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RESEARCH ARTICLE

Probiotic Drugs Impact on the Innate Immunity Factors

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Abstract

Probiotics are the drugs containing living microorganisms related to normal physiological intestinal flora. Recently probiotic drugs are made based on microorganisms' *pp. Bifidobacterium, Lactobacterium*, apathogenic strains *pp. Streptococcus, Enterococcus*, acquired from the humans and animals' gastrointestinal tract. These microorganisms have a certain impact on the innate immunity factors, interacting with the lymphoid intestinal system. The studies were aimed at examination of the probiotic drugs impact on the innate immunity factors. 60 newborn calves of black-motley breed and 60 piglets of large white breed of 45-day weaning became the object of the study. Blood draw of newborn calves and weaned piglets for immunological studies was performed before the study and then on the 10th, 20th and 30th day of the study. When examining the innate immunity factors there were defined such factors as lysozyme activity in blood serum, blood serum bactericidal factors value and phagocytosis system factors. It was stated that the animals treated with the probiotic drugs based on *L. plantarum 8P-A3* and synbiotic drugs based on *L. plantarum 8P-A3* as well as on the medicinal plant materials have the innate immune factors activated with age.

Key words: *L. plantarum 8P-A3, innate immunity, Chelidonium majus L., Berberis vulgaris, blood phagocytic activity.*

Introduction

Probiotics are the living microorganisms benefiting the host when taken in adequate amounts [1, 2]. *Lactobacillus* and *Bifidobacterium* are mostly used as probiotics, but *Saccharomyces boulardii* yeast and some kinds of *E. coli* and *Bacillus* can also perform this function. It was Mechnikov I.I. who defined the lactobacilli antagonism in relation with potentially pathogenic representatives of the intestinal microbiocenosis. The biomechanisms of the normal flora antagonistic effects are most studied based on the lactobacilli example and can be referred to the other normal biosis representatives.

The main function of the intestine lactobacilli is the biosynthesis of different kind's metabolites, suppressing the vital activity of potentially pathogenic representatives of the microbiome and removal of the latter from the intestine bacilli population [3].Numerous studies showed that [4,5,6,2,7] microorganisms *p. Lactobacterium* stimulate immunity T- and B-nuclei, activates production of specific IgA and IgM, non-specific IgG as well as the secretory pool sIgA, creating local immunity of the gastrointestinal mucosa. It is the secretory sIgA, that creates a mucous barrier, preventing the aggressive pathogens from penetration into the internal intestine walls and forms the live environment, which is critical for many pathogenic organisms [8].Bacterial component of probiotic drugs can serve as protection from potential pathogenic compounds, coming from the outside and created as a result of the organism life activity [9].

It is stated that animals without dysbiotic disorders of the gastrointestinal tract microbiota have higher activity of the phagocytosis system as well as of the immunity humoral factors in comparison with the animals suffering from intestinal

dysbacteriosis. Lactic acid bacteria contribute to the activation of the phagocytic cells and enhance the production of lymphocytic interferon. Probiotic drugs also contribute to the activation of the blood serum bactericidal system [10, 13]. Now the probiotic drugs are made based on microorganisms *pp. Bifidobacterium, Lactobacterium*, apathogenic strains *pp Streptococcus, Enterococcus*, acquired from the humans and animals' gastrointestinal tract.

These microorganisms have a certain impact on the innate immunity factors, interacting with the lymphoid intestinal system. The intestine lymphoid system consists of epithelial tissue, intestinal mucosa sheet, Peyer glands, mediastinal lymph nodes, specific intestine cells (M-cells), dentritic cells, polymorphonuclear leucocytal cells and lymphocytes.

However, it should be mentioned that the impact of different probiotic microorganisms on the intestine microbiome, intestine lymphoid system and local immunity is very diverse and not fully studied, that sometimes prevents from wide use of the probiotic drugs in medical and veterinary practice [14].The cavernous organs' mucosa makes a uniform cooperative system. It is the gastrointestinal tract colonization resistance that defines its barrier function.

The gastrointestinal tract colonization resistance is a symbiotic capability of the organism and its microflora to protect the endogenous cavities from the pathogenic agents [15].Formation of the anti-infective immunity begins with development of inflammatory reactions during interaction of pathogenic agents with *Toll*-like receptors.

