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Effects of bacterial inoculants in milk on the performance of intensively reared calves

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ABSTRACT

The aim of this study was to evaluate the effects of probiotics on the feed intake, body weight gain (BWG), feed efficiency (FE) and fecal microbiota of intensively reared calves fed milk inoculated with probiotic bacteria in a computerized milk feeder system. Thirty Holstein calves were allocated into three groups: group A, supplemented with an inoculum comprising *Lactobacillus casei* DSPV 318T, *Lactobacillus salivarius* DSPV 315T and *Pediococcus acidilactici* DSPV 006T; group B, supplemented with an inoculum comprising *Lactobacillus plantarum* DSPV 354T; and group C, control without probiotics. The calves were examined for 21 days. Inocula were added to the tank milk, and the daily dose for each calf was approximately 10 log CFU. The total *Lactobacillus*, inoculum strains, coliforms, yeast and enterococci in the stool and the total *Lactobacillus*, inoculum strains and pH in the tank milk were determined weekly. The lactic acid bacteria (LAB) in groups A and B produced enough acid to bring the milk to a pH lower ($P < 0.05$) than that of the control milk. Fermentation increased the shelf life of the milk, thereby avoiding the need to frequently discard the milk and reducing the costs for the use of the computerized milk feeder system. The probiotics-stimulated milk intake ($P < 0.05$) was compared with that of the control group. *L. casei* DSPV 318T, *L. salivarius* DSPV 315T, *P. acidilactici* DSPV 006T and *L. plantarum* DSPV 354T were present in the gastrointestinal tracts of the calves in the probiotics groups. In addition, the *Lactobacillus*/coliform ratio was greater than 1 in the probiotics groups and lower than 1 in the control group. BWG and FE were higher for group B than for group C. The presence of *L. plantarum* DSPV 354T and its dominance over coliforms in the fecal microbiota might have positive effects on the growth performance of calves.

Abbreviations: BWG, body weight gain; DMI, dry matter intake; FE, Feed efficiency; LAB, lactic acid bacteria.

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1. Introduction

Probiotics have been widely studied as prophylactics in intensive systems to maintain intestinal balance, improve performance and reduce the incidence of intestinal pathogens (Mokhber-Dezfouli et al., 2007; Signorini et al., 2012; Frizzo et al., 2011b). In these systems, probiotics are typically added to the milk or milk replacer immediately before it is consumed by the calf (Cruywagen et al., 1996; Ewaschuk et al., 2004; Higginbotham et al., 1998; Jenny et al., 1991). In these cases, the probiotics are individually dosed for each calf, generating more work on the farm for the implementation of this tool. Furthermore, when consumed, the bacteria are live but inactive because they are not activated prior to addition to the feed.

Group feeding conditions using computerized feeder systems increase the risk of disease transmission (Maatje et al., 1993). However, the use of computerized systems is becoming a more common rearing practice on dairy farms, primarily due to the reductions in labor requirements (Morrison et al., 2010). Morrison et al. (2010) previously reported on the use of probiotics in the group feeding of calves using a computerized feeder system, but there are no published data on the effects of probiotics for milk fermentation directly in the feeder tank.

The probiotic effect exerted on the host will depend on the composition of the inoculum. Each strain has unique characteristics (Ripamonti et al., 2011; Monteagudo-Mera et al., 2012), and thus, the effect on the parameters of interest must be evaluated for each particular inoculum. This study evaluates the effects of probiotic inocula on the performance of calves under group feeding conditions, in which the strains are metabolically active at the time of administration. The computerized milk feeder system could be a suitable method for the administration of probiotic inocula, ensuring that microorganisms are viable and metabolically active at the time of intake and that the delivered concentration is adequate for each animal. The hypothesis tested in this work is that supplementation of milk-fed calves with probiotic strains through an automated system will improve performance.

The aim of this study was to evaluate the effect of probiotic bacteria administered with milk on the feed intake, body weight gain (BWG), feed efficiency (FE) and fecal microbiota of intensively reared calves fed milk inoculated with probiotic bacteria in a computerized milk feeder system.

