Management of compensatory shift of immunohematological parameters in poultry of industrial crosses in the presence of feed antibiotics in the diets

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Abstract. A comparative analysis of the immunological parameters in chickens of industrial crosses, which were fed diets with antibiotics (group 1) and antibiotics in combination with a phytobiotic (group 2), is presented. After 30 days of feeding antibiotic hemoglobin concentration in the blood of laying hens remained unchanged – 87.3-93.2 g/l. On the 30th day of the experiment, in group 1 was noted an increase in the number of leukocytes up to 30.13±2.84·109/l and the proportion of eosinophils by 29.6%. In the same period, a decrease in the absolute number of immunocompetent cells – T-lymphocytes by 36.6% was observed and absorption capacity of phagocytes by an average of 27.9%. From the 30th to the 60th day of the experiment in the blood of the chickens of group 1, the dynamics of the increase in the CIC level was recorded (up to 143.8±12.1 c.u.). When antibiotic was introduced into the diet of laying hens in combination with a phytobiotic in group 2, only by the 60th day, the number of erythrocytes was increased by 17.4% compared to the "background values" (82.1±5.2·10^{12}/l). Hemoglobin fluctuation amplitude did not exceed 7%. On the 30th day of the experiment in laying hens of group 2, a decrease in the number of immunocompetent cells by 20.8% was registered compared to the background values. On the 45th day of the experiment after the abolition of antibiotic and phytobiotic, the content of T-lymphocytes did not significantly differ from the background values.

1 Introduction

In animal husbandry and poultry farming, the total volume of antibiotics used, according to the WHO, is 2.3 times higher than the volume of pharmaceutical products used in medicine. Discussions about the justification and expediency of using antibiotics in agriculture have been ongoing over the past decades at all levels of the scientific community [6, 14]. The interaction at the level of "antibiotic - pathogenic agent" and questions about the mechanisms of removing the antibiotic from the animal's body

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depending on the concentration and time of exposure are mainly considered [12]. The problem of the effect of antibiotics on animal's organism in veterinary aspect, in contrast to medicine, is not given enough attention. In studies of medical specialists, it was found that antibacterial drugs can have toxic and immunotoxic effects on the body [16].

Modern poultry technology is able to provide the population of the Russian Federation with proteins of animal origin, but its integral part is the widespread use of antibiotics as growth stimulants and for preventive measures [2]. Therefore, questions about the biological well-being of poultry, the production of edible chicken eggs and meat with high consumer properties, the use of feed antibiotics and antibiotic prophylaxis, as well as the development and search for alternative antimicrobial drugs remain open [1, 4].

2 Materials and methods

The object of the research was laying hens of the Lohmann-classic cross 400 days old, grown in one of the Ural poultry farms.

The formation of experimental groups of chickens was carried out according to the principle of analogues. Individuals were marked 7 days before the start of the experiment. Four experimental groups of 120 birds each were formed (Table 1).

Table 1. Scheme of introducing an antibiotic and a phytobiotic preparation into the diet of laying hens of the experimental and control groups during the experiment.

Experimental group	Monitoring period for laying hens			
	1st day	30th day	45th day	60th day
Group 1 "Antibiotic"	Feed antibiotic diet	Last day of use feed antibiotic diet	Diet for egg-laying hens	
Group 2 "Antibiotic + Phytobiotic"	Diet with feed antibiotic and phytobiotic	Last day of use diet with feed antibiotic and phytobiotic	Diet for egg-laying hens	
Group 3 "Phytobiotic"	Diet for egg-laying hens with a phytobiotic	Last day of use diet for egg-laying hens with a phytobiotic	Diet for egg-laying hens	
Group 4 " Control"	Diet for egg-laying hens			

In diet of laying hens in group 1 the antibacterial drug "Enroflon" 10% solution (LLC "NPF VIK", Russia) was added. The active substance of this antibiotic was enrofloxacin, the dosage was of 0.5 ml/l of drinking water for 7 days. This drug is still widely used for antibiotic prophylaxis in poultry. In group 2, laying hens received enrofloxacin in the same dosage as in group 1, as well as a phytobiotic preparation developed by us jointly with the Research Institute of Agriculture of the Crimea, group 3 received only a phytobiotic, group 4 (control) received only a standard diet without additives.

The phytobiotic preparation included sea buckthorn cake, red clover (85% and 15% wt.) and a mixture of cold-pressed oils: mustard (60%), sea buckthorn (30%), cedar (10%) with the ratio of the dry base and the oil component of 70%:30% of the mass. The oil component was applied to the base, mixed, kept for 24 hours at a temperature of +12°C to impregnate the base, then dried in a convective drying chamber at a temperature of +35°C for 2 hours and loosened. For hens of groups 2 and 3, the drug was added to the diet at the rate of 10 g/head per day for 14 days. Technological processes for the production of plant raw materials, its processing and the manufacture of individual components of the phytobiotic

were carried out on the basis and with the participation of specialists from the Research Institute of Agriculture of the Crimea.