This reaction promotes a cytokine cascade mechanism, responsible for enhancing the activity of phagocytic cells and other immunocompetent cells, immunoglobulins that increases activity of the intestine colonization resistance and helps to eliminate the pathogenic agent from the macroorganism [15, 16].

When using the probiotic microorganisms there is a higher possibility for a more active elimination of pathogenic agents on the gastrointestinal mucosa. It is caused by a group of the inflammatory processes contributing to the proinflammatory cytokines synthesis.

Besides, the researchers have noted the activation of the complementary blood system [17, 6].You should pay attention to the works devoted to study of probiotic bacilli impact on activity of the active phagocytic cells, enhancing the activity of the intestine lymphoid cells and immune system T- and B-cells [16, 1].The biomodels show that lactobacilli and bifidobacteria enhance the phagocytosis system activity [18, 12], increase NK killers number, as well as their viability [19].

Recently, it has been established that the probiotic bacilli the local immunity system, due to a rapid antagonistic interaction with pathogenic bacilli. If there are no changes in normal biocenosis, the microflora balance is maintained, and the immune system is not affected. However, the effects of using probiotic drugs in the immunosuppressive conditions may be reversed[20].But still, most of the literature sources prove that administration of the probiotic bacilli is accompanied with productive immune system alteration.

It happens because of the increase in the number of epithelial lymphocytes along with increase of their reproduction in the intestinal crypts [21].New blood vessels and intestinal epithelial cells are actively formed in the intestinal mucosa villi. As we can see from the abovementioned the probiotic commensal bacilli contribute to the activation of bactericidal and phagocytic blood systems, immunoglobulins synthesis and immunocompetent cells differentiation [22, 2, 7].Therefore, it is obvious that we need to study the dynamic factors of the natural resistance thoroughly when using the probiotic drugs. The objective of the study is to examine the impact of the probiotic drugs on the innate immunity factors.

Research Materials and Methods

The studies were performed on the basis of the Chair of Infectious Diseases, Pet Hygiene and Veterinary Sanitary Inspection of the Federal State Budget Educational Establishment of Higher Education Bashkir State Agrarian University. 60 newborn calves of black-motley breed and 60 piglets of large white breed became the object of the study. The animals have been selected for studies based on the paired analogues principle.

The following was used in the work:

- Liquid probiotic lactobacterin- microbial lactobacilli live weight (*L. plantarum 8P-A3*), grown on whey and milk medium;
- A synbiotic based on medicinal plant materials and lactobacilli - microbial lactobacilli live weight *(L. plantrum 8P-A3)*, grown on whey and milk medium, with addition of celandine grass aqueous extracts *(Chelidnium majus L.)* and common barberry fruits (Berberis vulgaris), with viable cells content of 7.4–9.3 × 109 CFU / ml.

The probiotic and synbiotic drugs were obtained from Immunopreparation FSUE NPO Microgen of the Ministry of Health of the Russian Federation. 1 ml of probiotic drugs contained (7.4-9.3) • 109 CFU/ml. The lactobacillus strain used for preparation of probiotics and phytoprobiotics is resistant to the antibiotics of the teracycline series, penicillin series, cephalosporins and can be used in conjunction with these antibiotics.

Besides, the synbiotic compositions have an evident inhibitory antagonistic activity in relation to the strains of *E. coli157, St. aureus 209, Pr. mirabilis 237, Pr. vulgaris 177* [23]. At the first stage of the scientific studies we examined the impact of probiotic based on *L. plantum 8P-A3* and synbiotic based on the medicinal plant materials and lactobacilli, on the innate immunity dynamics of newborn calves.

The calves of the control group (n = 20) were kept under the conditions of the adopted managing and feeding technology; the calves of the second (n = 20) and third (n = 20) test groups received liquid probiotic lactobacterini and synbiotic orally in a dose of 20 ml per head daily from the 1st to the 10th days from birth, and then from the 20th to the 30th days from birth. At the second stage of the scientific studies we examined the impact of probiotic based on *L. plantarum 8P-A3* and symbiotic based on medicinal plant raw materials and lactobacillin on the weaned piglets.