2. Materials and methods

2.1. Animals and housing

The experiments were performed on a farm in the province of Santa Fé (Argentina) during autumn (average temperature: 11.6 °C). Thirty female Holstein calves (*Bos taurus*) with an average age of 20 ± 2.5 days and an average initial body weight of 37 ± 3.1 kg were used. Each group was housed in a 480 m^2 barnyard. The animals were fed using a computerized milk feeder system (DeLaval CF150®), with a maximum of 6 L/d.calf of pasteurized milk and feed starter, with water available *ad libitum*. The equipment was cleaned every 12 h. For cleaning, the milk was removed from the feeder tank. Once the system was clean, the milk for the probiotics groups was returned to the tank, and the milk for the control group was discarded and replaced with fresh milk.

Animal care was provided according to the guidelines for the care and use of animals in research and teaching (FASS, 1998). The protocol used was approved by the Advisory Committee on Ethics and Security of the Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral (Santa Fe, Argentina).

2.2. Experimental design

The animals were divided into three groups of 10 animals (group A, group B, and group C (control)) using a completed randomized design based on the live weight. Calves were examined for a period of 21 days. Individual measurements of milk and starter intake were recorded on a daily basis. The ratio of milk to starter for each animal was controlled using a computerized system that regulates and records the individual dose of feed intake. Starter was given *ad libitum*. The body weight gain (BWG) of the animals was measured weekly, after 1, 2 and 3 weeks of inoculum administration.

The feed efficiency (FE) was calculated as using the following equation:

$$\mathrm{FE} = \frac{\text{kg of weekly weight gain}}{\text{kg of weekly dry matterintake}} \quad (1)$$

where dry matter (DMI) was calculated as follows:

$$\mathrm{DMI} = (\text{weekly milk intake (L)} \times 0.129) + \text{concentrate intake (kg)} \times 0.95 \quad (2)$$

where 0.129 is the solid concentration of the milk according to Alais (1985) and 0.95 is the solid concentration of the starter according to the supplier (Iniciador Terneros PLT, Boostermix Bovinos, Alimental®).

2.3. Microorganisms

The probiotic inoculum administered to group A comprised *Lactobacillus casei* DSPV 318T, *Lactobacillus salivarius* DSPV 315T and *Pediococcus acidilactici* DSPV 006T, with the following rDNA GenBank accession numbers: FJ787305, FJ787306

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Table 1
Microbial populations studied, media and culture conditions used.

Microorganisms	Media	Culture conditions
Lactobacillus spp.	LAMVAB	Anaerobiosis, 48 h, 37 °C
Inoculum	$LAMVAB_{rif}$	Anaerobiosis, 48 h, 37 °C
Coliforms	Violet red bile lactose (VRBL)	Aerobiosis, 24 h, 37 °C
Yeast	Fungi and yeast (F and Y) modified*	Aerobiosis, 48 h, 37 °C
Enterococci	Slanetz and Bartley (S and B)	Aerobiosis, 48 h, 37 °C

* With the addition of 20 g/L dextrose and 5 g/L pluripeptone.

and FJ787307, respectively. The probiotic inoculum administered to group B contained *Lactobacillus plantarum* DSPV 354T (GenBank accession number: FJ751793). These strains were selected using *in vitro* and *in vivo* probiotic properties tests (Frizzo et al., 2011b).

2.4. Selection of antibiotic-resistant mutants

The antibiotic resistance of the LAB strains of both inocula was determined from successive cultures in LAMVAB medium (Hartemink et al., 1997) from low concentrations up to 10 μg/mL of rifampicin (Kurzak et al., 1998; Demecková et al., 2002). An overnight culture was spread onto LAMVAB agar plates supplemented with rifampicin ($LAMVAB_{rif}$) and subsequently incubated for 48 h at 37 °C. Finally, a colony was obtained using the isolation method. The isolated rifampicin-resistant strains were used in the experiment.

