Multispecies Multistrain Probiotic Effects on Calves Development and Health

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Received September 10, 2012; revised October 16, 2012; accepted October 26, 2012

ABSTRACT

Experiment was designed to assess alimentary security or any beneficial effect on calves of a multispecies multistrain probiotic (MMP). An experiment with 36 calves, two day old, was conducted to assess the influence of probiotic on growth and health indicators. The treatment period was extended to 45 d. Group 1 received one daily dose of MMP (1.1 × 10^9 CFU per calf) during 20 d. Group 2 was the untreated control. On a weekly basis, every calf in each group was weighed to determine weight gain. Forty five days after the beginning of the experiment, blood samples were obtained from seven animals from Group 1 and six from Group 2, and peripheral blood neutrophils separated in order to determine metabolic and microbicidal activity. There was a significant increase in H_2O_2 production and NBT reduction test in MMP treated calves. The MMP not only lacks adverse effects when supplied as food additive, but showed health benefits. The prevention of infection and the highly significant increase of phagocytic activity in peripheral blood leukocytes seen in calves strongly suggest an efficient connection between the MMP and the immune system.

Keywords: Probiotic; Growth; Health; Immunology; Calves

1. Introduction

Raising calves in dairy farms represents a fragile stage for the primary milk production industry in Argentina [1]. The usual stress period after birth is worsened when newborn calves are separated from their mothers. Most of these calves are hypogammaglobulinemic due to failure of passive transfer of maternal gammaglobulines, having mild to severe immunological impairment [2]. Factors that often are cited as having an effect on passive transfer in the calf are the timing of colostrum ingestion the method and volume of colostrum administration, the immunoglobulin concentration of the colostrum ingested, and the age of the dam. [3] Passive immunity acquired via transfer of maternal immunoglobulins in colostrum provides newborn mammals with early protection against pathogens. This is particularly important in cattle because calves are born hypogammaglobulinemic and depend solely on transfer from colostrum for antibody-mediated immunoprotection [4,5]. Limited or complete failure of transfer of immunoglobulin can result in an increased incidence of morbidity, which can contribute to a failure to thrive as evidenced by suboptimal or diminished growth [6,7].

Normal intestinal microbiota seeding is also affected. Consequently, they suffer frequent intestinal and respiratory diseases with increasing morbidity and mortality, affecting weight gain and normal development [8].

Thus, the stressful conditions experienced by animals subjected to high production systems and environment hostilities negatively influence the composition and symbiotic interactions of gut microbiota [9]. The natural nutritional resources are changing, the composition of gut microflora must also change and incorporate higher biodiversity for adaptation to environmental perturbation [10,11]. Synthetic pharmaceutical formulations like antibiotics are extensively used as therapeutic or as preventive agents for intestinal diseases in dairy calves. These formulations induce the selection and proliferation of resistant bacterial strains; they are aggressive against host friendly flora deepening the fragility of gut homeostasis [12].

Probiotic represent a promising alternative as a disease
biocontrol strategy in raising dairy farm calves [13,14]. A probiotic is a “live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance” [15]. Thus, using a probiotic means an intervention on the intestinal ecosystem. Probiotic bacteria would have antagonistic impact against intestinal infectious bacteria just by competitive exclusion [16], modulating the intestinal medium, favoring growth of friendly bacteria or producing natural antibiotics or bacteriocines [17].

Perhaps the most intriguing phenomena associated with the consumption of probiotic focuses on their crosstalk capacity with host gut microbiota, observed as early as 1965 [18], host gut cells and host immune system cells [19,20] resulting in immune modulation. This would mean that the identification of probiotic soluble factors would explain the effect beyond intestine, general health improvement and higher resistance against systemic infectious invaders like bacteria, viruses, fungi observed in experimental animals [21]. Many studies, have shown that probiotics increase barrier function in terms of increased mucus, antimicrobial peptides, and slgA production, competitive adherence for pathogens, and increased tight junctions integrity of epithelial cells [22].