The piglets of the control group (n=20) were kept under the conditions of the adopted managing and feeding technology. The piglets of the second (n = 20) and third (n = 20) test groups received liquid probiotic drugs orally in a dose of 1 ml per 10 kg of live weight daily from the 1st to the 10th days after weaning. Before the study and then on the 10^{th}, 20^{th} and 30^{th} day we performed the blood draw to examine the innate immunity factors dynamics. We determined the blood serum bactericidal activity according to P.A. Emelyanenko (1980).

We used *Staphylococcus aureus* as a test-microbe. When performing the study, we received BCH optical density with culture and blood serum right after mixing and the optical density of the same mixture in 3 h of incubation in the thermostat. The measurements were taken using FEC-N in 5 mm thick cuvettes with a green filter against distilled water; the bactericidal activity of the test serum was expressed as a percentage.

We determined the blood serum lysozyme activity according to V.G. Dorofeychuk [24]. The method principle is based on the ability of blood serum lysozyme to induce lysis of a test microbe (*Micrococcus lisodeicticus*).Suspension was prepared in the phosphate buffer from a daily culture of the test microbe.

In order to study the neutrophils phagocytic activity we used 0.8 μm latex particles. The mixture of leukocytes with latex was kept in a humid chamber at 37° C for 30 min with gradual, slight agitation. Then we prepared the smears, fixed them in methanol for 5 min and stained with azur-II-eosin. The neutrophils absorption capacity was evaluated by: 1) phagocytic activity - the number of phagocytic cells. 2).

Phagocytic number-the average number of phagocytized latex particles absorbed by one neutrophil and 3) phagocytic index-was calculated by dividing the number of latex particles absorbed by the total number of leukocytes counted. Statistical processing of the experimental data was performed using the statistical analysis package for *Microsoft Excel*®. Statistical significance between the groups was evaluated using Student's t-test from $p \leq 0.05$ to $p \leq 0.001$.

Research Results and Discussion

The innate immunity is defined by cellar and humoral factors. The principal components of the macro organism natural immunity are as follows: neutrophils phagocytic activity, blood serum bactericidal activity and lysozyme activity.

The blood serum lysozyme activity is the activity of lysozyme protein, dissolving the polysaccharide of the prokaryote cellular membrane that contributes to cellular wall destruction, endosmosis change and death of bacteria [4].The blood serum lysozyme activity of the newborn calves of the control and test groups was rather evident and laid within the range of 21.8±0.36% - 22.0±0.42% (Fig. 1). The lysozyme activity of the calves in the control group changed from 21.8±0.36% до 24.8±0.33% during the studies.

The liquid probiotic added to the calves' diet enhanced the lysozyme activity. Thus, on the tenth day of the tests this indicator was smaller for the calves of the control group by 1.2%, and on the twentieth day it exceeded the previous value by 0.3%, on the thirty's day-by 0.65%. The blood serum lysozyme activity of the calves received the symbiotic drugs, changed more actively. Thus, if on the tenth day of the tests the lysozyme level exceeded the control value by 0.1%, then on the twentieth day- by 2.4%, on the thirty's day- by 3.4%.

