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Influence of the Probiotic Prolam™ at the Oxidase Activity of Blood Neutrophils of Newborn Calves

Korablyeva TR* and Senchuk IV

Academy of bioresources and environmental sciences, Crimean Federation University by V.I Vernadskyi, Russia

Research Article

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***Corresponding author:** Korablyeva Tatyana Rafailovna, Doctor of Veterinary Sciences, Head of the department of microbiology, epidemiology and veterinary-sanitary examination of Academy of Life and Environmental Sciences, Crimean Federation University by V.I. Vernadskiy, Russian Federation, Russia, Email: astemenkolp@gmail.com

Abstract

The aim of our research was to study the influence of different doses of the probiotic Prolam™ on the germicidal activity of neutrophils of calves' blood in a newborn period. Our studies have shown that the daily watering of probiotic Prolam™ to the calves within 10 days after birth in a dose of 5 ml did not have a material impact on the adaptive reserve of neutrophils having oxidase activity. The use of this probiotic in a dose of 20 ml optimized homeostasis that was evidenced by the reduction of the relative content of neutrophils in the blood of calves to the normative values and caused the rising of the oxygen-dependent germicidal activity of neutrophils of calves blood. The selection of effective doses of probiotics can be justified indicators oxidase activity of blood neutrophils of newborn calves.

Keywords: Probiotic; Homeostasis Neutrophils; Microbicidal Activity of Blood Neutrophils; Newborn Calves

Introduction

The antagonistic properties of bacteria are one of the mechanisms of formation and functioning microbiocenoses. In the safety and efficacy of probiotics on the basis of lactobacilli and bifidobacteria convince a sufficient number of publications. Lactobacillus bulgaricus (L. bulgaricus), Lactobacillus plantarum, Streptococcus thermophillus (S. thermophillus), Enterococcus faecium, Enterococcus faecalis, Bifidobacterium species, and Escherichia coli were listed [1,2].

In most cases, the antagonistic action of lactobacilli and Bifidobacterium mediated formation of specific metabolic products (toxins, antibiotics, lytic enzymes, bacteriocins) which may be synthesized in both monocultures and in the presence of a heterologous cell population [3].

The cattle industry has used various types of probiotics for many years [4]. Probiotics are a general category of dietary products that can be included in animal rations to enhance performance and/or reduce pathogenic bacteria [5]. The most commonly used probiotic bacterial strains remain Bifidobacterium and Lactobacillus [6].

One of the important places in the humoral-cellular organism protection from the infectious agents take polymorphonuclear neutrophil leukocytes PMNL. The aim

of our research was to study the influence of different doses of the probiotic Prolam™ of blood neutrophils of newborn calves.

Material and Methods

For achievement of the supplied goal on a dairy farm of educational, scientific and production center of the Academy of bio-resources and environmental sciences V.I. Vernadsky CFU was conducted a scientific and economic experience by the use of the probiotic Prolam™ (Biotechagro, Kuban, Russian Federation) for calves.

Characteristics of the probiotic Prolam™: it contains 5 strains of microorganisms (2 - of Lactobacillus, 2 - of Lactococcus and 1 - of Bifidobacterium), milk, sugar beet molasses, water, chalk, glucose, yeast. In $1cm^3$ of preparation contains at least $1*10^8$ colony forming microorganisms, preparation doesn't contain genetically modified microorganisms, it is used for prophylaxis and treatment of dysbacteriosis of farming animals.

For the experiments by paired analogs method were formed 3 groups of calves of red dairy breed (10 animals in each group): group № 1- control, group № 2 and № 3 - experimental. The calves of the control group didn't get the probiotic drug. The calves of the two experimental groups were given the probiotic Prolam™ for 10 days after the birth, with the only difference that the dose of probiotic formulations for the calves of the second group amount 20 ml, and for the animals of the third group - 5 ml. The calves were kept in the conditions suitable to a veterinary zoo-hygienic requirements, obtained an economic ration in accordance with the generally accepted norms. The experiments were conducted corresponding with the rules of work with the experimental animals. Blood samples were collected in the morning before the feeding, twice during the period of researches: before the application of the preparation and in 10 days from the start of application. The animals were clinically observed, accounting the body weight and growth rate.

