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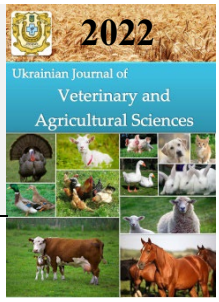

Blood indices and immune organs morphological structure of broiler chickens under the influence of different doses of probiotic feed additives

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Abstract

In the development of effective methods of prevention and treatment of poultry today, importance is given to probiotics based on microbial cultures, in particular, lactobacilli, bifidobacteria, yeast, etc. In contrast to antibiotics, the use of probiotics stimulates the immune response of animals, restores the microflora of the gastrointestinal tract, and ensures its optimal ratio. At the same time, livestock products remain safe for the consumer. The purpose of our work was to conduct comparative clinical trials of the efficiency of the probiotic feed additive Probion-forte in terms of productivity, blood indices, and histological structure of immune organs of chicken broilers during fattening. The clinical studies were carried out with broiler chickens of "Cobb-500" cross at the age of 2 days, which were formed into four groups (300 units in each one). In order to determine the efficiency of application, probiotic feed additives were given to chickens in different concentrations during the growing period, together with the main diet. The first group (1st) was given probiotic Probion-forte in the dose of 1 g/kg of feed, the second group (2nd) – Probion-forte in a dose of 0.5 g/kg, the third (3rd) one – a probiotic-analog "Bio plus 2B" in a dose of 0.4 g/kg; the forth (4th) group was a control group – chickens received an essential diet, without adding any feed additives. The compound feed was provided according to norms recommended for the cross "Cobb-500", considering age. On the 15th, 30th, and 43rd day of the test, 20 units were selected for hematological, pathomorphological and microbiological tests. The productivity of birds of all studied groups was evaluated by the average daily gains, safety, feed conversion, and slaughter output. The stabilized with EDTA chicken blood was used for morphological studies, and blood serum – for biochemical and immunological studies. Comparative clinical studies have shown that adding to the main diet of broiler chickens probiotic feed additive Probion-forte at a dose of 0.5 and 1.0 g/kg throughout the growing period increases the safety and improves feed digestibility, and increases the bird's European Efficiency Index. Based on the obtained hematological, biochemical, and immunological parameters of blood, it can be argued about the activating effect on the body of broiler chickens probiotic feed additives, namely the intensification of reanimation of free amino acids, which increases the content of the studied metabolites of lipid metabolism, which chickens use as energy and plastic material. Stimulating cellular and humoral protection, nonspecific resistance is established, confirmed by a high level of lysozyme activity in blood serum and significantly higher content of T- and B-lymphocytes, NK-cells, and γ-globulins. The macro- and microscopic structure of the studied immune organs is preserved in all groups of broiler chickens. Compared with the control group, morphometric examination in the immune organs of broiler chickens of the first and second groups revealed an increase in the area and density of lymphoid elements, an increase in the number of plasmablasts and plasma cells, much higher structural and functional capacity of cells, which was reflected at the ultrastructural level. The most significant difference was found in the 1st group of chickens fed with the feed additive Probion-forte at a dose of 1.0 g/kg of feed.

Keywords: clinical trials, chickens-broilers, probiotic feed additive, laboratory diagnostics, histostructure, immune system organs.

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1. Introduction

Probiotic drugs, in the form of feed additives, are increasingly used in the fattening of animals and poultry for therapeutic and prophylactic purposes (Martyshuk et al., 2022; Zawistowska-Rojek et al., 2022). The market of preparations of this group is actively developing and replenished with new drugs. In developing effective methods of prevention and treatment of poultry today, probiotics are given to microbial cultures, particularly lactobacilli, bifidobacteria,

yeast, etc. (Ashayerizadeh et al., 2009; Molnar et al., 2011; Wieërs et al., 2020; Wu et al., 2021; Wan et al., 2021). Their action is based on the high activity of these strains of microorganisms to pathogenic and opportunistic microflora (even insensitive to many antibiotics), the ability to activate macrophages, and the induction of interferons (Griggs & Jacob, 2005; Ushakova et al., 2012). The use of probiotics, in contrast to antibiotics, stimulates the immune response of animals, restores the microflora of the gastrointestinal tract, and ensures its optimal ratio, while livestock products remain safe for the consumer (Islam et al., 2004; Swiatkiewicz & Koreleski, 2007; Reshetnichenko et al., 2012).

In recent years, probiotics have been widely used to increase the productivity of poultry. The effectiveness of these drugs in the digestive process is due to their high enzymatic activity (amylolytic, cellulolytic, proteolytic), the ability to replenish the diet with essential amino acids, and vitamins, which in the process of digestion with bacteria are synthesized de novo. The new generation of probiotic feed additives includes sorbed forms of probiotics, which have high biological activity and are practical for use in the diets of various species of poultry (Patterson & Burkholder, 2003).

One of the keys and mandatory prerequisites for the creation and testing of new veterinary products, and feed additives, both in the context of compliance with national legislation and the requirements of the international community for their registration, is conducting clinical trials in compliance with Good Clinical Practice (GCP) requirements. Criteria for evaluating the efficiency and safety of the test products should not be limited only to safety and productivity but should also include characteristics of the morphofunctional state of the target animal species based on laboratory diagnosis (Anadon et al., 2006; Zhyla et al., 2012; Kotsyumbas et al., 2013).

The purpose of our work was to conduct comparative clinical trials of the efficiency of the probiotic feed additive Probion-forte in terms of productivity, blood indices, and histological structure of immune organs of chicken broilers during fattening.

2. Materials and methods

We used probiotic feed additives: Probion-forte, which includes *Bacillus subtilis*, not less than 1×10^8 CFU/g *B. coagulan*t, not less than 1×10^8 CFU/g, and Bio Plus 2B containing *B. subtilis*, not less than 2×10^8 CFU/g.

The clinical studies were conducted on the base of the private farm "Ego" (Zvertiv village, Zhovkivsky district Lviv region) with broiler chickens of "Cobb-500" cross at the age of 2 days, which were formed into four groups. Each group contained 300 units. In order to determine the efficiency of application, both probiotic feed additives were given to chickens in different concentrations during the entire cultivation period, together with the main diet. The first group was given the probiotic Probion-forte in a dose of 1g/kg of feed, the 2nd – Probion-forte in a dose of 0.5 g/kg, the 3rd – a probiotic-analog "Bio plus 2B" in a dose of 0.4 g/kg; the fourth one was a control group – chickens received an essential diet, without adding any feed additives. Compound feed was fed following the standards recommended by the company for the cross "Cobb-500". Chickens were kept on the floor with free access to food and water.

