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The Effect of Supplementation with Three Lactic Acid Bacteria from Bovine Origin on Growth Performance and Health Status of Young Calves

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Abstract: Good health in animals that are to be part of human diet during the production chain is key from both production and public health points of view. The use of indigenous microorganisms with probiotic capacity is an alternative for treating and preventing several bovine diseases. This study presents an assessment of the effect of a lactic acid bacteria inoculum from bovine origin integrated by *Lactobacillus casei* DSPV 318T, *Lactobacillus salivarius* DSPV 315T and *Pediococcus acidilactici* DSPV 006T on growth performance and health status of young calves. The 3-microorganism inoculum suspended in a solution of NaCl 0,15M was administered to an experimental group of calves at a daily dose of 10^9 UFC Kg^{-1} for 35 days. A control group was administered NaCl solution as placebo. The inoculum showed no significant effects, either positive or negative, on the animals´ performance that could have been measured in the conditions in which the study was developed. Perhaps, the lack of evident beneficial effects may have been due to the excellent health status of the animals, the appropriate environmental conditions of the test and the absence of stressing situations during breeding. All evidence shows that the advantages of using probiotics as regards growth performance, health improvement and calf survival could be more easily detected in farms presenting high morbidity and mortality rates mainly produced by diarrhoea syndrome.

Key words: Calves, diarrhoea, lactic acid bacteria, *Lactobacillus*, probiotics

INTRODUCTION

The gastrointestinal tract of healthy animals is colonized by a complex microbiota formed by many different species of microorganisms. When young calves are taken away from their mothers and housed in intensive systems, the possibility of acquiring the natural autochthonous microbiota is strongly reduced and, consequently, the intestine can be easily colonized by pathogens (Rosmini *et al*., 2004). In these intensive handling methods, farm animals are very susceptible to enteric dysbacteriosis, which leads to inefficient digestion and nutrient absorption processes delaying growth (Nousiainen and Setälä, 1998), particularly during the first 28 days of life, as afterwards calves´ intestines reach their full functional capacity. Therefore, the microbial balance in the microbiota of the digestive tract is important to promote efficient digestion and maximum nutrient absorption and at the same time to increase the host capacity for excluding pathogen microorganisms and thus, to prevent some diseases (Walter *et al*., 2003).

Gastrointestinal microbiota composition and metabolism affect farm animals´ performance, especially in young individuals that are subjected to stressing conditions (Nousiainen and Setälä, 1998). For this reason, it is interesting to incorporate indigenous microbial exponents into their diets in order to maintain microorganisms in balance. Lactic acid bacteria (LAB) are natural components of the normal intestinal microbiota in both, humans and animals (Raibaud, 1992; Smoragiewicz *et al*., 1993) and have been used as supplement in farm animals feed, especially in intensive production systems.

Probiotics are living microorganisms providing beneficial effects for the host when administrated in the adequate amounts (FAO/OMS, 2001). The use of autochthonous microorganisms with probiotic capacity is an alternative for the treatment and prevention of some animal diseases (Rosmini *et al*., 2004). Even though some strains have shown beneficial effects when therapeutically administered, the incorporation of microorganisms together with feed from birth for

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prophylaxis purposes allows for incorporating and establishing the selected strains together with calves´ microbiota. This early colonization of beneficial bacteria in the intestinal ecosystem would allow their action in physiological situations and consequently, would place calves in an advantageous position when they are invaded by pathogens (Frizzo *et al*., 2006).

The importance of using probiotic strains isolated from an animal of its own species is widely known due to the host's specificity effect (Fuller, 1997). Since, probiotic microorganisms performance may vary from one animal to another of the same species, it is convenient the inoculum to be administered be formed by a mixture of different strains (Gardiner *et al*., 2004), as the functionality of a multistrain probiotic inoculum could be more effective and consistent than that of a monostrain one (Timmerman *et al*., 2004). An advantage of inocula integrated by various strains is the possibility of complementing their effects by expressing their probiotic synergic properties. On the other hand, a complex ecosystem such as the gastrointestinal one is more probably colonized by multispecie probiotic inocula than by monostrain preparations.

