

Clinical and biochemical parameters of broiler chickens on the background of application of probiotic feed additive "BACELL-M®"

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Abstract. The paper presents the results of the study of morpho-biochemical, clinical-zootechnical and histological parameters of the organism of broiler chickens of KOB-500 cross against the background of application of probiotic supplement "BACELL-M®". The poultry population (n=10) was divided according to the principle of pair-analogs into experimental (n=5), with inclusion of probiotic additive in the diet and control (n=5) groups - diet without probiotic additive. Both groups were fed with "BEST®" brand mixed fodder. It is found that introduction of this additive into the diet of broiler chickens of the experimental group in the dosage of 100g of probiotic additive per 1 kg of mixed fodder improves general blood parameters, histological development of organs and directly affects productivity in the form of weight gain in comparison with the control group of birds. Due to the introduction of the studied additive in the diet of young poultry blood parameters were in the range of norm, in clinical and zootechnical parameters productivity was higher, as well as the additive improved the growth and development of the spleen. Thus, probiotic additive "BACELL-M®" can be recommended for use in the technology of growing broiler chickens.

1 Introduction

Poultry farming is currently a dynamically developing branch of agriculture in the Russian Federation. It provides the need of the country's population in proteins of animal origin at the expense of dietary meat and eggs [1]. Due to increased competition among producers in the industry in the domestic and foreign markets to obtain quality products, while increasing and improving the efficiency of production, is becoming problematic due to the high cost of feed components. This is reflected in the use of antibiotics on industrial farms together with cheap and sometimes low-quality feed as growth stimulants for poultry. This method leads to the problem of formation of natural resistant microflora [2]. To solve this problem search for alternative growth stimulants, more effective probiotic preparations and feed enzymes for industrial poultry farming are created.

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This paper considers a probiotic developed in the Russian Federation on the basis of bacteria of the genus *Bacillus* in the solution of urgent problems of Russian industrial poultry farming, and also analyzes the prospects for the use of bacteria of the genus *Bacillus* as the basis of biopreparation with phytase activity.

The founder of the idea of restoring health through the use of live microorganisms that improve the composition of intestinal microflora is the Russian scientist, embryologist, immunologist and bacteriologist, Nobel Prize winner in physiology and medicine I. Mechnikov (1845-1916). Studying the problems of aging, he found that with age the number of putrefactive microorganisms in the lower intestine increases, producing toxic substances that poison the body. In 1907, I.I. Mechnikov proposed to supplant putrefactive intestinal microflora with live lactic acid bacteria consumed with plain sourdough [3, 4].

The idea of intestinal microflora correction was developed in the 60-70s of the XX century. The concept of the composition of normal intestinal microflora and its functions in the vital activity of humans and warm-blooded animals was formed, changes in intestinal normoflora during the development of pathological processes and dysbacteriosis of various etiologies were characterized [5, 6]. Fundamental knowledge about the relationships between macro- and microorganisms was the basis for practical realization of the idea of bacteriotherapy: restoration of intestinal normoflora was achieved by using live bacterial cultures. The term "probiotic" was proposed to designate such a live microbial culture, which has a beneficial effect on the host organism by improving its intestinal microbial balance (from Greek "pro bios" - for life, in defense of life) [1, 2, 7, 8]. The first generation of probiotics was created on the basis of bifidobacteria (genus *Bifidobacterium*) and lactobacilli (genus *Lactobacillus*), which are representatives of the obligate intestinal microflora of humans and warm-blooded animals and predominate in it by number and physiological importance. These biological preparations were intended for the prevention and treatment of intestinal dysbacteriosis of various etiologies in children and adults, for the treatment of acute intestinal diseases, for use in the complex therapy of sepsis, pneumonia, and other infectious diseases [9, 10]. The first domestic therapeutic preparation from lyophilically dried biomass of live bifidobacteria *Bifidobacterium bifidum* - "Bifidumbacterin" - was developed in 1972 in the Research Institute of Epidemiology and Microbiology named after G.N. Gabrichevsky (Moscow) [11]. Later, the spectrum of probiotic microorganisms was supplemented with nonpathogenic *E. coli*, spore-forming bacteria, and yeasts. In general, probiotic microorganisms include [11, 12]:

- 1) bacteria producing lactic and propionic acids (*Lactobacillus*, *Bifidobacterium*, *Propionibacterium*, *Enterococcus*);
- 2) non-pathogenic bacteria of the genus *Escherichia* (*E. coli* M-17);
- 3) spore-forming bacteria (*Bacillus*, *Clostridium*);
- 4) yeasts (*Saccharomyces*, *Candida*);
- 5) thermophilic streptococci (*Streptococcus*).