2.5. Preparation and administration of LAB inocula

The bacteria were cultured in skim milk (10 g/L) for 18–20 h at 37 °C. The culture was dispersed into containers and frozen at −20 °C until further use in the artificial rearing of calves. The probiotic dose used was 30 mL of culture/L tank milk. The daily dose for each calf was approximately 10 log CFU.

A milk sample was collected weekly from the computerized milk feeder tank to determine the pH, the total *Lactobacillus* population, and the inoculum strains in LAMVAB and $LAMVAB_{rif}$. Each determination was performed in duplicate.

2.6. Counting the fecal microbiota

Fecal samples (approximately 5 g) were obtained from three calves of each group by rectal massage at the beginning of the experiment and after 1, 2 and 3 weeks of inoculum administration. The samples were weighed, diluted 1/100 in 1/4 Ringer solution and homogenized on a magnetic stirrer. Serial 10-fold dilutions of each sample were cultured with different media to count the microbial populations, as detailed in Table 1.

2.7. Statistical analysis

The initial weight of the calves was analyzed using one-way ANOVA. Each calf receiving treatment was considered as an experimental unit. The milk and starter intakes, FE, BWG and fecal populations (*Lactobacillus* spp., coliforms, *Enterococcus*, yeasts, probiotic inoculums, Inoculum/Total *Lactobacillus* and Total *Lactobacillus*/coliform ratio) were analyzed using ANOVA for repeated measures, with time (week) as the repeated measure, and Dunnett's test to compare the differences between treatments A and B and the control group. The total *Lactobacillus* load and pH of the tank milk were analyzed using one-way ANOVA and Dunnett's test. The number of *Lactobacillus* in the tank milk was analyzed using Student's *t*-test for independent samples. These analyses were performed using SPSS 11.0 for Windows software, with $P < 0.05$ representing a significant difference between the means.

3. Results

3.1. Feed intake, body weight gain (BWG) and feed efficiency (FE)

The milk intake was higher in the groups inoculated with LAB than in the control group, and no differences were observed in starter intake between groups (Table 2).

The initial weight was similar in all groups ($P = 0.177$): group A: 36.9 ± 3.70 kg; group B: 37.3 ± 3.33 kg and control group: 37.5 ± 4.40 kg. The BWG and FE showed tendencies toward differences between groups B and C. There were no differences observed between groups A and C (Table 2).

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Table 2
Milk intake, starter intake, body weight gain and feed efficiency for each experimental group.

	Groups			SEM	P[a]
	C	A	B		
Milk intake (L)/week	20.8	27.7[b]	27.1[b]	1.76	0.001
Starter intake (kg)/week	1.5	2.3	2.1	0.72	0.510
Body weight gain (kg)/week	1.6	2.5	3.6[b]	0.78	0.052
Feed efficiency (gain/DMI)	0.2	0.4	0.6[b]	0.18	0.112

A: Group supplemented with inoculum A; B: group supplemented with inoculum B; C: control group; DMI: dry matter intake.
[a] P-value ANOVA for effect of diet.
[b] Mean differs ($P < 0.05$) from the control group (Dunnett's test).

3.2. *Lactobacillus* count in milk from the computerized milk feeder tank

The total *Lactobacillus* load was lower in the milk of the control group than in the milk of the groups supplemented with probiotics, and the milk pH was lower for the probiotic groups. However, a large percentage of the *Lactobacillus* strains present in the milk of the probiotic groups were members of the inocula (Table 3).

The fermentation in the milk tank prevented the development of undesirable organoleptic characteristics (data not shown). Contrasting results were obtained for the control group; for this group, after some hours in the tank, the milk had to be discarded due to undesirable organoleptic characteristics and was replaced with fresh milk.

3.3. Fecal microbiota count

The bacteria in the probiotic inoculum were present in the feces of groups A and B from week 1. The counts were similar in both groups. In addition, the total *Lactobacillus* populations showed similar counts between the different groups. However, the inoculum *Lactobacillus*/total *Lactobacillus* ratios were close to 1 (between 0.92 and 1, Table 3).

The numbers of bacteria belonging to the other intestinal populations analyzed were not significantly different between the groups or over time (Table 3).

