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*Corresponding author

Fatima Nader Macias, Reference Center for Lactobacilli (CERELA-CONICET), Chacabuco 145, 4000, San Miguel de Tucumán, Argentina

Keywords

Lactic acid bacteria; Calves management; Probiotics; Fermented milk

Abbreviations

ISAPP: International Scientific Association for Probiotics and Prebiotics; WHO: World Health Organization; EFSA: European Food and Safety Authority

Research Article

Oral Administration of Probiotic Fermented Milk to Calves in Dairy Farms of Tucuman-Argentina: Effect on Diarrhea Incidence and Weight Gain

Maldonado NC[1], Rodriguez C[2], Arce OE[2], Mostajo JM[3] and Macías FN[1]*

[1]*Reference Center for Lactobacilli (CERELA-CONICET), San Miguel de Tucumán, Argentina*

[2]*Faculty of Agronomy and Zootechnics, National University of Tucumán, Argentina*

[3]*School of Agriculture and Saccharotechnics, National University of Tucumán, Argentina*

Abstract

A probiotic fermented milk designed with four homologous selected lactic acid bacteria by their beneficial, functional and technology properties was orally administered for seven days to newborn calves. A double mind randomized assay was performed in two farms located in Tucuman-Argentina. Hence, 22 newborn calves were enrolled to the Treated Group (TG) and 26 animals to the Control Group (CG). The animals were evaluated at 2, 7, 30 and 60 days of life, recording weight and signs of intestinal diseases. Weight gain was calculated between the following periods: 2-7, 8-30 and 31-60 days. No significant differences in diarrhea incidence were observed between the groups, but at the last period, no diarrhea was detected in the TG. Also, the average weight gain was significantly different at the end of the trial (31-60 days of life), being the weight gain 0.790kg/day in the TG and 0.600kg/day in CG. The length of the probiotic administration and the farm management could affect these parameters, referred to results previously obtained with 60 days probiotic administration in a different farm in Tucuman.

Introduction

In Dairy farms, new management systems such as intensive rearing and early weaning alter sanitary conditions and can lead to inefficient absorption of nutrients, immune system problems, retardation of growth and frequent intestinal infections in youth animals [1,2]. At birth, the immune system of calves is immature being susceptible to colonization by pathogenic microorganisms and higher diarrhea incidence [3]. The use of lactic acid bacteria as probiotics has been proposed as a novel alternative for the prevention of intestinal disorders and also as immunomodulatory to be included in animals feed [4,5,6]. Probiotics are defined as live microorganisms that exert a beneficial physiological effect on the host [7,8] and can be administered as adjunct therapy to reconstitute the intestinal microbiome. *Lactobacillus johnsonii* CRL1693, *Lactobacillus murinus* CRL1695, *Lactobacillus mucosae* CRL1696 and *Lactobacillus salivarius* CRL1702 were isolated from calf´s feces and selected by their beneficial characteristics for the design of a probiotic adjunct [9]. The bacteria fulfill the requirements established by different organizations such as the International Scientific Association for Probiotics and Prebiotics (ISAPP), the United Nations Food Organization (FAO), the World Health Organization (WHO) and the European Food and Safety Authority (EFSA) for probiotic bacteria, since the selection was supported on their homologous origin, functional and technological properties, and animal assays [4,9]. In a previous work, a multi-strain fermented milk was designed with the selected bacteria [9] and administered from the calves' birth up to their transition to solid food (60 days) [4]. The results showed a decreased in the incidence of diarrhea and mortality, higher weight gain and healthier animals [4]. Then, the objective of this work was to evaluate the effect of the administration of the probiotic fermented milk for shorter period of time (seven days from birth) on diarrhea incidence and weight gain in calves of two dairy farm in the NOA region located in Tucuman Argentina.

