (C-G) with an inoculum of lactic acid bacteria (LAB). No significant differences ($P>0.05$) were found. The indicated values are mean ± SD.

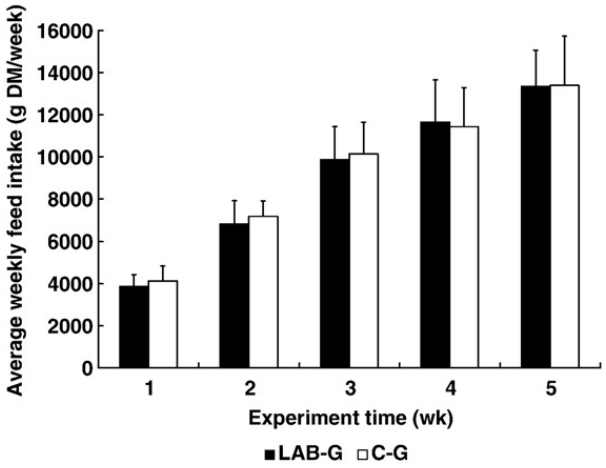

Fig. 3. Average weekly feed intake in calves either supplemented (LAB-G) or not supplemented (C-G) with an inoculum of lactic acid bacteria (LAB). No significant differences ($P > 0.05$) were found. The indicated values are mean ± SD.

4. Discussion

The intestinal microbiota of mammals is complex, numerous and strongly associated with the host's health (Vlková et al., 2006). In young calves, *Lactobacilli* is the dominant bacterial group, both in the digestive tract and in the fecal matter, with 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).

The absence of growth of the intestinal lactic flora in MRS_{rif} allowed us to track down the inoculum and study its colonization and translocation. Before starting the bacterial inoculum supplementation, results had revealed that the levels of lactic microbiota in the animals were equivalent to those reported by Vlková et al. (2006), and similar in both experimental groups, allowing us to verify differences attributed to the inoculum incorporation in the calves' diet. Moreover, the counts showed absence of growth in MRS_{rif} in mucosal scrapings collected from the three segments of the intestine studied. This absence was interpreted as a negative control that not only indicated that the rifampicine-resistant inoculum administered was not indigenous in the calves used in this experiment, but also confirmed the sensitivity of the model to track down the three strains. The inoculum used tolerated the adverse conditions of the first segment of the digestive tract and colonized the initial portion of the intestine, contributing to the colonization of the cecum from LAB-G animals, where the inoculum found an adequate environment for its development and establishment. The less restrictive environment of the large intestine permits a better development of the intestinal microbiota (Ozawa et al., 1983; Vlková et al., 2006).