For the analysis of the obtained results have been used hematological indices and parameters of an oxygen-dependent germicidal activity of neutrophils of calves blood. The amount of leukocytes in blood was counted in the Goryaev camera. Oxygen-dependent germicidal activity of neutrophils in blood was determined by NBT-test (+NBT %), based on the reduction of the absorbed soluble dye of nitroblue tetrazolium into the insoluble diphormazane. The examined blood was mixed with an equal volume of 0.1% solution of NBT (company «Sigma" USA) prepared on the phosphate buffer (Ph = 7.2). An activity of the oxidase systems of neutrophils (+NBT,%) was evaluated in 2 conditions: unstimulated-basal (NBTb) - in fresh blood stabilized with heparin, and stimulated (NBTs) - after putting in blood samples a heat-skilled of Staphylococcus aureus strain -209 P, that simulates the conditions of a bacterial infection and characterized adaptation reserves of germicidal and absorption ability of neutrophilic granulocytes. For the placing of stimulated NBT-test was used heparinized venous blood of calves, which was poured into a test - tubes, mixing each sample with a suspension of heat-killed at 80°C daily agar culture of Staphylococcus aureus strain 209-P (test system), prepared on a sterile saline and added 0.1% aqueous solution of nitroblue tetrazolium (NBT). For the statement of unstimulated NBT-test used heparinized venous blood of calves, which was poured into test tubes, each sample was mixed with a sterile saline solution and 0.1% aqueous solution of nitroblue tetrazolium. As a control, it is used 0.1 ml of heparinized blood taken away from the tested animals to which was added 0.2 ml of sterile saline. The arrangement of the NBT-test conducted using the identical reagents, glassware and equipment. The relative amount of blood neutrophils, potentially possesing by an oxidase activity and revealing it only after bacterial stimulation of the cells determined as the difference between + NBTs, % and + NBTb, %. The indicator of the reserve of oxidase activity of peripheral blood neutrophils (RON) determined using the formula:

$$RON = NBTs / NBTb$$

The use of animals for research purposes is regulated by the federal government. The research has complied with all relevant federal guidelines and institutional policies. The obtained digital data were processed by the method of variation statistics. To identify statistically significant differences was used a Student's t-test.

Results and Discussion

The analysis of blood samples of the experimental animals shown (Table 1) that before the beginning of test the maintenance of white blood cells corresponded to the highest standard values, after the finishing of the experiment the amount of it did not significantly changed. The relative content of neutrophils of all nuclear forms conformed to the highest standard values in the blood of calves in group's № 1 and № 2, and in a group of animals № 3 even slightly exceeded them.

Index	Before the experiment			After 10 days after using the probiotic		
	1 group (control)	2 group	3 group	1 group (control)	2 group	3 group
Total leukocytes count 109/L	9,14±0,601	10,19±2,48	10,88±1,25	10,32±1,42	8,99±1,04	11,14 ± 3,97
Neutrophils, %	31,13±4,12	29,92±6,74	33,03±1,14	50,50±10,16**	24,42±5,09*	29,48±1,28*
+ NBT b, %	13,55±3,92	11,50±2,80	16,13±3,25	41,90±5,81**	9,80±1,52*	13,30±1,87*
+ NBT s, %	29,35±2,06	24,73±3,21	26,70±4,20	19,80±1,74**	33,60±4,11*	26,60±2,41*
RON	2,18±0,33	2,63±0,71	1,59±0,41	2,12±0,32	3,43±0,50*	2,82±0,23**

Table 1: Microbicidal activity of neutrophils of blood from experimental calves.
Notes: [1] Mean values ± standard deviation
* - p <0.05 for group № 1,
**- p <0.05 before the experiment
(+NBTb) - oxidase activity of unstimulated neutrophils, (+NBTs)- oxidase activity of stimulated neutrophils.

The studying of the germicidal activity of neutrophils of calves blood taken from the experimental groups, showed that the relative number of neutrophils, exhibiting an oxidase activity (unstimulated NBT-test), was slightly higher than the standard values, before the experiment. At the end of the experiment were marked the opposite processes in allowance of neutrophils in the blood of test animals: at the group of calves № 1 was established a tendency of increasing of neutrophil levels by 162.22% (p<0,05).