The biomaterial for the study was the venous blood of laying hens. Blood samples were taken from the birds in the morning, from the saphenous axillary vein, on the first day (background value), day 30, days 45 and 60 of the experiment.

Hematological studies included determining the number of erythrocytes, leukocytes, hemoglobin concentration. To count the number of erythrocytes and leukocytes, the dye according to Fried and Lukacheva in the modification of I.A. Bolotnikova was used at a dilution of 1:200. Measurement of blood hemoglobin was performed by the Saly colorimetric method. The leukocyte formula was calculated in blood smears stained according to May-Grunwald-Giemsa. The results were recorded visually on Axio Score microscopes: A1 (Carl Zeiss, Germany), Micros MCX 100 (Austria).

The osmotic fragility test (OFT) was carried out according to the unified method of L.I. Idelson. The degree of hemolysis of erythrocytes was taken into account by optical density on photometer "KFK-3" at a wavelength of 414 nm. The digital value of the OFT (%) was calculated by method of Gorshkova M.A. et al. [11].

Immunological studies included the determination of the relative and absolute number of T- and B-lymphocytes; phagocytic activity of heterophile cells; the level of circulating immune complexes (CIC) in blood serum was carried out according to methodological recommendations [9]. The reactions of the cellular component of immune system were taken into account on a BX43 microscope (Olympus, Japan), the level of CIC was measured on an iMarkTM BIO-RAD photometer (Japan) by optical density. Determination of the adhesive ability of heterophile cells with the calculation of the adhesion index was carried out by the method of A.S. Sharonova et al., 2006 [15].

Biochemical studies included the determination of the activity of myeloperoxidase (MPO) in blood leukocytes according to the Greimm-Kiol method in the modification of Saidov M.Z. with co-authors [10]. Optical density was measured on an iMarkTM BIO-RAD photometer (Japan); the accumulation of lipid peroxidation products was determined by the concentration of malondialdehyde (MDA) in the blood serum according to methodological recommendations [7].

Statistical analysis was conducted using mathematical methods by a special Microsoft Office software package with the determination of arithmetic mean values and standard deviation.

3 Results and discussion

When determining hematological parameters in laying hens on the first day of the experiment (background value), it was found that they correspond to physiological values of these species (Table 2) [3].

Table 2. Blood parameters in laying hens of the experimental groups (1st day of the experiment).

Hematological index[3]	Group 1 "Antibiotic"	Group 2 "Antibiotic + Phytobiotic"	Group 3 "Phytobiotic"	Group 4 "Control"
Erythrocytes, N 1.5-3.0·10^{12}/l	2.24±0.21	2.18±0.24	2.21±0.15	2.27±0.22
Hemoglobin, N 77-115g/l	87.3±6.4	82.1±5.2	87.9±5.7	87.5±6.9
OFT H (0.9%)*	1.52±0.02	1.59±0.01	1.57±0.02	1.54±0.04
OFT H (0.45%)	27.04±1.31	26.72±1.21	27.61±1.47	26.52±1.27

Leukocytes, N 25-43·10^9/l	25.43±2.61	24.90±2.92	25.97±1.82	24.83±2.48
Heterophiles**, N 19-27%	27.50±2.37	27.28±2.48	26.80±2.52	27.57±2.18
Eosinophils, N 3-5%	3.10±0.35	3.25±0.41	2.75±0.67	3.29±0.31
Basophils, N 0-1%	0.72±0.08	0.65±0.06	0.75±0.05	0.68±0.08
Monocytes, N 2-5%	2.80±0.57	2.37±0.16	2.76±0.46	2.54±0.23
Lymphocytes, N 64-75%	66.82±5.60	65.40±6.48	65.97±5.64	65.87±5.84

* OFT – the osmotic fragility test, H (0.9%), H (0.45%) - the degree of hemolysis of erythrocytes in saline and 0.45% NaCl solution.
** Heterophiles – (synonym) "Pseudo-eosinophils"

Statistically significant differences between the determined immunological parameters in laying hens of the experimental groups on the 1st day of the experiment were also not registered (Fig. 1).