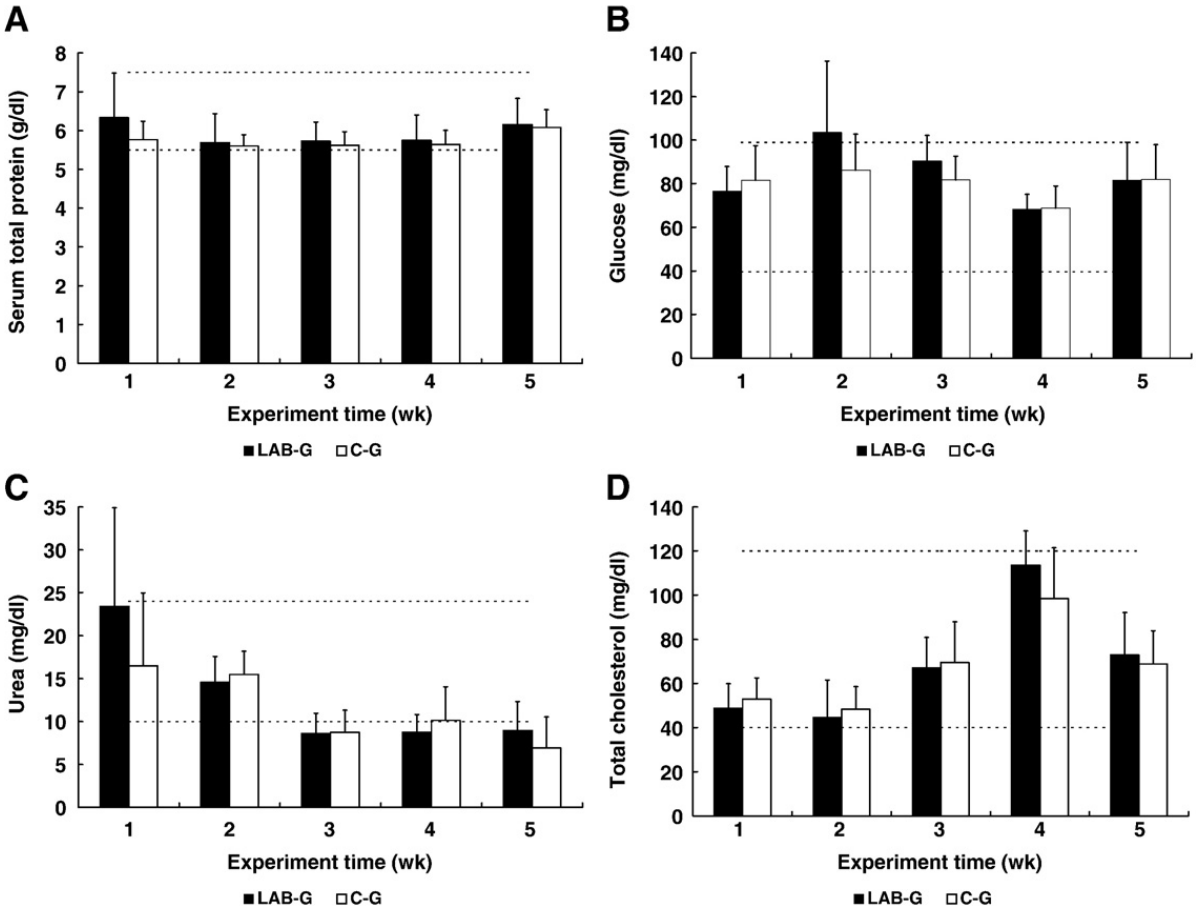

Fig. 4. Blood biochemical parameters in calves either supplemented (LAB-G) or not supplemented (C-G) with an inoculum of lactic acid bacteria (LAB). No significant differences ($P > 0.05$) were found. The indicated values are mean ± SD. Dashed lines represent previously reported reference values. A) Serum total protein. B) Glucose. C) Urea. D) Total cholesterol.