According to modern concepts, probiotics are preparations of live microorganisms, which, when introduced into the animal body, have a positive effect on physiological, biochemical and immune reactions of the host organism by optimizing the composition of its intestinal microflora [9, 13]. Probiotics have a complex action: exhibit antagonistic activity against pathogenic and opportunistic microorganisms due to the formation of antibiotics, bacteriocins, lysozyme, organic acids (lactic, acetic, succinic, formic), hydrogen peroxide, as well as due to competition for habitats and nutrients; participate in digestion by synthesizing hydrolytic enzymes - analogues of digestive enzymes of the macroorganism; produce amino acids, vitamins and other biologically active substances consumed by the macroorganism; have an immunomodulatory effect; destroy toxins, allergens; reduce the level of cholesterol in the blood; contribute to the removal of heavy metals (silver, strontium, cadmium, etc.) from the body [9, 12].

By inhibiting the growth of undesirable microorganisms, probiotics create conditions for the development of normal intestinal microflora, which plays an extremely important and multifunctional role in the vital activity of the host organism: it provides colonization resistance, carries out digestive, synthetic, immunomodulatory, detoxification functions [3, 9, 10]. Colonization resistance of the intestine is of particular importance, which is understood as a set of mechanisms that give individual and anatomical stability to the normal microflora and prevent the host from being colonized by foreign microorganisms [2]. The intestinal microbiota is considered as an independent "organ" that covers the intestinal wall with a biofilm that prevents the introduction of foreign microorganisms and plays an important role in intestinal homeostasis [4].

Microorganisms used as the basis for probiotics must meet certain requirements: 1) be non-pathogenic and non-toxic; 2) be resistant to the acids and bile of the gastrointestinal tract (GIT), survive passage through it; 3) attach to the intestinal epithelial cells; 4) multiply rapidly, colonizing the intestinal tract; 5) metabolize in the intestine; 6) stabilize intestinal normoflora; 7) remain viable during the process of obtaining lyophilized preparations, during their storage and use in production conditions [2, 14, 15].

The main indicator of metabolism in the body of birds, as in all animals, is blood. It participates in the processes of vital activity, delivering necessary nutrients and oxygen to the cells of organs, removes metabolic products. Any disturbance of these processes in blood or organs is often due to improper feeding and housing of poultry, which directly affects the animal's body [14, 15].

At the moment in Russia, both poultry industry and industrial production of mixed fodders and ready-made diets are developing [8]. There are a lot of ready rations such as: BEST, Purina, Tambov Rooster, PK-5, PK-6 and so on. Their composition may differ in many respects, affecting different productive qualities of poultry. There are also low-quality feeds, which in turn may contain mycotoxins that cause changes in the gastrointestinal tract, suppress immunity and can not only affect the functionality of internal organs, blood composition and overall performance of birds, but also lead to death [16, 17].

Unfortunately, in many compound feeds the composition is unbalanced, which directly affects the indicators of the bird's organism. Thus, the purpose of the study was to investigate the biochemical composition of blood, productivity on the basis of clinical and zootechnical indicators and histological structure of the spleen on the background of the application of probiotic feed additive BACELL-M in broilers cross KOBB-500.

In many cases with unbalanced feeding there are various disorders in the body, the balance of biochemical parameters of blood, growth and development of tissues and organs, productivity of birds is disturbed.

Especially in the presence of mycotoxins and other pathogenic fauna in the feed, which can lead to an increase or decrease of vital components in the blood, leading to stress and destruction in the body of birds tissues and organs, adverse effects and death.

Thus, the purpose of our research was to determine the role of a number of basic clinical and biochemical indicators of the organism of broiler chickens. The objective of the work was to study the morpho-biochemical composition of blood, histological structure of spleen and evaluation of clinical and zootechnical indicators when using in the diet probiotic active additive "BACELL-M®".

2 Materials and methods

BACELL-M® probiotic (registration number PVR-1-4.7\02100) compensates for the lack of nutrients, minerals and bioactive substances, as this supplement includes microbial mass of beneficial microorganisms, skim milk, distilled water, sunflower meal and beet molasses.

1 gram of such probiotic contains: *Bacillus subtilis* 945 (B-5225) in the amount of at least - 1×10^8 CFU/g (colony forming units), *Lactobacillus paracasei* (B-2347) in the amount of at least - 1×10^6 CFU/g, *Enterococcus faecium* M-3185 (B-3491) in the amount of at least - 1×10^7 CFU/g. The supplement strains were selected from natural sources, so they are not subjected to genetic transformation [18].