Good health maintenance in animals that will be part of human diet during the production chain is a key issue from both, production and public health points of view. Probiotics, as supplements of preventive feeding, are consistent with a global trend that fosters healthy and natural feeding with better nutritive quality and residue free.

In previous studies, lactic acid bacteria from young healthy calves that were fed with milk replacer have been isolated and identified (Schneider *et al*., 2004). Their *in vitro* probiotic properties and their capacity for protecting laboratory animals against *Salmonella dublin* have been studied (Frizzo *et al*., 2005, 2006, 2007). This research gives continuity to those tests that are intended to develop a bovine probiotic inoculum. In this case, the aim was to study the effect of supplementation with an LAB inoculum on growth performance and health status of young calves.

MATERIALS AND METHODS

Animals: This research was carried out in asector devoted to Artificial Breeding of Calves that belongs to the FCV-UNL. Twenty-four Holstein calves (*Bos taurus*) averaging 10 day old were used. Artificial breeding was done using "*en estaca*" on a floor covered with natural grass. "*En estaca*" means that each animal was confined with a chain tether to its individual feeder having a space to move limited by the chain length (3 m). Each week, the animals and their "*estacas*" were moved to a new area with the same characteristics and without dejections. Throughout the experiment, all animals were fed *Ad libitum* with commercial concentrate pelleted starter and twice a day with milk replacer (4 L day^{-1}) and water, directly rationed in the feeder. The milk replacer was reconstituted at 11% of dry matter (DM) and fed to calves at 6:00 a.m. and 6:00 p.m. at 38°C approximately. The animals´ care was performed taking into account the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1998).

Feed composition: All feed used in animal breeding was free of antibiotics. The milk replacer contained 21% protein, 40% lactose and its ethereal extract was 16%. The starter was formulated with the following ingredients: grinded maize, soy bean pellet, wheat bran, dicalcic phosphate, sodium chloride and a vitamin-mineral supplement. The starter also contained 90% DM, 18% protein, 2.9 Mcal Kg DM^{-1}, 80% total digestible nutrients (TDN) 5% fiber, 1.2% calcium and 0.8% phosphorous.

Experiment design: The animals were divided in 2 weight-homogeneous experimental groups, control group (C-G) and inoculated group (LAB-G), containing 12 animals each. Water consumption and diarrhoea frequency were daily measured doing macroscopic analyses of faeces and assigning values from 1-4 according to the particularities of faeces. The evaluation of such characteristics was performed following the parameters of faecal consistency and according to the scores proposed by Meyer *et al*. (2001): Normal: solid faeces, but not hard; its original form is slightly modified when falling; Soft, looser than normal, it has no form, it is set down in heaps and is slightly scattered; Fluid: is rapidly scattered forming 6-mm thick sheets; Aqueous: liquid consistency, diarrhoeal faeces. Those animals scoring 4 were considered as "animals with diarrhoea". The faecal consistency index (FCI) proposed by Meyer *et al*. (2001) was used as an indicator of intensity and duration of faecal excretions. The higher this score, the more intense and longer the faeces softening.

$$FCI = \frac{[(dE1\times1) + (dE2\times2) + (dE3\times3) + (dE4\times4)]}{Td\times4} \times 100$$

where, dE1, dE2, dE3 and dE4 represent the number of days with faecal consistency scoring 1, 2, 3 and 4, respectively and Td represents the total number of days involved in the experiment (Td = 35). Taking this formula as a basis, the weekly FCI was calculated (Td = 7).

Body weight (BW), wither height (WH) and heart girth (HG) data were weekly recorded. Feed and water consumption (Wc) was daily measured. 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). Blood samples were weekly taken to assess the blood biochemical profile and the general immunological status; and faecal samples were also taken to carry out coproparasitological analyses.