Experiments with animals followed the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (Strasbourg, 1986).

Chickens were vaccinated against infectious bronchitis, Gumboro disease, and Newcastle disease. On the experiment's 15th, 30th, and 43rd days, the body weight, average daily gains, and feed conversion were determined, and 20 birds from each group were removed from the experiment, selecting material for hematological and pathomorphological and microbiological studies. At the end of fattening in all studied groups of birds were evaluated performance indicators and calculation of the European Efficiency Index (EEI).

The stabilized EDTA blood of chickens were used for morphological and immunological studies, and blood serum – for biochemical studies. Blood from broiler chickens was taken from the axillary vein. The investigated parameters were determined according to generally accepted methods adopted in the Laboratory of Clinical Biological Research of the State Scientific Research Control Institute of Veterinary Medicinal Products and Fodder Additives, using appropriate instruments and test kits (Kotsiumbas et al., 2014).

After slaughter, the animals were subjected to complete pathologic anatomical dissection, with the selection of material for histological and morphometric studies. Internal organs were weighed, and their weight coefficients were determined. Material (pieces of duodenum, caecum, liver, thymus, spleen, and lymph nodes) was fixed in neutralized 10 % formalin, with the following paraffin pouring. Paraffin cuts were stained with hematoxylin-eosin.

Microscopy was conducted utilizing microscope OLIMPUS CX-41 and morphometric program DP-SOFT.

The obtained results were statistically processed, estimating the probability of difference of indicators on the Student's test. The arithmetic means (M), the mean error of the arithmetic mean (m), and the significance criterion (P) were determined. Numerical data were processed using Microsoft Excel XP and Statistica 10. The difference between values at which the probability of difference (P) did not exceed 0.05 was a statistically significant result.

3. Results and discussion

It was found that feeding chickens feed additives Probion-forte and Bio plus 2B contributed to better feed digestion and a gradual increase in body weight relative to the control group throughout the growing period (43 days). The difference in body weight between the experimental and control groups was observed starting from the 30th day and was most pronounced on the 43rd day in broiler chickens that received Probion-forte at a dose of 1.0 g/kg of feed. Thus, the body weight of chickens at the end of the experiment was more significant in the 1st group by 248.5 g ($P \le 0.001$), in the second and third groups, respectively, by 232.7 g ($P \le 0.01$) and 14.9 g, compared with the control group.

According to the average daily weight gains, the first and second groups of chickens were ahead of the control group by 6.0 g and 5.6 g, respectively. Feed conversion was the lowest in the 1st group and amounted to 1.78; in the second and third groups, it was 1.82 and 2.05, respectively, while in the 4th one – 2.07. Preservation of chickens in the 1st, second and third groups was higher, compared with the 4th group, respectively, by 6.0, 5.3 and 3.7 %. The carcass weight was higher in the birds of the 1st group by 20.4 % ($P \le 0.001$), in the 2nd group – by 18,2 % ($P \le 0.001$), and in the 3rd group, the difference was insignificant, compared to the control

group. The European Efficiency Index (EEI) was the highest in the 1st group and amounted to 314.4; in the 2nd, third and fourth groups – 303.2, 240.2, and 228.2, respectively.

In the analysis of morphological parameters of the blood of broiler chickens of the 1st, second and third groups receiving the main diet probiotic feed additives, they were within the physiological norm for a given age of birds throughout the fattening period increased with age (table 1). On the 15th and 30th day of the experiment in the blood of chickens of these groups, there was a tendency to increase the hemoglobin content and the number of erythrocytes compared with the control group. The analysis of leukocyte formula on the 15th and 43rd day showed an increase in the relative number of lymphocytes in the blood of chickens of the 1st group, as well as a significantly lower number of eosinophils in all groups on the 15th day of the experiment, compared with the control group (table 1).

Table 1

Morphological indices of broiler chicken's blood at the application of probiotic feed additives (M ± m, n = 10)

Indices	Groups	15th day of the test	30th day of the test	43rd day of the test
Haemoglobin, g/l	1	81.8 ± 4.0	110.1 ± 7.3	109.7 ± 4.2
	2	81.8 ± 3.1	112.2 ± 3.9	101.5 ± 5.5
	3	87.1 ± 4.3	113.8 ± 5.6	100.7 ± 5.0
	4	79.6 ± 3.5	106.7 ± 3.3	100.1 ± 6.0
Erythrocytes, T/l	1	2.3 ± 0.2	2.9 ± 0.1	3.6 ± 0.2
	2	2.0 ± 0.1	3.5 ± 0.1	3.5 ± 0.2
	3	2.1 ± 0.1	3.4 ± 0.1	3.0 ± 0.5
	4	1.9 ± 0.3	3.0 ± 0.1	3.0 ± 0.1
Hematocrit, %	1	23.0 ± 0.9	25.2 ± 0.8	30.5 ± 0.7
	2	27.3 ± 1.0	26.5 ± 0.9	27.5 ± 0.8
	3	23.0 ± 0.7	27.0 ± 1.2	29.1 ± 0.8
	4	23.8 ± 1.3	25.5 ± 0.8	30.3 ± 1.6
Leukocytes, G/l	1	23.9 ± 1.3	29.8 ± 1.2	42.7 ± 2.0
	2	30.0 ± 1.6	29.1 ± 1.6	40.0 ± 2.2
	3	28.0 ± 1.4	31.6 ± 1.5	39.7 ± 2.4
	4	25.9 ± 1.8	27.5 ± 1.4	37.9 ± 2.5
Basophils, %	1	0.8 ± 0.5	1.0 ± 0.4	0.4 ± 0.4
	2	0.8 ± 0.4	0.8 ± 0.4	0
	3	0.4 ± 0.2	0.4 ± 0.2	0.6 ± 0.4
	4	1.0 ± 0.4	0.8 ± 0.5	0.6 ± 0.4
Eosinophils, %	1	2.6 ± 0.6*	4.4 ± 0.7	4.4 ± 0.7
	2	2.6 ± 0.4*	3.6 ± 0.5	4.8 ± 0.8
	3	2.8 ± 0.5*	4.2 ± 0.7	4.8 ± 0.5
	4	5.2 ± 0.5	5.2 ± 0.5	5.2 ± 0.5
Pseudoeosinophils, %	1	36.6 ± 1.6	34.0 ± 1.4	26.8 ± 2.4
	2	39.2 ± 0.9	33.8 ± 1.7	28.0 ± 1.4*
	3	40.0 ± 2.1	35.0 ± 0.6	30.4 ± 0.7
	4	39.6 ± 1.5	30.8 ± 1.8	32.8 ± 1.0
Lymphocytes, %	1	53.6 ± 1.7*	55.0 ± 0.4	61.2 ± 3.0
	2	50.0 ± 1.4	56.0 ± 1.6	63.2 ± 2.1*
	3	49.2 ± 1.9	54.2 ± 1.2	58.4 ± 1.3
	4	46.0 ± 1.7	56.8 ± 1.6	56.0 ± 1.1
Monocytes, %	1	6.4 ± 0.7	5.8 ± 0.7	7.2 ± 0.5
	2	7.4 ± 1.2	5.8 ± 0.5	4.0 ± 0.6
	3	7.6 ± 0.8	6.2 ± 0.7	5.8 ± 0.7
	4	8.2 ± 0.5	6.4 ± 0.7	5.8 ± 0.7