In order to study the effect of the probiotic supplement BACELL-M, an experiment was conducted on broiler chickens of the cross KOB-500 in the conditions of IE "Krutova M. V.", village Borinskoye, Lipetsk district, Lipetsk region. The experiment was conducted according to the principle of pair-analogs, for this purpose two groups of broiler chickens at the age of 14 days were formed. When forming the groups, blood from the shoulder vein was taken from the chickens for background study.

Poultry was divided into two groups, control (n=5) and experimental (n=5) groups. The experimental group received probiotic with the main diet at a dose of 100g per 1 kg of feed. The control group was fed only the main diet (Table 1). On the fortieth day of the experiment all birds (n=10) were subjected to slaughter through decapitation. During this period, blood was taken from chickens to study biochemical parameters. The level of total protein, Gamma-GT, cholesterol, ASAT, ALAT and uric acid was determined in blood serum, as well as the mineral composition was determined.

Table 1. Composition of mixed fodder of the studied poultry.

Ingredients %	Percentage of mix		
	Start	Growth	Finish
Corn	20	20	8.96
Wheat	37.98	42.01	54.77
Bran	-	-	6
Full-fat soybean	-	8.8	4.63
Soybean meal (40-45%)	25	8.4	10.96
Sunflower cake	6.8	13.3	8.62
Feed yeast	3	3	3.25
Fish flour	1.8	-	-
Sunflower oil	0.7	-	-
Monocalcium phosphate	1.1	0.9	-
Chalk	1.8	1.8	1.02
Salt	0.1	0.1	0.1
Premix	1	1	1
Lysine	0.14	0.19	0.19
Methionine	0.22	0.14	0.14
Rovabio Enzyme	0.005	0.005	0.005
Sal-Carb	0.15	0.15	0.15
Bacell	0.2	0.2	0.2
100g of feed contains: DE, kcal	296	285	300
Crude protein, %	21.1	20.02	19.17
Crude fat, %	2.9	3.3	3.5
Crude fiber, %	4.3	4.36	4.5
Lysine, %	1.17	1.05	1.02
Methionine+cystine, %	0.87	0.8	0.78
Calcium, %	1.05	0.9	0.9
Phosphorus, %	0.77	0.77	0.71
Linoleic acid, %	1.46	2.34	2.73

Spleen samples were taken for morphological studies and fixed in 10-12% neutral formalin solution. Then the tissue samples were dehydrated in increasing concentration of ethyl alcohol, sealed with paraffin, paraffin blocks were used for preparation of 4-6 μ m thick

slices on microtome MPS-2 with blade R-35 from the manufacturer "Sakura Finetek Europe B.V." [7], which were stained with hematoxylin and eosin for histology and viewed in light-optical microscope.

3 Results

The following results were obtained when studying the biochemical indices of blood of the studied chickens (Table 2).

Table 2. Biochemical composition of blood of the studied birds.

Probe	Urea μM/l	Creatinine μM/l	AsAT u/l	AlAT u/l	Gamma-GT u/l	Protein g/l	Cholesterol mM/l
Norms	1.0-2.0	20-87	74.4-148.7	1.2-6.8	15-25	32-47	3.4-4.6
Experimental	1.58±0.1	79±5*	127.7±50.9*	6.3±1.4*	18.8±3.82*	37.3±7.40	3.4±0.7
Control	1.8±0.74	115±6.4*	189.7±45.8*	7 ±1.2*	26.6±3.52*	48.2±7.52*	2.9±0.59*

* -P<0.01.

When analyzing the data obtained during the study, it was found out that when adding feed additive BACELL-M to the diet in the control group of birds was found an increase in all biochemical indicators compared to the experimental group.

On the basis of comparison of biochemical indices it was found out that Urea indices in the control group are higher than in the experimental group by 13.9% respectively, which indicates the imbalance between the synthesis of urea in the liver and the rate of its excretion from the body.

The experimental group has a higher rate of Creatinine by 31.5%, indicating the intensity of kidney work, which positively affects the process of fecal matter formation and the work of the GI tract of birds as a whole [19].

Increased content of AsAt by 4.5%; AlAt by 84.4% in the experimental group indicates that the diet with the addition of feed additive BACELL-M contains a large amount of amino acids, the decomposition of amino acids releases a lot of energy, which contributes to the intensive growth and development of birds.

The lower content of the enzyme in the experimental group Gamma-Gt by 41.4%, indicates the normal process of acid metabolism and bile outflow in the body. Increased content of Protein 29.2% in the control group indicates an abundant growth of muscle mass, which has a positive effect on the productivity of this cross of poultry. Background indicators Cholesterol 14.7% better in the experimental group of birds, indicating the normal operation of the cardiovascular system.