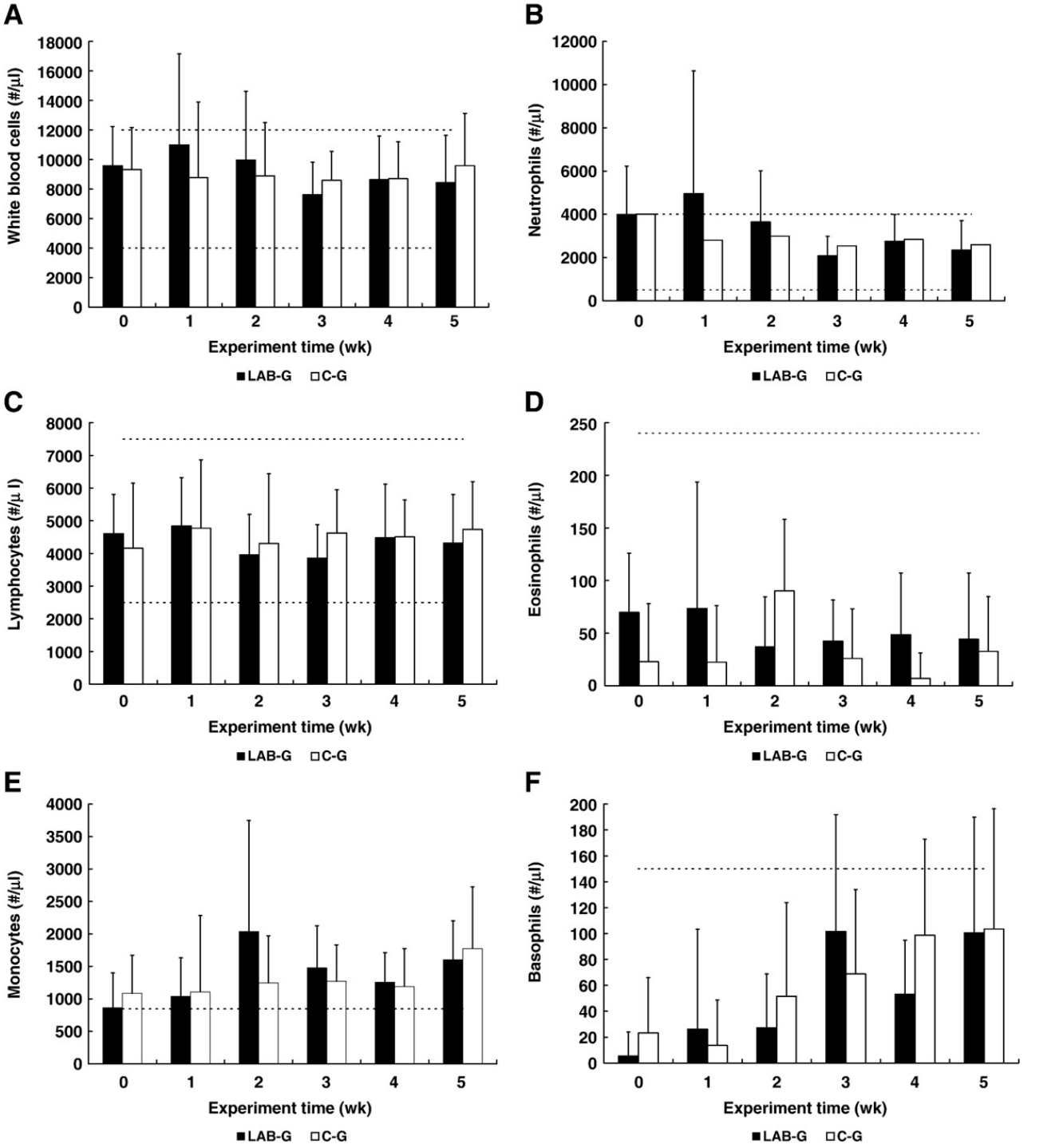

Fig. 5. Blood parameters in calves either supplemented (LAB-G) or not supplemented (C-G) with an inoculum of lactic acid bacteria (LAB). No significant differences ($P > 0.05$) were found. The indicated values are mean ± SD. Dashed lines represent previously reported reference values. A) White blood cells. B) Neutrophils. C) Lymphocytes. D) Eosinophils. E) Monocytes. F) Basophils.

In the duodenum, the microorganisms inoculated reached a level enough to keep stable the values found in the jejunum and cecum of the LAB-G animals. They also showed their competitive power, displacing and replacing the lactic flora residing in the duodenum, without increasing the total numbers of LAB. In the duodenum, the microorganisms only displaced part of the lactic flora, whereas in the jejunum and cecum they were able to significantly increase the total count of LAB. This situation, observed from the moment the inoculum was administered onwards, was evident by recovering a higher number of microorganisms from these intestine segments of treated animals. This result shows effects of both colonization and permanence represented by a high level of inoculum found in the intestinal tract throughout the experiment, a fact that seems to be sufficient enough to obtain some probiotic effect.

Generally, the presence of viable inoculum members in the intestinal tract is the result of different factors: the number of inoculated microorganisms able to survive the biological

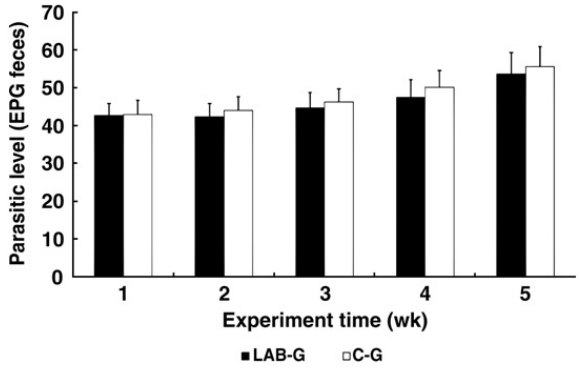

Fig. 6. Coproparasitological analysis in calves either supplemented (LAB-G) or not supplemented (C-G) with an inoculum of lactic acid bacteria (LAB). No significant differences ($P>0.05$) were found. The indicated values are the number of gastrointestinal parasite eggs detected per gram (EPG) in fresh fecal sample (mean ± SD).