Here and further: * — $P \leq 0.05$; ** — $P \leq 0.01$; *** − $P \leq 0.001$ compared to control

In the blood of chickens of the 1st group, there was a significant increase in the number of T- and B-lymphocytes and NK-cells on the 30th and 43rd day of the experiment, compared with the control group. In particular, the number of T-lymphocytes (CD-3) was 9.8 ± 0.7 G/l (P ≤ 0.01), B-lymphocytes (CD-19) – 6.8 ± 0.4 G/l (P ≤ 0.01) and NK-cells (CD-56) – 4.6 ± 0.3 G/l (P ≤ 0.05), and in the blood of chickens of the control group, these values were, respectively, 5.7 ± 0.2 G/l; 3.0 ± 0.7 G/l; 3.0 ± 0.6 G/l (table 2).

Table 2

The content of subpopulations of lymphocytes in broiler chicken's blood at the application of probiotic feed additives (M ± m, n = 10)

Indices	Groups	15th day of the test	30th day of the test	43rd day of the test
T-lymphocytes (CD-3), G/l	1	7.4 ± 0.7	8.8 ± 0.3*	9.8 ± 0.7**
	2	7.2 ± 1.2	7.8 ± 0.5	7.8 ± 0.6*
	3	7.1 ± 0.8	7.2 ± 0.7	6.9 ± 0.3
	4	6.8 ± 0.5	7.0 ± 0.5	5.7 ± 0.2
B-lymphocytes (CD-19), G/l	1	3.6 ± 0.6	4.9 ± 0.4*	6.8 ± 0.4**
	2	3.2 ± 0.2	4.8 ± 0.2*	5.7 ± 0.4**
	3	3.1 ± 0.1	4.1 ± 0.6	3.9 ± 0.5
	4	3.0 ± 0.5	3.8 ± 0.4	3.0 ± 0.7
NK-(CD-56), G/l	1	3.0 ± 0.4	3.2 ± 0.3*	4.6 ± 0.3*
	2	2.3 ± 0.1	2.8 ± 0.2	3.5 ± 0.3
	3	3.0 ± 0.4	2.0 ± 0.2	3.8 ± 0.2
	4	2.8 ± 0.3	2.5 ± 0.3	3.0 ± 0.6

On the 15th day of the experiment, in the serum of chickens of all experimental groups, the bactericidal activity and lysozyme activity of blood serum was relatively high. In the blood of chickens of the 1st group, lysozyme activity gradually increased and reached the maximum value on the 43rd day and was 13.5 % (P ≤ 0.05) higher than the control group. The bactericidal activity of the blood serum of chickens of the 1st, second and third groups was the highest on the 30th day. It gradually decreased on the 43rd day of the experiment, while in the control group, this indicator decreased after the 15th day. In the serum of chickens of the 1st and 2nd group on the 30th and 43rd day of the experiment, there was a significant increase in phagocytic activity of pseudoeosinophils and a tendency to increase the phagocytic index, compared with the control group (table 3).

Table 3

Indicators of nonspecific resistance of broiler chicken's blood serum at the application of probiotic feed additives (M ± m, n = 10)

Indices	Groups	15th day of the test	30th day of the test	43rd day of the test
Bactericidal activity, %	1	94.2 ± 3.7	95.3 ± 2.7	90.7 ± 2.1*
	2	93.0 ± 2.8	95.1 ± 1.5	86.5 ± 2.0
	3	97.8 ± 1.1	94.2 ± 1.6	89.0 ± 2.5
	4	95.5 ± 3.0	89.2 ± 2.9	87.0 ± 1.8
Lysozyme activity, %	1	37.7 ± 2.2	47.2 ± 2.1°	55.3 ± 1.1*
	2	43.0 ± 2.6	42.2 ± 1.5	52.5 ± 3.5
	3	44.4 ± 1.0	36.3 ± 3.1	53.5 ± 2.3
	4	44.9 ± 2.4	44.3 ± 1.4	48.7 ± 1.7
Phagocytic activity of pseudoeosinophils, %	1	19.4 ± 1.1	27.1 ± 1.0*	28.9 ± 0.5*
	2	17.4 ± 0.9	24.1 ± 0.8*	28.5 ± 0.9*
	3	18.2 ± 1.2	22.6 ± 1.0	23.3 ± 1.3
	4	18.0 ± 1.4	19.0 ± 0.7	18.8 ± 1.1
Phagocytic index	1	10.2 ± 0.8	12.0 ± 0.9	11.8 ± 1.1
	2	9.9 ± 0.6	11.8 ± 1.1	11.1 ± 0.7
	3	12.2 ± 0.9	10.3 ± 0.4	10.5 ± 0.5
	4	10.4 ± 1.0	10.7 ± 0.6	10.0 ± 0.9
Circulating immune complexes, U/100 ml	1	26.2 ± 1.7*	18.0 ± 2.8	10.8 ± 1.2*
	2	38.6 ± 2.5	19.8 ± 2.1	16.5 ± 2.0
	3	45.2 ± 3.1	16.5 ± 2.0	15.2 ± 6.4
	4	41.5 ± 5.7	27.7 ± 3.6	16.0 ± 0.5

The content of circulating immune complexes in the serum of chickens of all experimental groups decreased throughout the study period, which was associated with a gradual decrease in antigenic load in the chickens' bodies after vaccination. However, the most significant changes in the value of the circulating immune complexes were recorded on the 43rd day in the blood of birds of the 1st group. This indicator was lower by 32.5 % (P ≤ 0.05) than in the chickens of the control group and 2.4 times lower compared to the initial study period (table 3).