Microorganisms: Three bacterial strains from 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. They were isolated from healthy calves artificially bred by a work-team from "Departamento de Salud Pública Veterinaria (DSPV)". The isolated bacterial strains were kept at -80°C in a MRS medium (Biokar, France) with glycerol (35% v vG^1) and their identification was done using molecular techniques (Schneider *et al*., 2004).

Inoculum preparation and administration: Bacteria were grown in a MRS broth for 18-20 h at 37°C. Optic density was calculated at 560 nm of the cultures to build a calibration curve that was used to calculate bacterial concentration (Frizzo *et al*., 2006). Cultures were centrifuged at 3000 g for 10 min and were suspended in a NaCl solution. Afterwards, the three strains were mixed and leveled until reaching the final volume. The probiotic inoculum was formed by a 40 mL dose of a suspension of the three aforementioned microorganisms into a solution of NaCl 0.15M and then dosified with at least 10^9 UFC Kg BWG^1 (1 daily dose). This inoculum was administered to LAB-G calves by incorporating it to the milk replacer during the 35 day experiment period. The C-G was inoculated in the same way but with 40 mL of the 0.15M NaCl solution, serving as placebo.

Coproparasitological analyses of the faecal samples: With the aim of evaluating the possible influence of the parasitological load on animal health status and the possible effect of the inoculum on animals' parasitological load, coproparasitological analyses were weekly carried out. Faecal samples directly obtained from calf rectum were microscopically analysed to calculate the number of parasite eggs related to the complex gastroenteritis verminose, per gram of faeces using McMaster´s count method modified by Roberts and O´Sullivan (Núñez, 1987).

Biochemical blood profile and leukogram: Blood samples (15 mL) were taken every 7 day from the jugular vein using syringes with hypodermic needles. Sodium heparin as anticoagulant was added to 10 mL of the sample (Matsuzaki *et al*., 1997). A portion of this blood sample was used for the leukogram, in Neubauer´s chamber and the differential count (leukocyte formula) was performed in a Zeiss microscope from Giemsa-colored smears. Another portion was centrifuged to obtain the plasma, which was stored at -80°C until measuring uraemia, cholesterolaemia and glycaemia. The remaining 5 mL of the blood sample was processed without anticoagulant, with the same frequency, to measure total serum proteins. Once the coagulum was formed, a similar proceeding was used to obtain and store blood serum. Serum proteins and urea were measured with a spectrophotometer and Wiener Lab. reactive, applying biuret (540 nm) and urease (570 nm) techniques respectively. Total cholesterol was measured using an enzymatic method (cholesterol-oxidase/peroxidase, 505 nm) with Wiener Lab reactive. Glucose was measured with Wiener Lab reactive by means of an enzymatic method (505 nm).

Statistical analysis: An ANOVA test was carried out to evaluate if there were significant differences between the studied C-G and LAB-G variables (BW, WH, HG, FE, TFI, SI, LWG, Wc, total and weekly FCI, leukogram, parasite eggs count and glucose, cholesterol, urea and total serum proteins concentrations). Results as the arithmetic mean and with its standard deviations were expressed. Diarrhoea events were analysed by means of the *Chi*-Square test. Statistical tests were performed using the Statistix Program for Windows v. 1.0.

RESULTS

Effect of treatment on animal growth performance: Table 1 shows the values obtained in those variables related to calf growth performance. There were no significant differences ($p>0.05$) between groups in BW, WH and HG throughout the test. LWG, SI, TFI and Wc values did not differ from one group to the other ($p>0.05$). Therefore, FE was similar in both experimental groups. LWG average showed considerable variations each week. Calves gradually increased their capacity for gaining weight throughout the study. This was directly related to

Table 1: Growth performance of supplemented calves (LAB-G) and non supplemented calves (C-G) with lactic acid bacteria inoculum at a dose of 10^9 CFU Kg BW^{-1} during 35 day