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 in a complex ecological niche like the gastrointestinal tract from calves. This characteristic is quite important for microorganisms with probiotic potential (Rogelj et al., 2002).

Feed consumption, live weight gain and diarrhea occurrence have been used to evaluate acute toxicity by potentially probiotic strains (Zhou et al., 2000) and they are the most general and sensitive indicators of calf health. In the present work, the administration of the bacterial inoculum showed no adverse effects on general health, growth or development, thus indicating that the inoculum has no acute oral toxicity.

The results obtained in the blood biochemical parameters, leukogram, coproparasitological analysis and spleen weight index were within the range of the reference values (Dubreuil and Lapierre, 1997; Mohri et al., 2007). This information reinforces the statement that the animals showed no toxicity signs and presented an adequate health state.

Bacterial translocation is an indicator recommended to evaluate the safety level of a probiotic (Locascio et al., 2001)

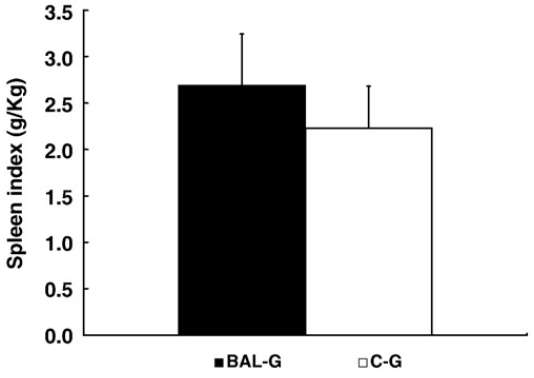

Fig. 7. Spleen weight index in calves either supplemented (LAB-G) or not supplemented (C-G) with an inoculum of lactic acid bacteria (LAB). No significant differences ($P>0.05$) were found. The indicated values are the spleen weight (g)/body weight (kg) (mean ± SD).

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). 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 could be detected. It is reasonable to think that the analyzed strains do not have the ability to survive outside the animal's intestine. Likewise, an unalterable spleen weight in the treated animals indicates that the administered LAB strains do not cause or induce systemic infections and are not invasive, thus strengthening the hypothesis that they are safe to be used in calves' diets.

In agreement with that observed by Zhou et al. (2000), growth in some microbial population shows that the organs in the internal medium were not microbial-free. Translocation occurred in both experimental groups and its intensity was not altered by the treatment. This result indicates that the inoculum was not responsible for the translocation of the microbial populations studied because the counts were obtained both before and during the probiotic administration. As mentioned by Zhou et al. (2000) and Trevisi et al. (2007), the hypothesis that a relatively constant small number of commensal bacteria persist in the host's lymphoid organs to maintain the immune system active during the weaning transition phase cannot be excluded. The effect is not attributable to the inoculum, but is related to a basic microbial population that the animal tries to evacuate continuously. Translocation of lactic acid bacteria could be found even in nonsupplemented animals, and might normally occur (Trevisi et al., 2007). Since at the dose used in this work the inoculum did not translocate to the internal medium, further studies with higher doses should be carried out to obtain beneficial effects. Additionally, these new tests would confirm, in a larger number of animals, the hypothesis on the no translocation of microorganisms studied and, thus, strengthen the hypothesis presented on this point which was established from a limited number of animals.

During the weaning period, the incorporation of a milk replacer may start an inflammatory response that could produce anatomic and functional disorders (Davis and Drackley, 2001). Occasionally, some bacteria could pass through the intestinal mucosa up to the mesenteric lymph node and other tissues (Trevisi et al., 2007). Translocation incidence and dissemination are indicators of the intestinal mucosal barrier integrity (Lee et al., 2000), and, in the intestine of young calves, immaturity 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.

Many of the difficulties in the performance of young weaned calves are directly related to the nutrient absorption and digestion deficiencies caused by pathogenic bacteria that produce diarrheas. The inoculum used here neither interfered in the animals' growth nor produced diarrhea during a critical stage of the calves' growth in either group. Because of the latter we were unable to verify a possible effect of the inoculum as regards diarrheas. Further studies should be carried out to evaluate the inoculum performance when challenged by pathogens that produce diarrhea.