The *Lactobacillus*/coliform ratio was lower than 1 for the control group and higher than 1 for groups A and B (Table 3).

4. Discussion

Some probiotic inocula have shown positive effects on calf performance (Hossaini et al., 2010). The effects depend on the inoculum composition and the breeding system in which the inoculum is administered (Signorini et al., 2012). This study evaluated the effects of the supplementation of milk with two probiotic inocula in computerized feeder systems on some microbial populations in the intestines of calves and on feed intake, BWG and FE.

The supplementation of the tank milk with probiotic inocula for animal consumption increased the milk intake of the probiotic groups compared with that of the control group. This increased consumption may be due to several reasons. First, the acidity of the food may have improved feed palatability (Keith et al., 1983). Second, the difference in consumption

Table 3
Fecal populations counts (*Lactobacillus* spp., probiotic inocula, coliforms, yeast and enterococci for each experimental group), inoculum/total *Lactobacillus* and total *Lactobacillus*/coliform ratio. Bacterial counts (*Lactobacillus* spp. and probiotic inocula) and pH of milk tanks.

	Calf groups log (CFU/g or mL)			SEM	p[b]
	C	A	B		
Fecal counts					
Lactobacillus spp.	6.4	6.7	6.6	0.48	0.787
Probiotic inocula	ND	6.4	6.6	0.33	0.695
Colifoms	6.8	6.6	6.6	0.31	0.747
Enterococcus	5.3	5.9	5.1	0.38	0.156
Yeast	6.0	5.8	5.4	0.38	0.279
Inoculum/Total *Lactobacillus*		0.95	1.00	0.01	0.014
Total *Lactobacillus*/coliform	0.95	1.04	1.01	0.03	0.578
Milk tank counts					
Lactobacillus spp.	4.1	7.0[a]	6.8[a]	0.50	0.002
Probiotic inocula	ND	6.1	6.7	0.29	0.143
pH	6.7	4.9[a]	6.7	0.20	<0.001

A: Group supplemented with inoculum A; B: group supplemented with inoculum B; C: control group. ND: not detected.
[a] Mean differs ($P < 0.05$) from the control group (Dunnett's test).
[b] P-value ANOVA.

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between groups might reflect the number of visits to the feeder. Borderas et al. (2009) observed that the number of visits to the feeder varies with the health of the calf. In this study, no signals of illness were observed, as rearing was performed under adequate sanitary and environmental conditions. However, some stress factors might have affected the calves and their intakes. The stress of the first days of life due to different factors associated with artificial rearing is known (Davis and Drackley, 1998). In this case, competition to reach the feeder was an additional factor of stress. Achieving an appropriate intestinal microbiological balance can avoid the impact of stress. In this study, the probiotics-supplemented calves overcame the stress impact of feed competition quicker than the control calves. This effect was observed in the stimulation of milk intake. The computerized system provides a limited and equal ration to calves at each feeding; these results suggest that calves with higher milk intake approached the feeder more times. The stress of the first days of life might affect the probiotic groups less significantly because the balance of the microbial ecology was reached more quickly in this group.

The probiotics' effects on the balance of the microbial ecology can also be considered when explaining the differences observed in BWG between group B and the control group. Factors affecting the growth performance of the control calves during this period did not exert this effect on group B, potentially reflecting improved adaptation to the stress factors conferred by probiotic supplementation during the first weeks of life, which stimulated milk consumption and improved BWG (Frizzo et al., 2011b).