The results of biochemical studies on the 30th day of the experiment showed a significant increase in the total protein content in the serum of chickens of the first and second groups by 22.3 and 17.9 % (P ≤ 0.05), and on the 43rd day –

by 29.9 and 21.3 % ($P \leq 0.05$), respectively, compared with the control group. In the analysis of the serum protein spectrum of 30-day-old broiler chickens, an increase in the content of γ-globulins was found: in the 1st group – by 34.6 % ($P \leq 0.05$). The 2nd group – by 41.2 % ($P \leq 0.05$), and the third one – by 12.7 %, compared with the control group (table 4).

The bird's serum from the 1st group revealed significantly higher activity of AST on the 15th and 30th day of the experiment, respectively, by 7.5 and 13.9 % ($P \leq 0.05$), the activity of ALT – by 21.2 % ($P \leq 0.05$) in the 2nd group on the 30th day and 17.8 % ($P \leq 0.05$) in the 1st group on the 43rd day, compared with chickens from the control group (table 4). In the bird's blood of the 3rd group, the activity of these enzymes had a similar trend, but the difference was not significant. AP activity was relatively high in the chicken serum of all experimental groups during the initial periods of the study, which is associated with the processes of intensive growth of young birds. On the 15th day in the chicken blood of the 1st, second and third groups, the activity of this enzyme was higher, respectively, by 15.2, 12.6, and 10.6 % ($P \leq 0.05$), compared with the control group. On the 30th day of the experiment, the activity of AP in the chicken's blood remained high, and on the 43rd day – it significantly decreased compared to previous periods of the experiment.

Table 4

Biochemical parameters of broiler chicken's blood serum at the application of probiotic feed additives (M ± m, n = 10)

Indices	Groups	15th day of the test	30th day of the test	43rd day of the test
Total protein, g/l	1	31.5 ± 1.2*	30.7 ± 0.6*	36.5 ± 1.8**
	2	27.5 ± 1.1	29.6 ± 0.5*	34.1 ± 1.1*
	3	29.1 ± 1.5	28.1 ± 1.0	32.8 ± 1.3
	4	26.3 ± 1.3	25.1 ± 1.1	28.1 ± 1.5
Alanine aminotransferase (ALT) μkat/l	1	0.45 ± 0.01	0.51 ± 0.02*	0.53 ± 0.01*
	2	0.42 ± 0.02	0.52 ± 0.02*	0.49 ± 0.02
	3	0.47 ± 0.03	0.49 ± 0.01	0.49 ± 0.02
	4	0.41 ± 0.03	0.43 ± 0.02	0.45 ± 0.02
Aspartate aminotransferase, (AST) μkat/l	1	0.86 ± 0.01*	0.90 ± 0.02*	0.90 ± 0.03
	2	0.82 ± 0.01	0.84 ± 0.03	0.93 ± 0.02
	3	0.88 ± 0.03	0.89 ± 0.05	0.94 ± 0.02
	4	0.82 ± 0.02	0.79 ± 0.02	0.85 ± 0.03
Alkaline phosphatize (AP) μkat/l	1	1.19 ± 0.05*	1.19 ± 0.04	1.05 ± 0.04
	2	1.16 ± 0.04*	1.19 ± 0.02	1.09 ± 0.05
	3	1.14 ± 0.04*	1.20 ± 0.01	1.05 ± 0.04
	4	1.03 ± 0.02	1.15 ± 0.05	0.95 ± 0.06
Total lipids, g/l	1	6.20 ± 0.18	6.80 ± 0.70	3.71 ± 0.52
	2	5.92 ± 0.15	4.10 ± 0.40	3.48 ± 0.45
	3	6.56 ± 0.20	5.20 ± 0.60	3.37 ± 0.50
	4	5.76 ± 0.10	6.24 ± 0.62	2.34 ± 0.38
Triacylglycerols, mmol/l	1	1.87 ± 0.24*	1.91 ± 0.18*	1.84 ± 0.10*
	2	1.21 ± 0.06*	1.31 ± 0.13	1.39 ± 0.10*
	3	1.26 ± 0.08*	0.84 ± 0.12	0.93 ± 0.11
	4	0.93 ± 0.06	1.02 ± 0.16	1.00 ± 0.12

The results of the study of lipid metabolism in 15-day-old chicken serum of the 1st group indicated an increase in the concentration of total lipids by 25 % ($P \leq 0.05$) and the content of triacylglycerols – by 2.2 times ($P \leq 0.05$), compared with the control group. On the 43rd day, the content of triacylglycerols in the chicken blood of the first and second groups was higher, respectively, by 1.8 ($P \leq 0.05$) and 1.4 times ($P \leq 0.05$) than indicators of chickens in the control group.

On the 15th. 30th and 43rd day of the experiment, a diagnostic slaughter of broiler chickens (20 heads from each group) was conducted with a complete pathologic anatomical dissection and selection of material for laboratory studies. External examination of the chickens revealed no damage to the body. Visible mucous membranes and skin derivatives were pale pinks. Poultry of all study groups had good fattening according to age.

An internal examination revealed that the location of the organs of the thoracic-abdominal cavity was anatomically correct. Serosal and mucous membranes are smooth, shiny, and moist. Skeletal muscles are light red, with a characteristic structure and elastic consistency; subcutaneous adipose tissue was more developed in chickens on the 43rd day.

The thymus of the studied broiler chickens was well formed in all three age groups. Particles of the thymus were light pink, lamellar, and elastic consistency. The bird's thymus mass increased with age in all groups. However, it was significantly higher in the first and second experimental groups, compared with the control

group, during the entire observation period. The broiler chicken's spleen of all groups was round, dark red, and elastic; the structure in the organ section was preserved, the edges of the section converged, and the pulp scraping was insignificant. The mass of the spleen naturally increased with the age of the birds. There was a significant increase in weight in the first and second groups of chickens, especially on the 43rd day of the experiment. The caecal tonsil is represented by a slight thickening at a distance of 3–5 mm from the branch of the large intestine and laterally from the central axis.