	Treatment		
Parameters	C-G	LAB-G	P-level[1]
Calves (#)	12	12	
Initial weight (kg)	43.9±3.33	42.8±3.07	NS
Final weight (kg)	61.5±6.51	60.7±5.80	NS
Average live weight gain (kg d^{-1})	0.5±0.2	0.5±0.1	NS
Average live weight gain (kg wk^{-1})	3.5±1.2	3.6±1.0	NS
Week 1	0.2±2.0	-0.5±1.8	NS
Week 2	2.1±1.2	2.3±2.1	NS
Week 3	3.9±2.0	2.8±2.8	NS
Week 4	5.5±1.9	6.3±3.3	NS
Week 5	6.0±3.8	7.0±2.5	NS
Total feed intake (g DM wk^{-1})			
Week 1	4133.9±706.6	3859.1±572.6	NS
Week 2	7174.0±737.9	6816.2±1106.8	NS
Week 3	10138.7±1508.6	9881.0±1556.4	NS
Week 4	11435.2±1851.3	11652.7±2005.3	NS
Week 5	13400.9±2336.0	13359.3±1699.5	NS
Starter intake (g DM wk^{-1})			
Week 1	1493.9±706.6	1219.1±572.6	NS
Week 2	4094.0±737.9	3736.2±1106.8	NS
Week 3	6178.7±1508.6	5921.0±1556.4	NS
Week 4	8355.2±1851.3	8572.7±2005.3	NS
Week 5	10320.9±2336.0	10279.3±1699.5	NS
Feed efficiency (kg DMI kg $gain^{-1}$)	2.9±1.2	2.7±0.5	NS
Feed efficiency (g gain kg DMI^{-1})	376.3±114.8	385.5±62.9	NS
Water intake (L wk^{-1})			
Week 1	12.3±5.6	13.5±5.6	NS
Week 2	12.3±5.1	12.6±6.2	NS
Week 3	12.7±5.6	13.1±6.1	NS
Week 4	16.4±5.6	18.0±4.5	NS
Week 5	12.7±6.0	14.4±5.0	NS
Initial heart girth (cm)	80.0±2.0	79.3±2.4	NS
Final heart girth (cm)	85.4±2.2	84.8±2.7	NS
Initial wither height (cm)	80.9±2.4	80.8±2.0	NS
Final wither height (cm)	90.8±2.7	90.9±3.3	NS

No significant differences were found (p>0.05). The values indicated are mean±SD. [1]No significant difference, defined as p>0.05, is denoted by NS. BW = body weight

Table 2: Faecal consistency index, coproparasitologic analyses and diarrhoea frequency of supplemented calves (LAB-G) and non supplemented calves (C-G) with lactic acid bacteria inoculum with a dose of 10^9 CFU kg BW^{-1} during 35 day

	Treatment		
Parameters	C-G	LAB-G	P-level[1]
Calves (#)	12	12	
Faecal consistency index (%)			
Week 1	55.7±7.7	53.0±5.7	NS
Week 2	48.5±4.4	48.2±7.1	NS
Week 3	50.3±6.5	48.8±4.7	NS
Week 4	41.1±6.2	39.6±4.2	NS
Week 5	47.9±4.4	48.2±3.2	NS
Week 1-5	48.7±3.5	47.6±2.7	NS
Coproparasitologic analysis (EPG)			
Week 1	43.0±3.70	42.8±3.07	NS
Week 2	44.1±3.56	42.3±3.58	NS
Week 3	46.2±3.52	44.6±4.11	NS
Week 4	50.1±4.45	47.4±4.77	NS
Week 5	55.6±5.36	53.7±5.63	NS
Diarrhoea frequency (event)	0	0	

No significant differences were found (p>0.05). The values indicated are mean±SD. [1]No significant difference, defined as p>0.05, is denoted by NS. BW = body weight

the constant feed intake during growth. Total feed intake was increased from 3.9-13.4 kg in the LAB-G and from 4.1-13.4 kg in the C-G between the 1st and 5th week of the study. Feed efficiency values show that the animals in the C-G were able to gain 376 g of BW per kg of consumed feed, while the animals in the LAB-G gained 386 g with a similar consumption. This represents a consumption of 2.9 and 2.7 kg of food per kg of BW in each group, respectively. Throughout the study, weekly water consumption showed values in a range of 12.3-16.4 and 12.6-18.0 L for the C-G and the LAB-G, respectively (Table 1).