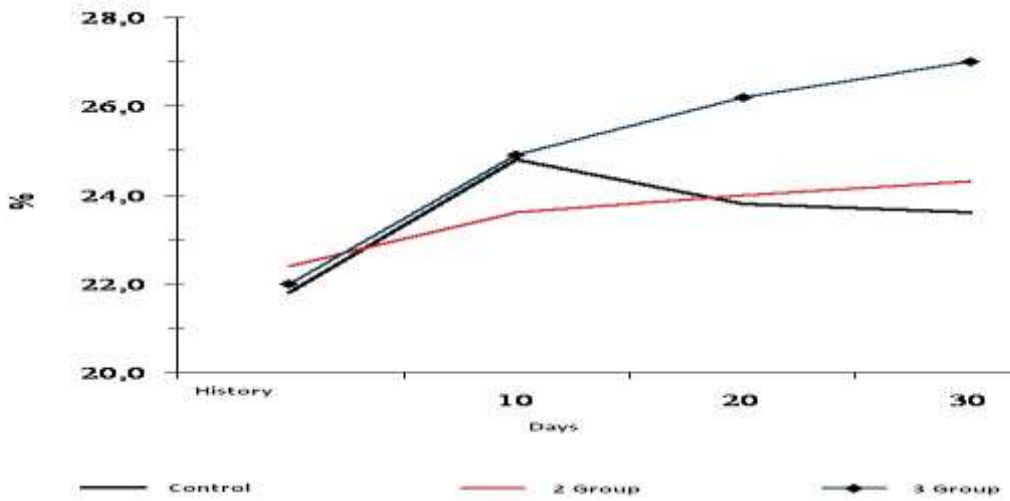

Figure 1: Lysozyme activity dynamics of the calves' blood serum, %

The lysozyme activity of the piglets in the test groups was within the range of 41.5±0.7 – 42.5±0.1%. The blood serum lysozyme level of the first group piglets changed from 41.5±0.72 to 42.5±0.64% during the studies. The blood lysozyme of the second test group animals increased in comparison with the control values on the tenth day of the tests by 1.2%, on the twentieth day- by 2.8% and on the thirty's day- by 4.4%. We also recorded dynamic increase of lysozyme for the piglets from the third test group.

Thus, on the tenth day of the tests the blood serum lysozyme activity exceeded the control values by 2.8%, on the twentieth day- by 5.2% and on the thirtieth day-by 7.0%. The blood serum bactericidal system consists of the biological components of the serum, which, when interacting with a pathogenic agent, either destroy it or inhibit its activity. As we can see in Figure 2 the blood serum bactericidal activity of the test calves was within the range of (31.9±0.5% - 34.1±0.4%) (Figure 2).

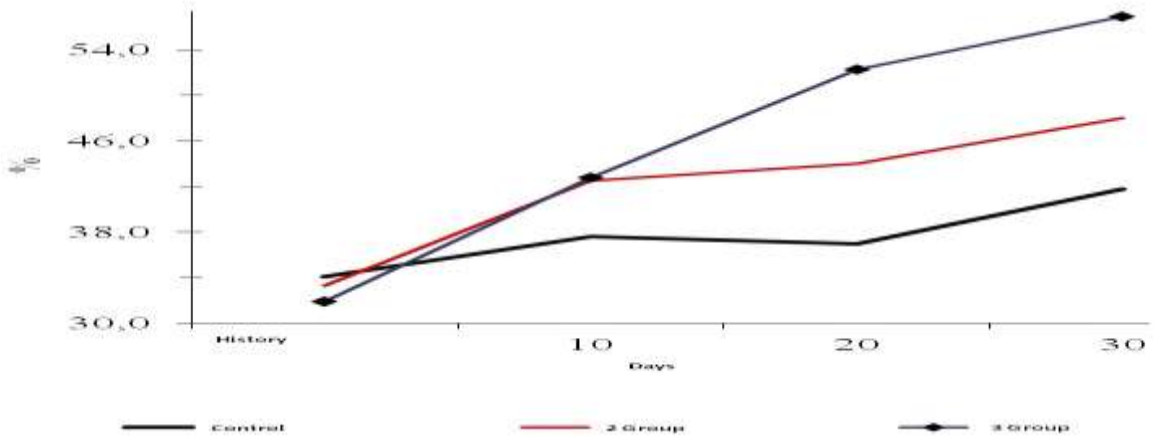

Figure 2: Bactericidal activity dynamics of the calves' blood serum, %

The indicators of the blood serum bactericidal activity for the second test group calves in the course of the studies were higher than the control values on the tenth day-by 4.9%, on the twentieth day-by 3.0% and on the 30-th day-by 6.2%. The highest blood serum bactericidal activity was recorded for the third test group calves. Thus, on the thirtieth day of the studies it exceeded the first and second groups by 15.8% and by 8.9%, respectively.

The changes of the blood serum bactericidal activity for the first control group piglets ranged from 42.6-43.2%. The piglets received liquid probiotic drugs had a higher level of the blood serum bactericidal activity in comparison with the control group. Thus, the studied indicator was higher than the control values on the tenth day by 3.0%, on the twentieth day – by 4.4% and on the thirtieth day – by 6.9%.