The increase in feed intake led not only to an increase in BWG but also to an increase in the FE of group B. Although the bacterial loads of both inocula were similar, the effects exerted on the host were different. Improvements in the BWG and FE of group A were not observed with respect to the control, suggesting that the observed effects were strain dependent. The improved performance in group B was generated through the improved utilization of the nutritional capacity of the feed. Different studies have shown that probiotic bacteria improve the use of the nutrients that reach the intestine. This improvement might reflect the exogenous production of hydrolytic enzymes (lipases, proteases and amylases) by probiotic bacteria, which convert the unusable food into nutrients available for uptake and utilization (Wang and Gu, 2010; Frizzo et al., 2011a). In addition, the reduction of colonization by pathogens due to the synergistic effects of LAB might also contribute to the observed improvements (Signorini et al., 2012). Moreover, in this study, probiotic bacteria were in contact with the food not only in the intestine of the animals but also during the period in which the milk remained in the tank. During this period, the milk fermented, and the food was transformed into substances assimilable by the animal during storage in the tank (Davis and Drackley, 1998). In turn, the acidification of milk during fermentation delays the breakdown of food (Davis and Drackley, 1998); thus, the nutritional use of fermented milk would be more appropriate than the use of fresh milk. The feeding system used in this experiment has disadvantages, as the milk tanks were not refrigerated. This limiting factor not only represents an economic loss because of the need to discard residual milk but could also cause a decrease in production yields due to diarrhea and deaths resulting from the consumption of unsafe feed if the milk is not discarded. The milk stored in non-refrigerated systems is an optimal culture medium for many microorganisms, including pathogens. This problem is increased greatly during the warm months, when milk must be replaced more often or inhibitors of microbial growth need to be incorporated. In this study, the addition of LAB to the milk resulted in fermentation, which was evidenced as a decrease in pH. This acidity decreased the risk of spoilage or the growth of pathogenic strains, making the milk safer. As a result, it was not necessary to discard the milk because it could be maintained in the tank until complete consumption, preventing economic losses. Thus, we can conclude that the addition of LAB to the milk tanks improves this feeder system in addition to having effects on the performance of calves.

As in the milk tank, in the intestines of calves supplemented with LAB, many of the *Lactobacillus* present were derived from the probiotics administered. The total counts of *Lactobacillus* in the gut per gram of stool did not differ between the probiotic groups and the control group. The difference between the microbiota of the groups involved the quality, but not the quantity, of *Lactobacillus*. Although the three groups of calves showed the same counts of *Lactobacillus*, the effects were different. Replacing a part of the autochthonous *Lactobacillus* with *L. plantarum* DSPV 354T might explain the better results for growth performance. The lactobacilli strains present in the control group were derived from the environment and did not produce the same beneficial effects as the probiotic strains. These results confirm the importance of supplementing the animals with LAB strains previously selected for their probiotic characteristics (Frizzo et al., 2006; Adams et al., 2008; Soto et al., 2010). Importantly, this improvement in performance was observed in healthy calves, which is of great importance because the probiotic effect is easier to obtain under conditions of disease or nutritional stress (Frizzo et al., 2011a). In cases of illness, the potential probiotic effect would be much more evident because calves supplemented with probiotics would maintain or increase their BWG, and this in turn could enhance resistance to disease (Frizzo et al., 2011b).

The *Lactobacillus*/coliform ratio was altered in probiotic calves compared with control calves. According to Abu-Tarboush et al. (1996), animals with diarrhea have a ratio lower than 1, while healthy animals have a ratio greater than 1. In this study, this ratio was higher than 1 for the probiotic groups and lower than 1 for the control group, showing that supplementation with probiotics generated a favorable microbial relationship (Frizzo et al., 2011a).

Both probiotic inocula produced positive effects in calves. Notably, in this particular system of automatic feeding, the feed was milk, and the animals had to search for the feed by themselves. In this study, we observed higher FE after supplementation of the milk with probiotics, and these effects could vary if the feeding were made with milk replacer, as the effects of probiotics change depending on the feed (Signorini et al., 2012). Therefore, the effects of the probiotic inocula should be experimentally studied in other farming systems.

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5. Conclusions

L. plantarum DSPV 354T supplementation modified the intestinal microbiota in calves, affecting the composition of populations of *Lactobacillus* and the *Lactobacillus*/coliform ratio. This modification of the intestinal balance had beneficial effects on milk intake, BWG and FE in the supplemented calves. Furthermore, the fermentation of milk by the probiotic inocula in the computerized feeder allowed more efficient use of the milk. The use of *L. plantarum* DSPV 354T improved this feeder system and calf performance, but further studies are needed to evaluate the effectiveness of this microorganism in other farming systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.anifeedsci.2013.12.004.

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