Effect of treatment on diarrhoea frequency, faecal consistency index, faecal parasite eggs count and mortality rate: No diarrhoea events occurred in any of the studied animals participating in the essay and, thus, it was not possible to find significant differences between both experimental groups (Table 2). The FCI calculated for the whole study was similar (p>0.05) for both C-G (48.7%) and LAB-G (47.6%). Weekly FCI values did not show differences (p>0.05) between the groups. Even though the number of parasite eggs in faeces increased as the study progressed, no significant differences were found (p>0.05) between groups throughout the experiment (Table 2). It was not possible to prove any effect of the inoculum administered on the animals parasite load. Calves' health status was excellent throughout the study. No signs of disease were found and no animal deaths were reported in any the studied groups.

Biochemical blood profile and leukogram: The results obtained from the leukogram and the variables measured to assess the biochemical blood profile are shown in Table 3 and 4, respectively. No differences have been found (p>0.05) between the experimental groups as regards the variables under study.

Table 3: Blood cells of supplemented calves (LAB-G) and non supplemented calves (C-G) with lactic acid bacteria inoculum

	Treatment		
Parameters	C-G	LAB-G	P-level[1]
Calves (#)	12	12	
White blood cells (10^6 LG[1])			
Week 1	8.8±5.1	11.0±6.2	NS
Week 2	8.9±3.6	10.0±4.7	NS
Week 3	8.6±2.0	7.6±2.2	NS
Week 4	8.7±2.5	8.6±2.9	NS
Week 5	9.6±3.5	8.4±3.2	NS
Neutrophils (%)			
Week 1	28.8±12.4	36.5±21.0	NS
Week 2	36.3±12.3	34.8±11.5	NS
Week 3	29.0±8.5	26.8±7.3	NS
Week 4	30.6±11.8	30.8±7.0	NS
Week 5	27.0±5.4	26.4±7.7	NS
Eosinophils (%)			
Week 1	0.4±1.0	0.8±1.1	NS
Week 2	1.0±0.7	0.4±0.5	NS
Week 3	0.3±0.5	0.6±0.5	NS
Week 4	0.1±0.3	0.7±0.8	NS
Week 5	0.3±0.5	0.5±0.7	NS
Basophils (%)			
Week 1	0.2±0.4	0.3±0.9	NS
Week 2	0.7±0.9	0.4±0.7	NS
Week 3	0.9±0.9	1.3±1.0	NS
Week 4	1.3±0.9	0.7±0.5	NS
Week 5	1.1±0.9	1.1±0.8	NS
Lymphocytes (%)			
Week 1	59.1±14.2	50.9±17.1	NS
Week 2	48.4±11.4	44.4±14.8	NS
Week 3	54.6±11.7	51.1±6.0	NS
Week 4	53.4±11.7	52.3±6.3	NS
Week 5	51.6±10.8	52.1±6.6	NS
Monocytes (%)			
Week 1	11.1±6.8	10.8±5.3	NS
Week 2	14.0±4.6	18.6±7.3	NS
Week 3	14.6±4.9	19.4±4.9	NS
Week 4	14.0±6.3	14.8±4.2	NS
Week 5	18.0±4.8	19.6±5.7	NS

No significant differences were found (p>0.05). [1]No significant difference, defined as p>0.05, is denoted by NS

Table 4: Blood biochemistry parameters of supplemented calves (LAB-G) and no supplemented calves (C-G) with lactic acid bacteria inoculum