The dynamics of the mass coefficients of the immune system of the studied groups of chickens during the experiment correlated. As can be seen from table 5, the thymus mass coefficient of chickens from the 1st, second and third groups on the 15th day were significantly higher compared to the control group. The same trend was found on the 30th day. On the 43rd day, the thymus mass coefficients remained probably higher in the chickens of the first and second groups. The chicken's weight coefficients of Bursa of Fabricius tended to increase in the first and second groups, especially on the 15th and 30th day of the experiment. On the 15th day, there was a tendency to decrease the chicken's liver mass coefficients in all experimental groups, compared with the control one. However, on the 30th and 43rd day of the experiment, the coefficients of liver mass increased slightly (table 5).

Table 5

Dynamics of broiler chicken's mass coefficients of individual internal organs at the application of probiotic feed additives (M ± m, n = 10)

Indices	1st group	2nd group	3rd group	4th group
		15th day of the test		
Thymus	0.45 ± 0.02*	0.40 ± 0.04*	0.37 ± 0.05	0.29 ± 0.03
Spleen	0.09 ± 0.04	0.08 ± 0.03	0.08 ± 0.04	0.06 ± 0.03
Bursa of fabricius	0.23 ± 0.01	0.25 ± 0.02	0.23 ± 0.06	0.21 ± 0.02
Liver	2.81 ± 0.09	3.03 ± 0.11	3.06 ± 0.12	3.11 ± 0.13
		30th day of the test		
Thymus	0.48 ± 0.03*	0.44 ± 0.07*	0.46 ± 0.06	0.35 ± 0.01
Spleen	0.10 ± 0.04	0.10 ± 0.04	0.08 ± 0.01	0.14 ± 0.03
Bursa of fabricius	0.22 ± 0.02	0.20 ± 0.03	0.19 ± 0.02	0.18 ± 0.03
Liver	2.72 ± 0.12	2.83 ± 0.21	2.46 ± 0.17	2.71 ± 0.16
		43rd day of the test		
Thymus	0.40 ± 0.05	0.41 ± 0.03*	0.24 ± 0.08	0.22 ± 0.05
Spleen	0.14 ± 0.06	0.13 ± 0.08	0.11 ± 0.76	0.10 ± 0.1
Bursa of fabricius	0.08 ± 0.03	0.07 ± 0.03	0.06 ± 0.05	0.08 ± 0.01
Liver	2.46 ± 0.11	2.49 ± 0.14	2.24 ± 0.09	2.35 ± 0.10

The characteristic thymic lobular structure was preserved in birds of all groups. Thymic lobules are divided into the cortex and medulla (Fig. 1–2).

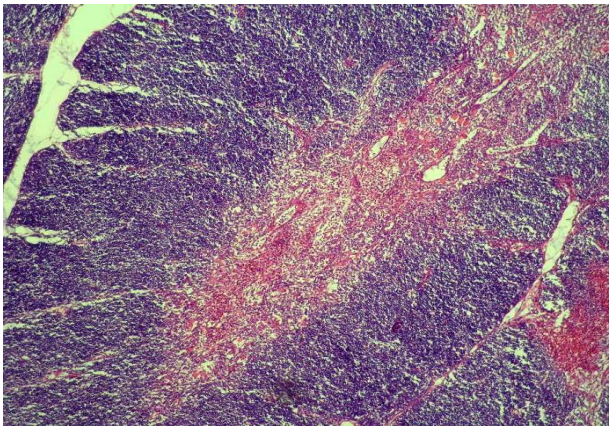

Fig. 1. The structure of the broiler chicken's thymic lobule is preserved in the 1st group on the 30th day of the test. H&E. x 100

The Gassal's bodies were round, of different sizes, and localized in the medulla (Fig. 3). Microscopic examination of the broiler chicken's thymus from the control group revealed enlargement of the medulla due to the narrowing of the cortex (the borders not continuously contoured) and congestive hyperemia in the parenchyma of the organ (Fig. 4). Interlobular connective tissue layers are thickened, in some places are fibrous. The thymic lobule cortex is densely populated with thymocytes, mostly of medium and large size.

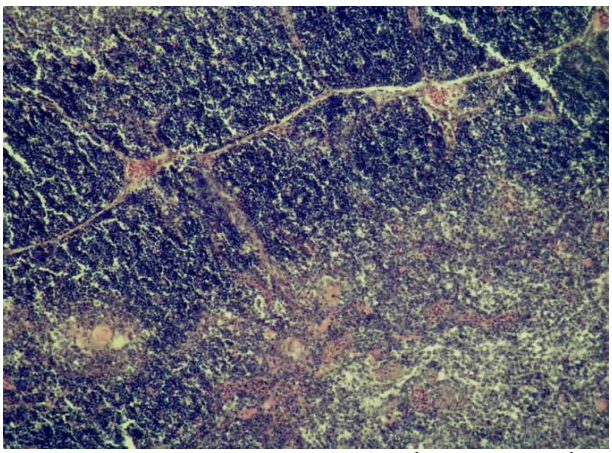

Fig. 2. Broiler chicken's thymus, the 2nd group, the 30th day of the test. The division into the thymic cortex and medullar is well expressed. H&E. x 100

The density of their placement in the field of view was higher in the 1st, 2nd, and 3rd group on the 30th day and was, respectively, 140.2 ± 19.2; 131.7 ± 17.0; 112.4 ± 14.9, compared with the control group (98.7 ± 13.2). On the 15th and 30th day of the experiment, the predominance of the thymic

lobule cortex over the medulla was established. In contrast, the width of the medulla was more significant than the cortex on the 43rd day, as indicated by the cortex-medulla ratio (table 6).