5. Conclusion

An important result of this study was that the lactic acid bacteria inoculum of bovine origin was capable of overcoming the biological barriers of the intestinal tract, remaining in the calves' intestine without translocating to the organs in the internal medium. The generation of rifampicine-resistant mutants makes possible the enumeration and isolation of the inoculum members, facilitating their differentiation from the intestinal indigenous microbiota. Furthermore, it was possible to verify the absence of the inoculum in extra-intestinal sites. The administration of the inoculum did not produce adverse effects on the animals' general health, growth and development, a situation that demonstrates the absence of acute oral toxicity. The absence of diarrhea and the normal survival in individuals, together with the absence of translocation to the internal medium of the microorganisms studied, suggest that the strains used are not pathogenic and probably safe to be added as food additive in calves' diet. Because of its bovine origin, it is possible to administer these microorganisms as a probiotic, although further studies should be carried out to test their effectiveness in controlling bacterial pathogens. In future studies, an interval between inoculum administrations should be accurately determined, in order to allow the maintenance of a reasonable bacterial load and the permanence of the microorganisms in the intestinal tract after the end of the treatment.

Acknowledgements

This research was financed by the *Universidad Nacional del Litoral* (Project CAI + D). L.S. Frizzo and L.P. Soto are fellows of the *Consejo Nacional de Investigaciones Científicas y Técnicas* (CONICET, Argentina). We thank the team *Crianza Artificial de Terneros* (CAT) at the FCV-UNL for their indispensable contribution.

References

Berg, R.D., 1995. Bacterial translocation from the gastrointestinal tract. Trends Microbiol. 3, 149–154.

Davis, C.L., Drackley, J.K., 2001. Desarrollo, nutrición y manejo del ternero joven. Intermédica, Buenos Aires.

Demecková, V., Kelly, D., Coutts, A.G.P., Brooks, P.H., Campbell, A., 2002. The effect of fermented liquid feeding on the faecal microbiology and colostrum quality of farrowing sows. Int. J. Food Microbiol. 79, 85–97.

Dubreuil, P., Lapierre, H., 1997. Biochemistry reference values for Quebec lactating dairy cows, nursing sows, growing pigs and calves. Can. J. Vet. Res. 61, 235–239.

FAO/WHO, 2001. Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Expert consultation report: Córdoba, Argentina: Food and agriculture organization of the United Nations and World Health Organization, 1–4 October.

FASS (Federation of Animal Science Societies), 1998. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, First rev. ed. Federation of Animal Science Societies, Savoy IL.

Frizzo, L.S., Peralta, C., Zbrun, V., Bertozzi, E., Soto, L., Marti, E., Dalla Santina, R., Sequeira, G., Rosmini, M.R., 2005. Respuesta de ratones inoculados con bacterias lácticas de origen bovino a un desafío con *Salmonella dublin*. Rev. FAVE-Cienc. Vet. 4, 41–53.

Frizzo, L.S., Soto, L.P., Bertozzi, E., Sequeira, G., Martí, L.E., Rosmini, M.R., 2006. Evaluación *in vitro* de las capacidades probióticas microbianas orientadas al diseño de inóculos probióticos multiespecie para ser utilizados en la crianza de terneros. Rev. FAVE-Cienc. Vet. 5, 61–72.

Frizzo, L.S., Zbrun, M.V., Bertozzi, E., Soto, L.P., Sequeira, G., Martí, E., Lajmanovich, R., Rosmini, M.R., 2007. *Lactobacillus casei* DSPV 318T capacity to colonize and remain in mouse gastrointestinal tract. J. Anim. Vet. Adv. 6, 1158–1166.

Fuller, R., 1997. Introduction. In: Fuller, R. (Ed.), Probiotics 2: Applications and Practical Aspects. Chapman & Hall, London, pp. 1–9.

Karney, T.L., Jonson, M.C., Ray, B., 1986. Changes in the lactobacilli and coliform populations in the intestinal contents of calves from birth to weaning. J. Anim. Sci. 63 (Suppl. 1), 446–447.