	Treatment		
Parameters	C-G	LAB-G	P-level[1]
Calves (#)	12	12	
Serum total protein (g dL^{-1})			
Week 1	5.8±0.5	6.3±1.1	NS
Week 2	5.6±0.3	5.7±0.7	NS
Week 3	5.6±0.3	5.7±0.5	NS
Week 4	5.6±0.4	5.8±0.7	NS
Week 5	6.1±0.5	6.2±0.7	NS
Glucose (mg dL^{-1})			
Week 1	81.6±15.9	76.5±11.3	NS
Week 2	86.2±16.5	103.5±32.7	NS
Week 3	81.8±10.9	90.5±11.7	NS
Week 4	68.8±10.1	68.3±6.9	NS
Week 5	82.0±15.9	81.5±17.4	NS
Urea (mg dL^{-1})			
Week 1	16.5±8.5	23.4±11.5	NS
Week 2	15.5±2.7	14.6±3.0	NS
Week 3	8.7±2.6	8.6±2.3	NS
Week 4	10.1±3.9	8.8±2.0	NS
Week 5	6.9±3.6	9.0±3.4	NS
Total cholesterol (mg dL^{-1})			
Week 1	52.9±9.6	48.8±11.1	NS
Week 2	48.3±10.3	44.7±16.8	NS
Week 3	69.5±18.4	67.1±13.8	NS
Week 4	98.5±23.0	113.6±15.5	NS
Week 5	68.9±15.0	72.9±19.2	NS

No significant differences were found (p>0.05). [1]No significant difference, defined as p>0.05, is denoted by NS

DISCUSSION

Evolution in calves´ weight showed the usual characteristics for this stage of animal growth and development in both groups throughout the study. Weight gain was continuous and constant from the second week of the study and no decrease or peak was observed during the study. This behaviour is the one that frequently occurs when sanitary conditions are optimal, starting after the second week of breeding. At that moment, animals had overcome the stressing period of transport and settling down. Weight gains were similar to those reported by Abe *et al*. (1995) for Holstein calves bred in similar conditions. Even though the difference between the variables that constitute the performance of both groups could not be demonstrated, a poor dose of the inoculum or an insufficient duration of the study may account for the obtained results and at the same time, may open the doors to do another study in which these new conditions should be considered. Also, the use of prebiotics together with microorganisms, in these circumstances, could improve animal performance, which would allow the inoculum to settle more efficiently at intestinal level.

Calves are vulnerable to diseases, particularly gastroenteric diseases, during the first weeks of life and BW maintenance during this stage might improve resistance against these sufferings (Cruywagen *et al*., 1996). In particular, calves fed with milk replacer frequently undergo a BW loss during the first weeks of life (Jaster *et al*., 1990). Many of the difficulties in young calf performance are directly related to failures in nutrients digestion and absorption processes caused by diarrhoea-causing pathogenic bacteria. The microbial inoculum being used was not able to produce BW significant increases in adequate sanitary conditions, in which diarrhoea was not present. The effect of probiotics may not be significant when the health status of calves is good (Jenny *et al*., 1991). It would be interesting to test this microbial inoculum in an experimental model in which calves with nutritional origin diarrhoea are used. The use of whey or lactose in high quantities together with milk replacer would cause an imbalance at intestinal level and the probiotic microorganisms might demonstrate their beneficial action in these circumstances.