Table 6
Dynamics of morphometric parameters of the broiler chicken's thymus at the application of probiotic feed additives (M ± m, n = 10)

Indices	Groups	15th day of the test	30th day of the test	43rd day of the test
Cortex width, µm	1	143.2 ± 5.2	171.3 ± 8.1**	103.7 ± 4.5
	2	152.2 ± 3.1*	163.9 ± 6.1**	98.4 ± 4.2
	3	134.5 ± 4.5	152.8 ± 8.8*	89.3 ± 3.5
	4	135.7 ± 4.1	137.6 ± 7.2	78.4 ± 3.4
Medulla width, µm	1	101.8 ± 5.2	119.7 ± 4.1*	132.4 ± 6.7*
	2	104.2 ± 3.2	120.2 ± 4.0	139.5 ± 5.1
	3	106.6 ± 3.8	135.4 ± 4.0	145.9 ± 3.1
	4	111.3 ± 6.1	129.1 ± 3.2	153.7 ± 5.1
CW/MW ratio	1	1.41:1	1.43:1	0.78:1
	2	1.46:1	1.35:1	0.71:1
	3	1.25:1	1.12:1	0.60:1
	4	1.22:1	1.08:1	0.51:1

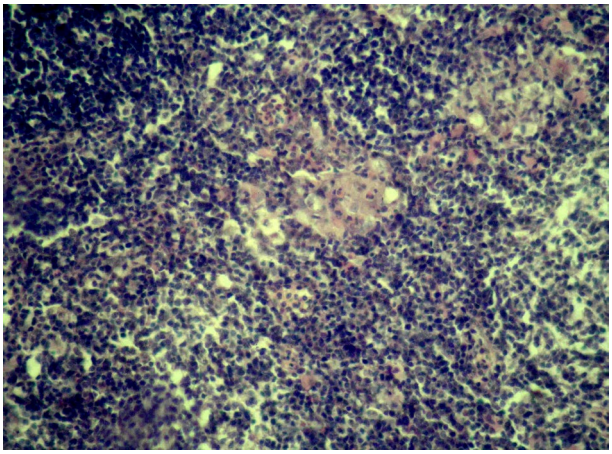

Fig. 3. Gassal's bodies in the medulla of the broiler chicken's thymic lobule, the 1st group, the 30th day of the test. H&E. x 200

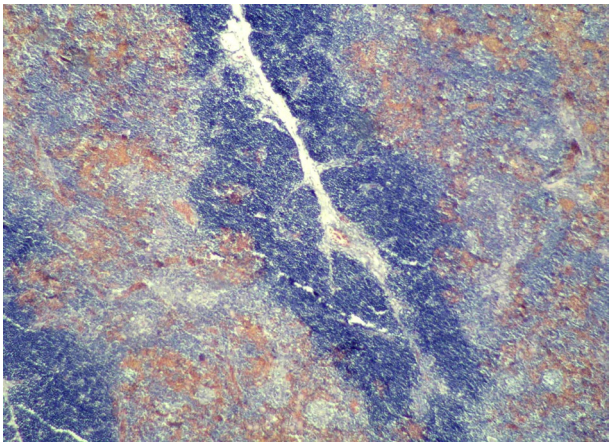

Fig. 4. Enlargement of the medulla, stagnation in the broiler chicken's thymus, the 4th group, the 30th day of the test. H&E. x 100

The microscopic examination of chicken's Bursa of Fabricius of the 1st. 2nd. 3rd group, on the 15th and 30th day of the test, reveals that the lobules (follicles) are evenly developed, polygonal, rarely elongated shape, and the boundary between the cortex and medulla is well expressed. The mucous membrane's epithelium is evenly developed, the nucleus of the epithelial cells is located near the basal surface, and the apical layer is transparent. The connective tissue layers are narrow, separating one follicle from another in relief (Fig. 5). The length of the largest lobules of 30-day-old chickens is 380 µm to 450 µm, and the cortex is compact, represented by 7–10 rows of cells. The medulla is rarefied, represented by lymphocytes, lymphoblasts, and cells with pyroninophilic cytoplasm.

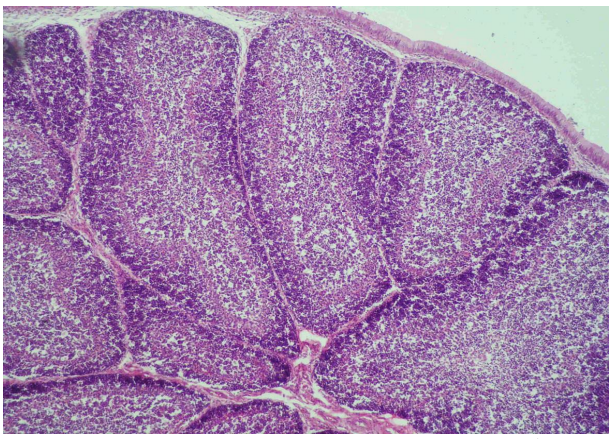

Fig. 5. Broiler chicken's Bursa of Fabricius, the 1st group, on the 30th day of the test. Lobules have a polygonal shape, and the division into cortex and medulla is preserved. H&E. x 100

The staining of histological sections by the Brache method revealed a high RNA content in the cytoplasm of plasmocytes, especially on the 15th and 30th day of the experiment, which indicates active processes of immunogenesis. On the 30th day, the most Bursa of Fabricius's follicles of chickens from the 4th (control) group were rounded, the boundaries between the cortex and medulla were not always clearly contoured, the connective tissue layers were thickened, and in some places, they were fibrous. The length of the largest lobules ranged from 305 µm to 390 µm. Plasmatization of the organ's parenchyma is less pronounced in comparison with chickens of all experimental groups, and

RNA content in the cytoplasm of plasmocytes is moderate (Fig. 6–7).

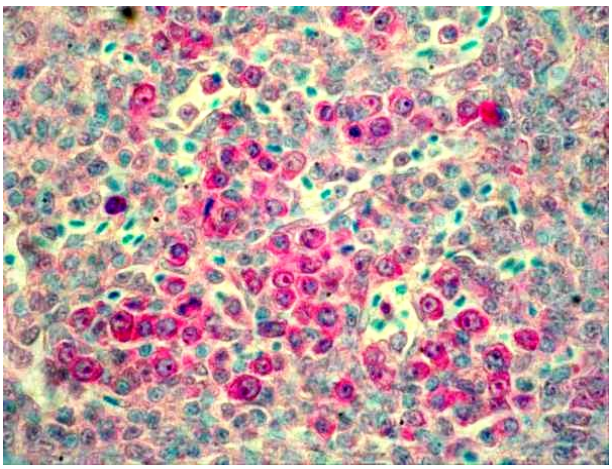

Fig. 6. Moderate pyroninophilicity of chicken's Bursa of Fabricius plasmocytes cytoplasm, the 4th (control) group, the 30th day of the test. Brache. x 400