Kurzak, P., 2000. Development of pathogen suppressive poultry feed supplements containing lactic acid bacteria from ducks. Ph.D. thesis. Technische Universitat Munchen.

Kurzak, P., Ehrmann, M.A., Vogel, R., 1998. Diversity of lactic acid bacteria associated with ducks. Syst. Appl. Microbiol. 21, 588–592.

Lee, D.J., Drongowski, R.A., Coran, A.G., Harmon, C.M., 2000. Evaluation of probiotic treatment in a neonatal animal model. Pediatr. Surg. Int. 16, 237–242.

Locascio, M., de Ruiz Holgado, A.P., Perdigón, G., Oliver, G., 2001. Enteric bifidobacteria: isolation from human infants and challenge studies in mice. Can. J. Microbiol. 47, 1048–1052.

Lukás, F., Koppová, I., Kudrna, V., Kopecný, J., 2007. Postnatal development of bacterial population in the gastrointestinal tract of calves. Folia Microbiol. 52, 99–104.

Matsuzaki, M., Takizawa, S., Ogawa, M., 1997. Plasma insulin, metabolite concentrations, and carcass characteristics of Japanese Black, Japanese Brown, and Holstein Steers. J. Anim. Sci. 75, 3287–3293.

Meyer, P.M., Vaz Pires, A., Vagadlo, A.R., Correia de Simas, J.M., Susin, I., 2001. Adiçao de probiótico ao leite integral ou sucedáneo e desempenho de bezerros da raça holandesa. Sci. Agric. 58, 215–221.

Mohri, M., Sharifi, K., Eidi, S., 2007. Hematology and serum biochemistry of Holstein dairy calves: age related changes and comparison with blood composition in adults. Res. Vet. Sci. 83, 30–39.

Nousiainen, J., Setälä, J., 1998. Lactic acid bacteria as animal probiotics. In: Salminem, S., Von Wright, A. (Eds.), Lactic Acid Bacteria: Microbiology and Functional Aspects. Marcel Dekker Inc., Nueva York, pp. 437–473.

Ozawa, K., Yabu-Uchi, K., Yamanaka, K., Yamashita, Y., Nomura, S., Oku, I., 1983. Effect of *Streptococcus faecalis* BIO-4R on intestinal flora of weanling piglets and calves. Appl. Environ. Microbiol. 45, 1513–1518.

Roberts, F.H.S., O'Sullivan, P.J., 1950. Methods for egg counts and larval cultures for strongyles infesting the gastro-intestinal tract of cattle. Aust. J. Agric. Res. 1, 99–102.

Rodríguez Armesto, R., 2004. Necropsia: su importancia diagnóstica a campo. En CD room. FCV-UNL.

Rogelj, I., Matijas, B.B., Majhenic, A.C., Stojkovic, S., 2002. The survival and persistence of *Lactobacillus acidophilus* LF221 in different ecosystems. Int. J. Food Microbiol. 76, 83–91.

Rosmini, M.R., Sequeira, G.J., Guerrero-Legarreta, I., Martí, L.E., Dalla-Santina, R., Frizzo, L., Bonazza, J.C., 2004. Producción de probióticos para animales de abasto: importancia del uso de la microbiota intestinal indígena. Rev. Mex. Ing. Quím. 3, 181–191.

Schneider, R., Rosmini, M.R., Hermann, M., Vogel, R., 2004. Identificación de bacterias lácticas componentes de la microbiota típica de los terneros criados en condiciones artificiales. FAVE-Cienc. Vet. 3, 7–15.

Trevisi, P., De Filippi, S., Modesto, M., Mazzoni, M., Casini, L., Biavati, B., Bosi, P., 2007. Investigation on the ability of different strains and doses of exogenous Bifidobacteria, to traslocate in the liver of weaning pigs. Livest. Sci. 108, 109–112.

Vlková, E., Trojanová, I., Rada, V., 2006. Distribution of Bifidobacteria in the gastrointestinal tract of calves. Folia Microbiol. 51, 325–328.

Zhou, J.S., Shu, Q., Rutherfurd, K.J., Prasad, J., Gopal, P.K., Gill, H.S., 2000. Acute oral toxicity and bacterial translocation studies on potentially probiotic strains of lactic acid bacteria. Food Chem. Toxicol. 38, 153–161.