Many researchers have studied supplementation with probiotics in calves and have provided opposite results. Some of them present improvements in growth performance (Bechman *et al*., 1977; Gilliland *et al*., 1980, Schwab *et al*., 1980; Abe *et al*., 1995; Meyer *et al*., 2001; Timmerman *et al*., 2005), while others have shown no beneficial effects (Hatch *et al*., 1973; Morrill *et al*., 1977; Ellinger *et al*., 1980; Jenny *et al*., 1991; Higginbotham and Bath, 1993; Abu-Tarboush *et al*., 1996; Cruywagen *et al*., 1996; Gonçalves *et al*., 2000; Oropeza Aguilar *et al*., 1998). The main factors that account for the differences between

the studies, without taking into account the microbial inoculum itself, are related to the health status, stress level of animals and degree of exposure to pathogens during breeding. All shows that the benefits provided by the use of probiotics as regards growth performance, health improvement and calves' survival could be more easily found in farms with high morbidity and mortality rates mainly due to diarrhoea syndrome. This idea, which is surely associated to a potential healing effect, should not encourage its use just for therapeutic purposes and leave aside the strategic importance of its use for prophylaxis. The beneficial action of probiotics should be always aimed at keeping indigenous intestinal microbiota in balance so that the animal may be ready to successfully respond to an eventual colonization and invasion of a pathogen.

Variability in growth rate and starter acceptance during the early stage of calves´ development does not allow beneficial effects of probiotics to be seen, especially, in types of breeding where stressing factors are reduced and the intestinal microbiota is balanced (Jenny *et al*., 1991). The inoculum showed no significant, either positive or negative, effects on the animals´ performance that could have been measured in the conditions that the study was done. These results may be related to a good health status of the animals due to adequate environmental and health conditions. Healthy animals present a balanced intestinal microbiota that allows them to develop adequately. However, when they are under stressing conditions, an imbalance occurs in the microbiota where lactobacillus and bifidobacteria populations are reduced and pathogenic microorganisms can be increased. The use of probiotics prevents the imbalance in the intestinal tract and avoids diarrhoea occurrence (Fuller, 1989), or significantly reduces its prevalence in calves (Abe *et al*., 1995). No diarrhoea cases were reported for both groups and therefore, the inoculum behavior could not be tested. The results of this experiment coincide with those obtained by Higginbotham and Bath (1993), who did no observe cases of diarrhoea or death in calves treated with *Lactobacillus acidophilus* and *Streptococcus faecium* during breeding. It is necessary to do new studies where to evaluate in detail the effects of the inoculum in the presence of a challenge with diarrhoea-causing primary pathogens.

To ensure the normal growth of pre-stomach tissues, calves should be stimulated to eat dry food at an early age, since the development of the pre-stomach and absorptive papillae responds to fiber and grain consumption. Most of the weight gain in young calves fed with limited quantities of milk plus a starter is due to the intestinal tissue growth and to its filling (Davis and Drackley, 2001). In this study, milk replacer consumption was intentionally provided similarly and constantly to all calves to stimulate the rapid starter intake. Even though no greater or earlier consumption was observed in the animals of the LAB-G, an important starter intake was observed throughout the study. This intake would allow reducing, partially or totally, milk replacer from the diet by the end of the 4th week. The apparent digestibility of proteins improves over age, which is related to maturation of protein digestion systems (Davis and Drackley, 2001).

It is improbable that the treatment with microbial inoculum would cause any adverse effect on the animals' health because feed intake and WG were adequate and the health status of animals was excellent. The results obtained from blood biochemical parameters and the leukogram showed values that were within the reference value range (Debreuil and Lapierre, 1997; Mohri *et al*., 2007). This reinforces the statement that the animals remained in good health status.

CONCLUSION

The use of the inoculum, in the conditions in which the study was carried on, had no influence on growth performance. The good health status of the animals and the absence of stressing conditions during breeding might have been the causes for the absence of evident beneficial effects of the inoculum. Anyway, this microbial inoculum from bovine origin could act as an antagonist once it is settled in the intestine and, thus, it would be interesting to study the effect of the probiotic treatment on colonization and translocation of a primary disease-causing pathogen in young calves to verify its possible protecting effect. Besides, a maximal interval between inoculations should be specified so as to allow keeping a reasonable bacterial load and the permanence of microorganisms in the gastrointestinal tract after finishing the treatment. Even though specific tests should be carried out, the inoculum being used showed some degree of harmlessness since it does not cause adverse reactions from a clinical point of view.

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