Materials and Methods

Microorganisms and growth conditions

The fermented milk was prepared with *Lactobacillus johnsonii* CRL1693, *Lactobacillus murinus* CRL1695, *Lactobacillus mucosae* CRL1696, and *Lactobacillus salivarius* CRL1702 [9], isolated from calf´s feces and selected by their beneficial, functional and technological properties [10,11,12]. The detailed elaboration of the fermented milk was published in a previous work [9]. The protocol was applied in two dairy farms in Trancas department (Tucumán province-Argentina, NOA dairy region) to 48 newborn Holstein animals from September 2017 to January 2018. The treated group (TG; 22 animals) received the fermented milk (109 CFU/day/animal) during 7 days, while the control group (CG; 26 animals) received the same feeding without probiotics. When enrolled, calves were randomly assigned to receive the fermented milk (TG) orally once a day during 7 consecutive days after the second day of life. The detailed administration of the fermented milk was published previously [4]. The rearing system of the calves was individual raised outdoors (stake system), entering in the protocol from the second day of life. At birth and the first day of life, the newborn was feed with natural colostrums from their mother. Animal feed included liquid diet (milk-based milk), and solid diet: pellet (Molino Trigotuc SA, Tucumán, Argentina) from 3rd of life and alfalfa straw from day 30. Data from the mothers (first-calving or multiparous cows) were recorded and the colostrum level in calves assayed. The level of immunoglobulins in blood serum was determined on the second day of life with an optical refractometer (Sino Tech. Model: RHCN-200/ATC, Fujian, and China). Animals were evaluated at 2, 7, 30 and 60 days of life, recording weight and signs of intestinal diseases. Weight gain was calculated between the following periods: 2-7, 8-30 and 31-60 days.

Statistical analysis

Mixed models were used for the statistical analysis. The experimental design was completely randomized within each farm. The dairy farm`s effect and the animal´s effect nested within the farm were included as random effects. In the statistical model

applied, these two effects generate data without independence, which were included in the model in order to estimate the adjusted least squares means. The assumptions of the model were verified, both for the residuals and for the random part. The proportions of males-females was considered, and the calves of first-calving or multiparous cows were homogeneous in the GT and GC (chi square test of homogeneity). The colostrum level between cattle and between cows and heifers was also analysed, and no significant differences between the two groups (t-test) was detected. The two groups were found to be comparable. Weight gain was analyzed in each period independently and the adjusted means were compared using Fisher's test. The incidence of diarrhoea was analyzed with the Cochran-Mantel-Haenzel test using the drum. Aggregated and separated data were evaluated in each period. The farms applied the usual therapies to animals with diarrhoea (rehydration and antibiotics) with no modification in the treatment of diarrheal scheme between the groups. The significance level used was 5%.

Bioethics

Animal protocols were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No.8023, revised 1978). The protocol was approved by the CERELA-CONICET Bioethics Committee (Centro de Referencia para Lactobacilos-Consejo Nacional de Ciencia y Tecnología) and the CICUAL (Comité Institucional Para el Cuidado y Uso de Animales de Laboratorio) of UNT (Universidad Nacional de Tucumán) (N024/17).

Results and Discussion

The total number of animals with diarrhea symptoms did not show significant differences between the two groups (TG and CG), but the results indicated there were no diarrhea in the TG at the end of the trial, data evaluated between 30 and 60 days of life (Figure 1). This result could be explained by the low number of animals with this symptoms. The incidence of diarrhea in the CG (n=10/26, 38%) was lower than the value registered 69.2%) in a previous work when the calves received the same probiotic fermented milk for 60 days under similar conditions (mode of administration and animal feed). In that work, significant differences were obtained in the incidence and the duration of diarrheas between the control and treated groups [4]. The lower diarrhea of the calves in this work can be explained by the differences in the management and animal health in the farms. The location of the farms of the two trials is the same region of Argentina (NOA dairy region), but the two farms included in this work registered a lower diarrhea incidents and no mortality. The diarrhea rates obtained at this time were similar than those reported by González Pereyra et al. [13] in Buenos Aires, Argentina (21% enteritis prevalence in 4359 suckling calves). But they report 29% disease-fatality rate, contrasting with 0% fatality rate obtained in this work.