The activity of the bactericidal blood systems for the third test group animals changed more intensively. The values of this group exceeded the control ones on the tenth day by 5.1%, on the twentieth day-by 4.4% and on the thirtieth day- by 6.9% and the value of the second test group by 2.0%, 3.7% and 6.2% respectively. Therefore, the humoral factors of the innate immunity represent the peptide components of the blood serum and other liquids of the macroorganism. The principal factors are the bactericidal activity components, namely, lysozyme protein and complementary activity. Our studies showed that the probiotic drugs contribute to increase of the blood serum bactericidal activity throughout the entire studies. The neutrophils phagocytic activity is the number of the actively phagocytic leukocytes per 100 cells.

This factor indicates the ability of neutrophilic leukocytes to phagocytose foreign agents, including pathogenic microorganisms. Reduction of phagocytosis can occur due to decrease in blood serum factors and destruction of the phagocytes themselves [25]. Phagocytes are mainly leukocyte cells that absorb exogenous pathogenic agents and destroy them. In the course of the performed studies we determined that the phagocytosis activity of the control and test groups calves ranged from 32.3±0.5% до 34.6±0.6%, the phagocytic number did not exceed 4.9±0.6 units, phagocytic index 2.3 ± 0.03 - 2.5 ± 0.007 units (Table 1).

Table 1: Dynamics of the phagocytosis values for calves

Animal groups	**Research days**							
	Background		10		20		30	
	Statistical index							
	M±m	P	M±m	P	M±m	P	M±m	P
Phagocytic activity of neutrophils, %								
Control	32,3±0,5		34,5±0,6		37,5±0,6		40,8±0,8	
The first group	34,6±0,6		38,8±0,7	***	40,4±0,7	***	44,5±0,5	***
The second group	33,2±0,6		45,6±0,5	***	52,1±0,1	**	56,3±0,8	***
Phagocytic number, units.								
Control	4,7±0,1		5,4±0,03		4,2±0,03		4,8±0,06	
The first group	4,9±0,6		5,2±0,08	***	5,2±0,06	***	5,3±0,053	***
The second group	3,9±0,02		4,6±0,03	***	5,1±0,06	***	5,8±0,09	***
Phagocytic index, units.								
Control	2,4±0,05		1,6±0,03		1,6±0,03		1,5±0,03	
The first group	2,3±0,03		1,8±0,02		2,3±0,03	**	2,4±0,035	***
The second group	2,5±0,07		2,6±0,04	*	2,6±0,03	***	2,7±0,041	***

The phagocytic activity of the first group animals ranged from 32.3±0.5% to 40.8±0.8%. The blood serum phagocytic activity of the second test group calves changed throughout the study when using the probiotic drugs. The number of the active phagocytic neutrophils was higher than the control value on the tenth day by 4.3%, on the twentieth day – by 2.9% and on the thirtieth day- by 3.7%. The number of the active

phagocytic neutrophils and the phagocytosis index increased throughout the term of the test. These indicators exceeded the identical values of the control animals on the thirty's day of the test by 0.5 unit and 0.9 unit. The level of the neutrophils phagocytic activity changed more intensively for the animals received liquid symbiotic product. The macrophages phagocytic activity exceeded the control values and the data of the second test group calves by the end of the studies by 15.5% and 11.6% ($p<0.01$), respectively.

Besides, the calves of the third test group received symbiotic drugs, had a higher phagocytic number and index. Thus, the phagocytic number of the calves on the thirtieth day was higher than the control data by 1.0 unit while the phagocytoc index increased by 1.2 unit. Figure 3 shows the studies' results of the piglets' blood macrophages phagocytic activity. The phagocytosis activity of the control group piglets was within the range of 33.4±0.2 - 33.8±0.4 % (Figure 3).

Conclusions

The phagocytosis system of the piglets received the probiotic drugs was activated. Thus, the macrophages phagocytic activity exceeded the indicators of the control animals on the tenth day of the studies by 1.8%, on the twentieth day-by 3.8% and on the thirtieth day- by 5.6%. The phagocytosis activity of the piglets, received the synbiotic drugs was also higher than the level of the control animals on the tenth day of the studies by 6.0 %, on the twentieth day-by 9.7% and on the thirtieth day-by 15.6% and exceeded the level of the second group by 4.2%, 5.9% and 10.0%, respectively. It should be noted that the changes in the indicators of natural resistance of the organism indicate the activation of the nonspecific link of innate immunity when applying probiotic and synbiotic drugs.