The aim of this work was assess alimentary security and any beneficial effect on calves of a multispecies multistain probiotic

2. Materials and Method

The experiment was carried out in a commercial dairy farm which had a nursery field for its own calves. Calves were exposed to weather conditions without any protection and with no chance to move freely. The regular feed and general management in the nursery were also applied to the selected experimental animals. The animals were followed for 65 day of age. Thirty-six Holstein calves (two days old) were selected for this experiment and separated in two groups of 18 animals per group. The calves were fed twice at day with milk plus colostrum (four L/d per calf). Group 1 received one daily dose of probiotic (MMP) per calf during 20 d. One MMP dose was 330 mg biomass containing 1.1 × 10^8 CFU. Group 2 was the untreated control. The MMP is composed by at least seven lactic acid bacteria and two yeast species, comprising agents from separated dominium like Eukaria and Bacteria (L. helveticus, L. fermentum L. paracasei, L. casei, L. parabuchneri, Lactobacillus gasseri and Lactobacillus panis and Pichia Kudravzevii and Saccharomyces cerevisiae) [23].

All calves in Group 1 and Group 2 were observed daily and any physical and/or clinical change registered. On a weekly basis, every calf in each group was weighed to determine weight gain. Forty five days after the beginning of the experiment, blood samples were obtained from seven animals from Group 1 and six from Group 2 and peripheral blood neutrophils separated [24] in order to determine metabolic and microbicidal activity as follows: Nitroblue tetrazolium (NBT) reduction test: The NBT is a yellow dye that, when incorporated into the phagolysosome, is biochemically transformed in a blue formazan crystal. The more metabolically active cells are the more NBT is incorporated. Two hundred µL NBT solution (0.11% in HBSS), 200 µL of neutrophils (4 × 10^7) were mixed with 40 µL opsonized zymosan (OpZ) or 100 µL of 10 µg/mL phorbol 12-myristate 13-acetate (PMA). Duplicate tubes were prepared without stimulant. All tubes were incubated at 37°C for 15 min, the reaction was stopped by addition of 3 ml of 0.5N HCl. Tubes were centrifuged at 1000 × g for 10 min, and cells were washed twice with 3 mL of 0.5N HCl for 5 min. The sediment was re-suspended in 3 ml of dimethylformamide (DMF) and heated in a boiling water bath for 10 min, then 2 ml of 10N KOH was added and mixed thoroughly, centrifuged and the upper DMF layer was read in OD 710 nm. The results are expressed as Δ OD/2 × 10^6 neutrophils/15 min [25]. All samples were analyzed in triplicate.

Production of hydrogen peroxide (H_2O_2): The test was carried out three times in each isolated cell sample. The production of H_2O_2 was determined by the method described by [26] with slight modifications. Two hundred µL of neutrophils suspension (4 × 10^7 cells), were mixed with 40 µL OpZ (125 µg/mL) or 100 µL of 10 µg/mL PMA. Negative controls were prepared without stimulant. All tubes were incubated under an atmosphere of 5% CO_2, 95% air at 37°C. After 1 h of incubation, a solution of phenol red and 0.2 µM horseradish-peroxidase was added to the medium to quantify the hydrogen peroxide content. After 10 min, the reaction was stopped with 100 µL of 1N NaOH and the amount of hydrogen peroxide formed was measured spectrophotometrically at 620 nm. Results were expressed as nmol H_2O_2/10^6 cells.

Statistical Analyses

Dates were analyze using GLM procedure of SAS. The initial body weight was used as a covariate for analysis. Mortality, the occurrence of diarrhea, distress respiratory and the animals needing therapeutic treatment were evaluated by means of a χ^2 test. The student t test was used to compare the values of NBT reduction test and H_2O_2 production in MMP-treated and untreated calves. A value of p < 0.05 was considered significant [27].