The results of the administration of probiotic fermented milk during the first 7 days of life show a significant increase in the weight gain of calves, registered on day 60 of the protocol (average weight gain in 31-60 age range, $p < 0.05$). The weight of each animal is shown in (Figure 2), and the average weight gains according to the age range in (Figure 3). The increased weight was observed after 30 days´s life, but the fermented milk was administered only 7 days. When the milk was administered for 60 days [4], the nutritional parameters: height, weight, and body performance improved when compared with control group. In the present work, the average daily gain was higher: TG 0.790kg/day, CG 0.600kg/day, when compared with weight gains obtained previously (Treated 0.276kg/day, Control 0.102kg/day. These results can be related to the lower incidence of diarrhea and animal mortality in this assay. Is important to point out that the present work was carried out for a short period (only 7 days) of fermented milk administration, with a small number of animals (n=48), during the summer time, and after the application of different strategies in the farms related to calf rearing, animal health and welfare (data non published). The administration of a probiotic product for a short period of time means a lower cost and facilities requirements in the farm, when comparing with 60 days administration. Renauld et al. [14] evaluated the administration of a commercial multispecies probiotic bolus (MSP; Revive, Partnar Animal Health, Ilderton, ON, Canada) in calves with symptoms of diarrhea (determined by fecal consistency). Although the trial was carried out on animals of different ages, the administration period was 7days, the same than in this work. They observed no difference in the increase of the average weight gain between the groups consuming probiotic and placebo in two weeks. Similar results were obtained in this work at 30 days. However, at the last stage of the study (30-60 range of age) differences in the average weight gain were observed, which is a very interesting result.

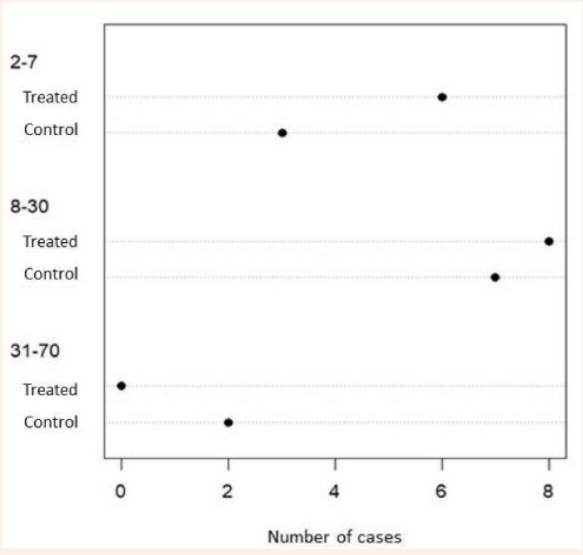

Figure 1: Incidence of diarrhea according to the age ranges of calves.

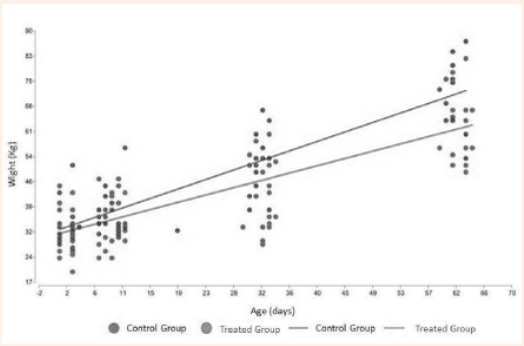

Figure 2: Individual weights of animals during the study.

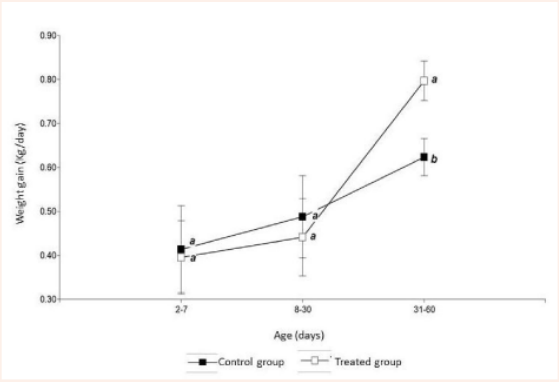

Figure 3: Weight gain according to the age range of the animals, expressed as means ± standard error. Different letters (vertically) indicate significant statistically differences according to the Fisher test ($p < 0.05$).

Conclusion

The administration of probiotic fermented milk for seven days to newborn calves improves the animal weight mainly after 30 days life, even though no differences in diarrhea symptoms were observed. Further studies should be performed to determine the effect of the administration of the probiotic in higher number of animals. Also, animal´s management, welfare and health care in the farm should be considered as a factor in the diarrhea incidence to complement the evaluation of the probiotic effect.

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