In the experimental group of broiler chickens the content of this component in blood serum was 1564.6±, which is 60% higher than in the control group, indicating the intensity of bone tissue growth in birds.

The level of bilirubin in blood serum in the control group of broiler chickens was higher by 22.9% than in the experimental group, which may indicate disease (hypatosis) or liver dysfunction.

Table 3. Biochemical parameters of blood serum of broiler chickens.

Probe	Calcium mM/l	Phosphorus mM/l	Sh-phosphatase u/l	Bilirubin μM/l	Uric acid μM/l
Background	1.86±	2.48±	2183.29±	8.6±	462.32±
Control	1.39±	2.34±	1564.6±	9.52±	460.9±
Experimental	2.21±	2.57±	2647.2±	7.74±	463.6±

Phosphorus and calcium in the organism of birds are one of the main structural components of the organism. The content of these components in the control group was lower by 33.9%, which may indicate dehydration of the organism and increased excitability of nervous tissues.

Sh-phosphatase correlates with the level of total protein and albumin in the body.

Uric acid is the main metabolic product of nitrogen-containing compounds in birds. Elevated uric acid levels are usually caused by kidney disease. Analysis of the data obtained showed that

The content of uric acid in both groups was within the physiological norm, the difference between the experimental and control groups was 1.58%, which indicates that the experimental group has better protein digestibility due to probiotic supplement "BACCELL-M®".

At clinical and zootechnical researches it is established that at chickens of 40 days old, the average weight of the experimental group was 3100.5±15.24, however in the control group the weight is less up to 2200.5±18.51. The spleen in the experimental group had a mass of 0.66±0.0459, and in the control group, the mass of the organ was less by 1.6% and amounted to 0.65±0.035 (Table 4).

Table 4. Weight of chicken and spleen at 40 days of age.

Indicators	Control	Experimental
	Weight, grams	Weight, grams
Chicken	2200.5±18.51	3100.5**±15.24
Spleen	0.65±0.048	0.66±0.0459

Morphologic studies of the spleen of 40-day-old chickens in the control group (Figure 1) revealed that the spleen parenchyma was loaded with erythrocytes. Most of the vessels are dilated. Arterial walls are thickened, endothelium is swollen and protrudes into the vessel lumen. In the red pulp cellular elements are discharged, white pulp is active and was at different stages of development. In experimental animals at the age of 40 days white pulp is active. Forming large couplings. In the T zone lymphocytes form a wide zone tightly lying around the artery. Lymphoid nodules are large, reactive. In them the processes of proliferation and differentiation of cellular elements of lymphoid tissue are expressed. Nucleation of new lymphoid couplings is expressed. In the splenic thrusts, near the pulp vessels foci of plasmatisation. Plasma cells at different stages of differentiation. T-dependent zone of the coupling is wide, the cells form a dense spherical mass around the central artery. In B-zone processes there are proliferation and differentiation processes.

Medium and large lymphoid couplings are well defined, an increase in the total area of germinative centers with a clear increase in their diameter was revealed. Thus, the obtained morphological data of the immune organ in the early postnatal period is an important indicator for determining the state of the organism during its formation and adaptation in the environment. In the experimental group at the age of 40 days the spleen was in the state of physiological maturity, follicle reproduction centers were activated, there was an increase in

the area of white pulp and activation of germinative reproduction centers, which indicates the formation of the spleen as an immune organ.