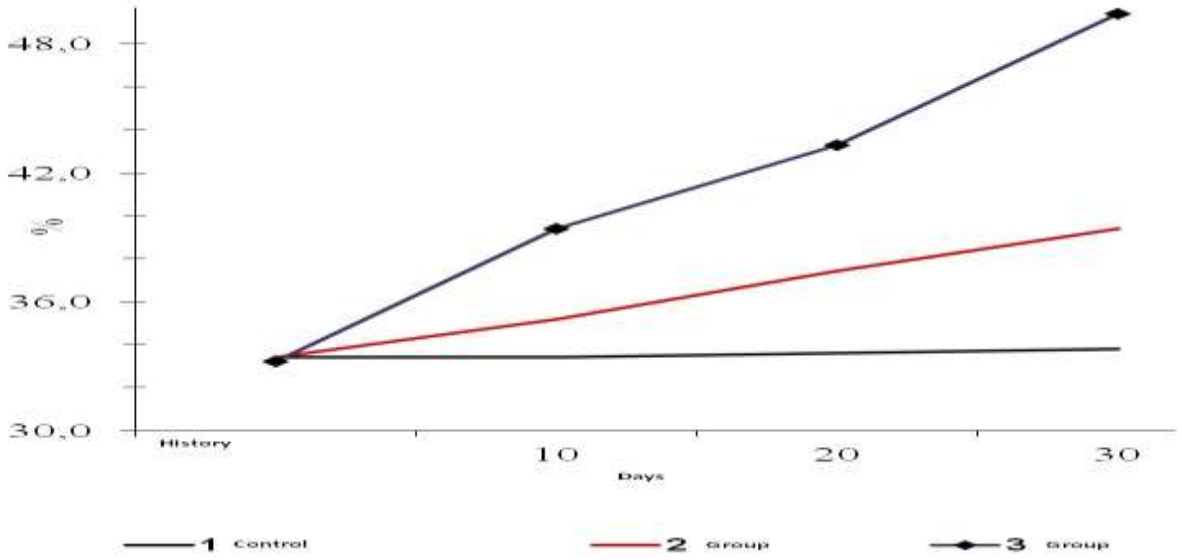

Figure 3: Neutrophils phagocytic activity of the piglets' blood (%)

The number of the phagocytic cells of the control group piglets' blood ranged from 4.0 to 4.3 units. The level of the phagocytic number of the second group animals' blood, received the probiotic drug throughout the studies exceeded the indicators of the control animals by 0.25, 0.44 and 0.59 units, respectively. The number of the active phagocytic cells in the blood of the third test group piglets, received the symbiotic drugs, changed more evident. The phagocytic number in the piglets' blood of this group was higher than the control values on the tenth day of the studies by 0.6 units, on the twentieth day-by 1.20 and on the thirtieth day- by 1.95.

The phagocytosis level of the control group animals ranged from 1.3 to 1.35. The test showed increase of the phagocytic index of the second group piglets in comparison with the control figures on the tenth day of the studies by 0.08 units, on the twentieth day-by 0.24 units and on the thirtieth day-by 0.37 units. The phagocytic index of the piglets, received liquid symbiotic drugs changed more actively. Thus, the mentioned indicator was higher than the control figures and the data

of the second test group on the tenth day of the studies by 0.3 units, on the twentieth day-by 0.72 units and on the thirtieth day – by 1.05 units and by 0.28, 0.49 and 0.67 units, respectively. In the course of the studies we found out that the neutrophils phagocytic activity is one of the indicators of the microorganism immune response when taking the probiotic bacilli. The phagocytic immune component changed the most actively when taking probiotic and synbiotic drugs. Therefore, the use of the probiotic drugs activates the innate immunity factors.

The blood serum bactericidal activity of the newborn calves and weaned piglets increases by 1.29 and 1.35 times, respectively, the lysozyme activity- by 1.29 and 1.14 times; the phagocytic macrophages percentage increase by 1.3 and 1.37 times with increase of the phagocytic number and index. We should note that the changes, defined in the indicators of the organism natural resistance indicate the activation of the innate immunity non-specific component when taking probiotic and symbiotic drugs.

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