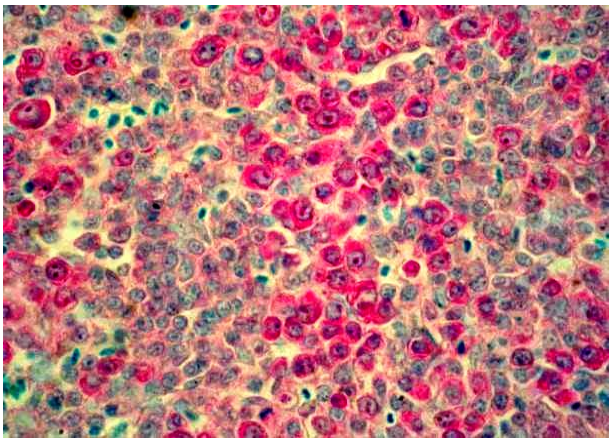

Fig. 7. High RNA content in the chicken's Bursa of Fabricius plasmocytes cytoplasm, the 1st group, the 30th day of the test. Brache. x 400

At the ultrastructural level in chickens from the 1st group, plasmocytes with dilated tubules of the endoplasmic reticulum filled with ribosomes were found more often than in the control group. The nuclei in the vast majority were with open nuclear pores, which indicated an increase in contact between the nucleus and the cytoplasm and a well-defined synthesizing function of plasma cells (Fig. 8–9). Lymphocytes had a pronounced perinuclear zone of enlightenment, loose nucleus, and the presence of a well-formed endoplasmic reticulum, Golgi apparatus.

Histomorphometrically in the broiler chicken's Bursa of Fabricius from 1st second and third experimental groups on the 43rd day, there was a decrease in the size and number of lobules, an increase in connective tissue thickness and delimitation of the parenchyma, which indicated the development of age involution of the organ. Such processes were more expressed in chickens from the control group.

Microscopically, the chicken's caecal tonsil was represented by lymphoid nodules, localized at the submucosal base (Fig. 10–11) and inhabited mainly by lymphoblasts and plasmocytes.

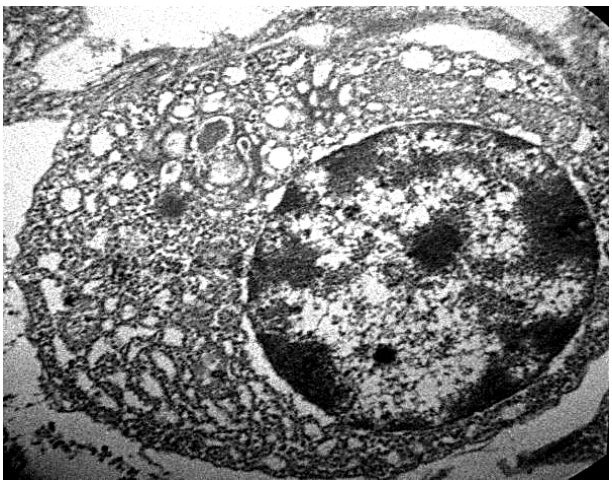

Fig. 8. Electrogram of broiler chicken's plasmocytes, the 4th (control) group, the 30th day of the test. Expansion of endoplasmic reticulum tubules, degranulation. x 8000.

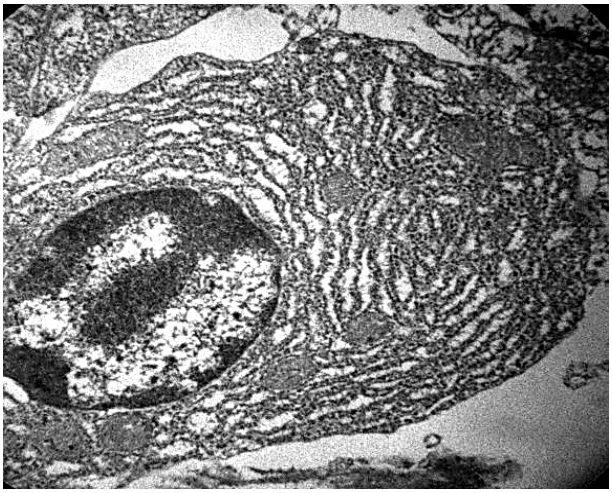

Fig. 9. Electrogram of chicken's plasmocytes, the 1st group, the 30th day of the test. The presence of ribosomes in the tubules of the granular endoplasmic reticulum. x 8000

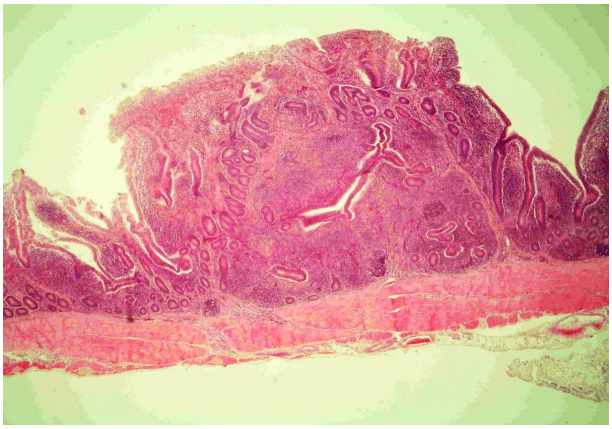

Fig. 10. Broiler chicken's caecal tonsil, the 1st group. thec30th day. H&E. x 50

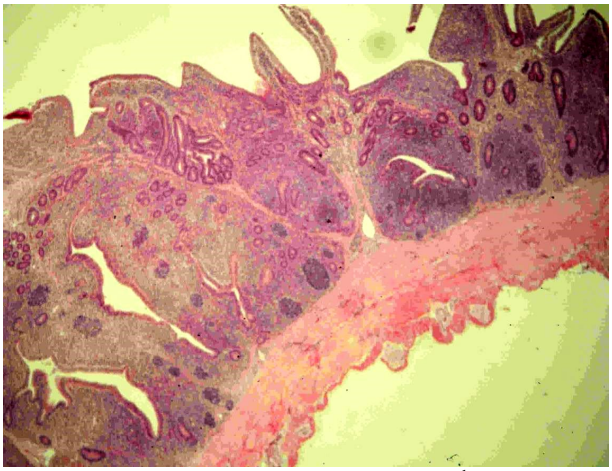

Fig. 11. Broiler chickens caecal tonsil, the 2nd group, the 30th day. H&E. x 50

Morphometrically, a significant increase in the length and height of the caecal tonsil and the size of lymphoid nodules was established in chickens that were fed probiotic feed additives, in contrast to chickens that were on the main diet. The most significant difference was found in chickens of the 1st group, compared with birds of other groups (table 7).