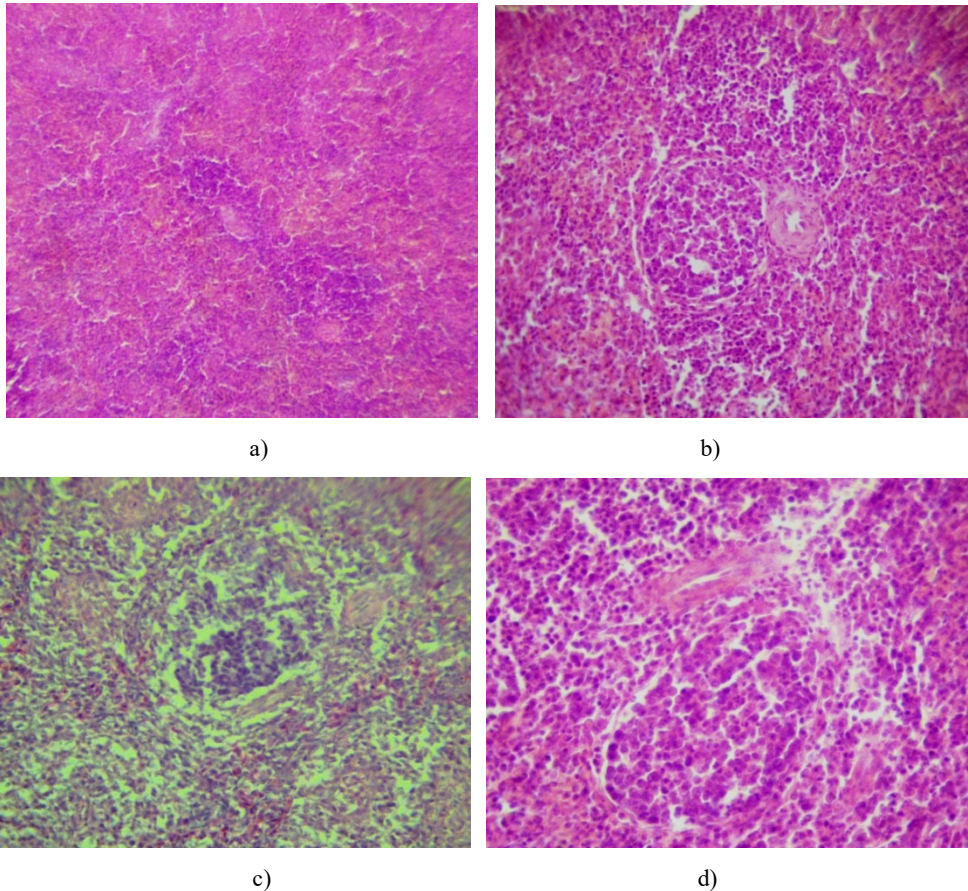


Fig. 1. Histologic structure of the spleen of broiler chickens at 40 days of age: a), b)- spleen of the experimental group of broiler chickens; c), d)-control group of broiler chickens. White pulp, follicle reaction centers a), b), primary and secondary follicle c), d), Hematoxylin eosin staining. a), b), c), d) - magnification 40x.

4 Discussion

An urgent problem in the production of broiler breeds is the maintenance of poultry immunity to preserve the stock, improve productivity and, consequently, meat quality. In this case, the most important factor is the organization of high-grade feeding of poultry. In poultry farming recently there is a tendency to use various preparations that contribute to the improvement of digestion of birds and can replace feed antibiotics [14, 18, 19]. The search for biologically active substances that can stimulate the development of natural microflora of broiler chickens in order to suppress pathogenic bacteria by increasing the number of bifido- and lactoflora have a great interest in science and practice. Various probiotic preparations have similar properties. Certain species of bacteria from the genus *Bacillus*, used as probiotics, which help to improve the assimilation of nutrient components of feed and control the growth of pathogenic strains of gastrointestinal bacteria such as *Clostridium perfringens* [1, 2, 8, 15, 19], deserve great attention.

It is known that probiotics, including bacteria of the genus *Bacillus*, are able to synthesize various exogenous enzymes such as lipase, protease, cellulase, xylanase. They participate in the decomposition of feed molecules, thereby improving the digestion of nutrients, contribute to the reduction of intestinal viscosity when fed starch-free polysaccharide diets, reducing the nutrient environment conducive to the growth of pathogenic bacteria. The use of probiotics, which include strains of *Bacillus* spp. bacteria, increases protein digestibility. Reduces the entry of undigested protein into the large intestine, negatively affects protein fermentation of pathogenic proteolytic bacteria, which are causative agents of diseases of the gastrointestinal tract of birds [1, 2, 5].

The positive effect of the use of various multifunctional additives is primarily reflected in the productivity of birds, therefore, when studying the effectiveness of probiotics, it is important to conduct comprehensive studies to establish the quality indicators and commercial and technological characteristics of meat [3, 4, 10].

5 Conclusion

The conducted studies have shown a positive effect of using probiotic active feed additive "BACCELL-M®" on productive qualities, biochemical parameters of blood and on histological development of spleen as an immune organ of broiler chickens of 40 days of age. The release of nutrients from the structural fiber of the feed due to the enzymatic activity of a special probiotic strain of *Bacillus bacillus* contained in the supplement increases the digestibility and assimilation of feed nutrients. Control of blood parameters of the experimental group fed in the diet with probiotic supplementation showed superiority over the control group (diet without probiotic) in all studied parameters. The use of probiotic active feed additive "BACCELL-M®" had a significant effect on the clinical and biochemical composition of the organism of broiler chickens and histological development of the spleen.

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