This study was approved by the Animal Care Committee of the Facultad de Ciencias Veterinarias de Universidad Nacional del Centro de la Provincia de Buenos Aires, Argentina.
3. Result

No negative side effects, like death, depression, emaciation or nervous signs were registered in the treated calves during the experiment. The weight gain showed a tendency to be higher compared to the untreated group: 76.07 kg (SD: 6.37) vs 70.33 kg (SD: 9.18). The number of dead calves (0-1) calves with scour (8 - 13, p = 0.09), calves with respiratory distress (0 - 3) and calves with antibiotic treatments (3 - 5) were for treated and control groups, respectively. Subjectively, the hair of the treated calves was brighter and their state of alert was higher.

The Table 1 shows the results obtained by testing the metabolic and microbicidal activity of neutrophils in calves from both groups.

4. Discussion and Conclusions

In no case the inclusion of the MMP produced some problems to the health for the calves also if showed health benefits in calves. Brightness of the hair, the state of alert and a tendency to an increased weight gain observed in MMP-treated calves may indicate an improvement at intestine level allowing a better absorption of nutrients induced by probiotic. The MMP treatment shown a tendency in favor of the lower number of calves whit diarrhea (p > 0.09). This outcome agrees with that of other studies in calves [28,29,14]. The MMP reduced the number of calves whit distress respiratory and the calves whit therapeutic treatments against diarrhea and respiratory disorders, although had not difference significant. Apart from their positive effects on gastrointestinal infections, probiotic may be used to prevent non intestinal infectious conditions, such as respiratory tract infections [30,31]. The present results indicate that this MMP may reduce the mortality and antibiotic treatment in veterinary practice as was observed by Timmerman et al. (2005) [14]. The most impressive observation made in treated calves was the significant increase in metabolic and microbicidal activity in neutrophils. The observation that digestive and respiratory infections diminished in this MMP group when compared to the untreated group may indicate a good connection between the MMP and the immune system. This situation has been demonstrated in other animal species [32-34].

Host specificity is regarded as a desirable property for probiotic bacteria and therefore recommended as one of the selection criteria [35,36]. This criterion was not considered by us during this probiotic development [23]. Biological diversity and symbiosis strengthen adaptability and these three concepts constitute the most impressive characteristics of this probiotic, suggesting that these are significant factors for the success in favoring gut health in calves. Acting as a unit enabling multifunctional coordinated actions, a diversities, symbiotic and adaptive community would have better chances to survive, colonize or have a longer residence in gut than a monostrain probiotic. Concerning crosstalk, advantages of the new probiotic would be obvious. As a consequence, up to this date, we only can speculate about the probiotic mechanism of action to achieve the documented beneficial effects in intestine.

The decrease of incidence of respiratory diseases and diarrhea and the highly significant increase of phagocyte activity in peripheral blood leukocytes seen in calves strongly suggest an efficient connection between the probiotic and the immune system. Studies examining gut colonization and dialogue between community members and host are under way.

5. Acknowledgements

This work was financially supported by grants PICT06-2106 from FONCYT (Argentina), from CIC (Buenos Aires—Argentina) and from Secretaría de Ciencia, Arte y Tecnología de la Universidad Nacional del Centro de la Provincia de Buenos Aires.

Table 1. Functionality and microbicidal activity of neutrophils in calves treated with the probiotic and in control calves.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NBT</th>
<th>H2O2 (OpZ)</th>
<th>H2O2 (PMA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>5.5 ± 3.51</td>
<td>12.4 ± 7.59</td>
<td>4.3 ± 1.73</td>
</tr>
<tr>
<td>Probiotic (n = 7)</td>
<td>27.3 ± 2.12</td>
<td>35.3 ± 11.83</td>
<td>23.4 ± 8.63</td>
</tr>
</tbody>
</table>

p: number of calves; NBT: nitro blue tetrazolium; Δ DO/1 × 10^6 PMN; PMA: phorbol myristate acetate; OpZ: opsonized Zymosan, H_2O_2: nmoles/1 × 10^6 PMN.

REFERENCES