Watering a probiotic Prolam™ to the calves within 10 days after the birth caused an increasing of the number of oxidase activity of unstimulated neutrophils on in control groups, In connection with this an exponent of oxidase activity reserve of neutrophilic granulocytes of calves from the control group in the end of the experiment was lower than at the animals from the second experimental group on 130.41% (p< 0,05).

After watering the probiotics was registered a tendency of decreasing of the relative number of cells revealing an oxidase activity in basal conditions in the animals of groups № 2 and № 3, respectively, compared with the beginning of the experiment, that indicates the optimization of homeostasis of the experimental animals.

At this period the adding of Staphylococcus aureus as a stimulator of metabolic activity to the blood samples, taken from calves of № 2 and № 3 groups, for raising the stimulated NBT - test, caused a reliably significant increasing of the maintenance of oxidase active neutrophils at 169.69 and 134.34 %, respectively, compared with the basal levels, what points to the increasing of the adaptive reserve of this protective mechanism. At the calves of control group the value of the adaptation reserve of cells, possessed an oxidase activity was significantly lower than in animals which were applicated probiotic. Indicators of the reserve of an oxidase activity of blood neutrophils of calves from the 2 and 3 experimental groups, after the end of the experiment, were significantly increased compared with the beginning of the experiment. After watering the probiotics preservation calves in the control group was 60% for the experimental animal's croups 2 and 3 of 100% and 80% respectively.

Conclusion

It is known that the oxygen-depending cytotoxicity appliance of blood cells conditioned by a functional activity of segmented neutrophil forms. The process of absorption and elimination of pathogenic bacteria is accompanied by a dramatic increase in the rate of absorption of molecular oxygen PMNL and generation of reactive oxygen species (ROS): superoxide anion radical (O^-), hydrogen peroxide, hydroxide radical and other forms, which are the components of the oxygen-dependent germicidal PMNL system. ROS formed by an activation of the PMNL come both in phagosomes and in the extracellular space, causing damages to themselves (PMNL) and to the tissues which they infiltrate. It has been shown that the increased production of ROS by phagocyting PMNL observed under a number of bacterial and autoimmune diseases. The predecessor of ROS generated by phagocyting PMNL is a (O^-) defined by a number of reduced formazan solution of nitroblue tetrazolium (NBT) [7]. According to our studies, at the end of the experiment, compared with the beginning, the adaptational reserve of oxidase active neutrophils significantly increased only in the animals of the experimental group № 2, which have been watering the probiotic Prolam™ in a dose of 20 ml. In the calves of the experimental group № 3, which have been watering Prolam™ in a dose of 5 ml was established a statistically reliable trend to the increasing of the

number of these cells.

The use of this probiotic in a dose of 20 ml optimized homeostasis that was evidenced by the reduction of the relative content of neutrophils in the blood of calves to the normative values and caused the rising of the oxygen-dependent germicidal activity of neutrophils. Preservation calves in the second groups was 100%.

As is known probiotics can enhance both the specific and nonspecific immune response, possibly by activating macrophages, increasing levels of cytokines, increasing natural killer cell activity, and/or increasing levels of antibody. In spite of limited testing in humans, these results may be particularly important to the elderly, who could benefit from an enhanced immune response. The immune system effects of probiotics are to enhance specific and nonspecific immune response, inhibit pathogen growth and translocation, and reduce chance of infection from common pathogens.

We believe the selection of effective doses of probiotics can be justified indicators oxidase activity of blood neutrophils of newborn calves.

Conflict of Interests

He authors declare that there is no conflict of interests regarding the publication of this paper.

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Korablyeva Tatyana Rafailovna - Doctor of Veterinary Sciences, Head of the department of microbiology, epidemiology and veterinary-sanitary examination of Academy of Life and Environmental Sciences «Crimean Federation Yniversity by V.I. Vernadskyi», e-mail: astemenkolp@gmail.com, 295492, Agrarnoe Senchuk Ivan Victorovich - Candidate of Veterinary Sciences, Associate Professor Department of therapy and obstetrics of Academy of Life and Environmental Sciences «Crimean Federation Yniversity by V.I. Vernadskyi», ivansenchuk1981@mail.ru, 295492, Agrarnoe

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