According to a histomorphometric study of spleen structures, an increase in the area of white pulp in chickens fed Probion-forte was found, which was most marked on the 43rd day of the experiment. Thus, in this period, the red pulp of chickens from the first and second groups occupied 60.4 ± 2.1 %; 59.1 ± 1.9 %, respectively. White pulp – 33.4 ± 1.3 %; 32.2 ± 1.1 %, respectively, the stroma – 6.3 ± 0.9 %; 7.8 ± 0.6 %, the ratio between parenchyma and stroma was 14.9: 1 and 12.7:1.

Table 7

Dynamics of morphometric parameters of broiler chicken's caecal tonsils at the application of probiotic feed additives (M ± m, n = 10)

Groups	Caecal tonsil length, μm	Caecal tonsil height, μm	The average size of lymphoid nodules, μm
15th day of the test			
1	1430 ± 15.3***	740 ± 3.2**	101 ± 7.2
2	1298 ± 10.2**	655 ± 8.9*	95.5 ± 3.1
3	1377 ± 18.3***	738 ± 7.25**	98.3 ± 9.4
4	1059 ± 14.2	686 ± 9.4	85.9 ± 4.8
30th day of the test			
1	2772 ± 18.8***	968 ± 10.0***	174 ± 5.1*
2	2595 ± 17.3***	1002 ± 16.3***	152 ± 13.7
3	2679 ± 16.2***	998 ± 9.7**	168 ± 25.9
4	2281 ± 14.6	901 ± 11.83	143 ± 14.6
43rd day of the test			
1	3342 ± 12.5***	1609 ± 13.5***	291 ± 7.8**
2	3025 ± 11.7***	1352 ± 8.4***	280 ± 11.1**
3	2950 ± 19.0***	1443 ± 14.6***	177 ± 8.7
4	2379 ± 18.9	1073 ± 17.0	176 ± 12.6

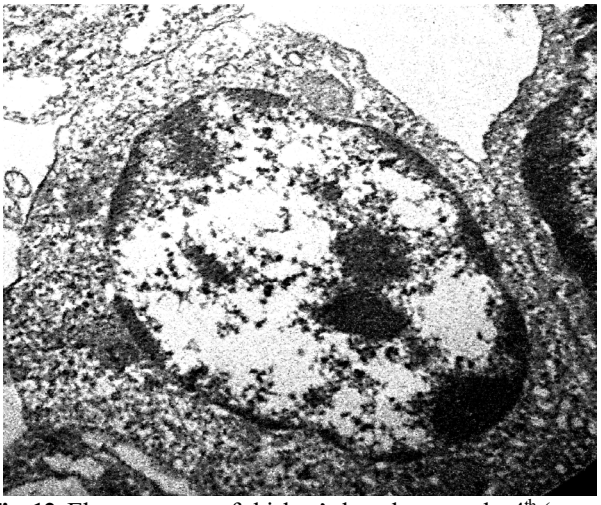

Fig. 12. Electronogram of chicken's lymphocytes, the 4th (control) group, 30th day. x 8000

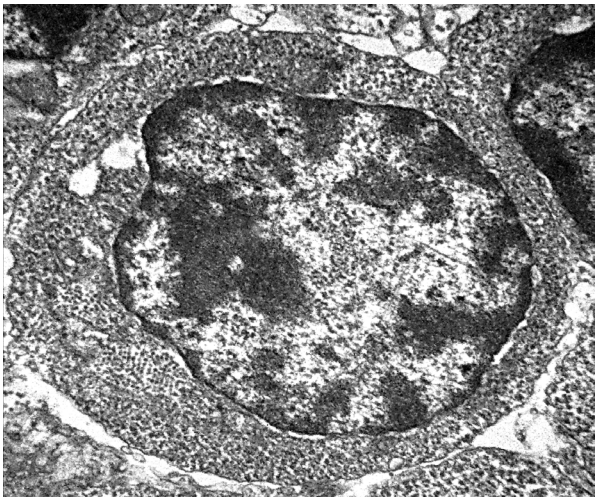

Fig. 13. Electronogram of chicken's lymphocytes, the 1st group, 30th day of the test. The nucleus is contoured, with a perinuclear zone of enlightenment, rich in chromatin. x 8000

In chickens of the control group, the red pulp accounted for 62.1 ± 1.2 %; white pulp – 24.7 ± 1.0 %; stroma – 13.8 ± 0.7 % of the total volume of the organ, the ratio between parenchyma and stroma was 10.1:1. The cellular composition of white pulp is represented mainly by small and medium lymphocytes, lymphoblasts, macrophages, and plasmocytes. In the first and second groups of chickens, the ultrastructure of lymphocytes, compared to birds of the control group, is represented by more chromatin-rich nuclei with a pronounced perinuclear zone, evenly distributed nuclear pores, and densely populated cytoplasm: ribosomes, polysomes, and mitochondria. Plasmocytes have well-developed granular endoplasmic reticulum, the tubules containing a significant amount of protein, indicating their high functional activity.

4. Conclusions

Comparative clinical studies have shown that adding to the main diet of broiler chickens probiotic feed additive Probion-forte at a dose of 0.5 and 1.0 g/kg throughout the growing period increases the safety and improves feed digestibility, and increases the bird's European Efficiency Index. Based on the obtained hematological, biochemical, and immunological parameters of blood, it can be argued about the activating effect on the body of broiler chickens probiotic feed additives, namely the intensification of reanimation of free amino acids, which increases the content of the studied metabolites of lipid metabolism, which chickens use as energy and plastic material. Stimulating cellular and humoral protection, nonspecific resistance is established, confirmed by a high level of lysozyme activity in blood serum and significantly higher content of T- and B-lymphocytes, NK-cells, and γ-globulins.

The macro- and microscopic structure of the studied immune organs is preserved in all groups of broiler chickens. Compared with the control group, morphometric examination in the immune organs of broiler chickens of the first and second groups revealed an increase in the area and density of lymphoid elements, an increase in the number of plasmablasts and plasma cells, much higher structural and functional capacity of cells, which was reflected at the ultrastructural level.

The most significant difference was found in the 1st group of chickens fed with the feed additive Probion-forte at a dose of 1.0 g/kg of feed.

Prospects for further research: the study of the effect of different doses of probiotic feed additives on the microflora of the duodenum and cfecum of chickens-broilers.

Conflict of interest

The authors declare that there is no conflict of interest.

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