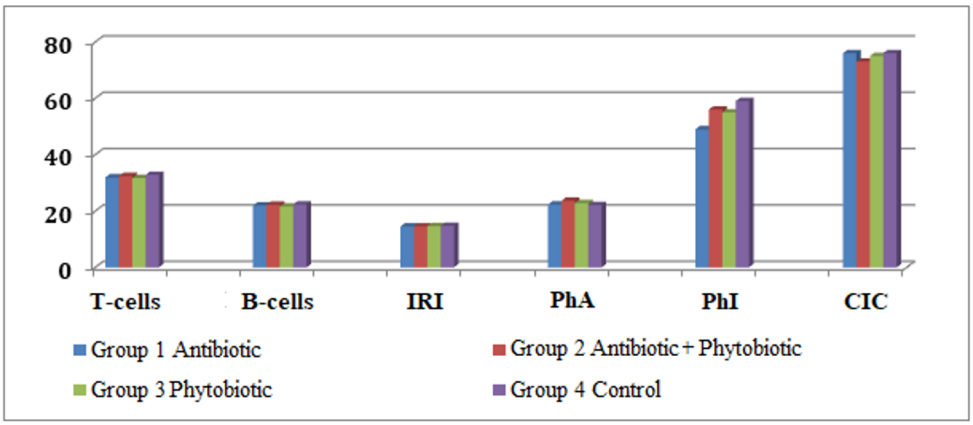

Fig. 1. Immunological parameters of the blood of laying hens of the experimental groups on the 1st day of the experiment (T-cells in%; B-cells in %; IRI - immunoregulatory index T-cell / B-cell, in c.u. · 10; PhA – phagocytic activity of heterophils in %; PhI – phagocytic index in c.u. · 10; CIC – circulating immune complexes c.u.)

The results of studies of the biomaterial taken on the 1st day of the experiment were used as background values. The results of laboratory diagnostics of the blood of laying hens on the 30th, 45th and 60th day of the experiment were compared with the background values.

In the experimental group 1 changes in hematological and immunological blood parameters were registered (Table 3).

Table 3. Dynamics of changes in immunohematological parameters in laying hens of group 1 (with addition of the antibiotic enrofloxacin to the diet)

Defined indicator	Background values	30 day	45 day	60 day
Erythrocytes, N 1.5-3.0·10^{12}/l	2.24±0.21	2.61±0.28 ↑17.1%	2.66±0.32 ↑19.4%	2.87±0.22 ↑23.2%

Hemoglobin, N 77-115g/l	87.3±6.4	76.4±5.2 ↓11.6%	93.2±4.7	89.2±5.4
OFT H (0.9%)	1.52±0.02	2.89±0.04	1.61±0.07	1.55±0.01
OFT H (0.45%)	27.54±1.37	34.43±1.52	29.61±1.72	26.71±1.27
Leukocytes, N 25-43·10^9/l	25.43±2.61	30.13±2.84 ↑15.9%	21.03±1.74 ↓14.9%	20.62±1.58 ↓16.9%
Heterophiles**, N 19-27%	27.50±2.37	28.08±2.10	28.33±2.42	27.67±2.18
Eosinophils, N 3-5%	3.10±0.35	4.15±0.38 ↑29.6%	3.45±0.67	4.89±0.51 ↑62.9%
Basophils, N 0-1%	0.72±0.08	0.65±0.09	0.71±0.07	0.74±0.08
Monocytes, N 2-5%	2.80±0.57	2.67±0.26	2.72±0.41	2.64±0.53
Lymphocytes, N 64-75%	66.82±5.60	65.70±5.28	65.97±4.64	65.29±5.74
T-lymphocytes, (abs.) 10^9/l	7.32±0.67	7.10±0.92	5.42±0.77 ↓25.9%	4.64±0.58 ↓36.6%
B-lymphocytes (abs.) 10^9/l	4.53±0.41	5.23±0.74 ↑15.5%	3.66±0.51 ↓19.2%	3.48±0.59 ↓23.2
PhA, N 12-25%	21.65±2.47	21.37±1.38	19.80±1.97	23.6±2.35
PhI N2.5-6.0 c.u.	4.93±0.41	4.65±0.46	3.55±0.57 ↓27.9%	3.89±0.48 ↓21.1%
Macrophage adherence c.u.	3.7±0.2	5.3±0.3	3.8±0.2	3.1±0.1
CIC, c.u.	74.6±9.4	94.8±10.4 ↑26.7%	76.7±8.9	143.8±12.1 ↑**92.7%**
MDA[8], N1.5- 2.5 μM/l	1.67±0.23	1.97±0.51 ↑17.9%	2.32±0.45 ↑38.9%	2.72±0.64 ↑**62.8%**
MPO of phagocytic cells, ng/ml	34.5±2.7	32.1±2.4	26.9±2.8 ↓22.8%	26.6±1.9 ↓22.9%

** Heterophiles – (synonym) "Pseudo-eosinophils"

As can be seen from the data presented in Table 3, the immunohematological parameters were within the reference physiological parameters of these species. However, when comparing these data with the background values of group 1 and the immunohematological parameters of control group 4, it was shown that on the 30th day of the experiment, the number of erythrocytes increased by 17.1%, on the 45th day of the experiment by 19.4%, and on the 60th day – by 23.2%. Such changes indicate the presence disorder of the main function of erythrocytes – transport. In addition, changes in the physicochemical properties of the erythrocyte membrane were recorded. On the 30th day of the experiment, the degree of hemolysis of erythrocytes increased by an average of 1.3 times. Changes in the permeability and plasticity of the erythrocyte membrane can potentiate the deterioration of the microrheological and hemodynamic functions of erythrocytes, and as a result, lead to impaired blood circulation in the capillaries [5]. Indirectly, changes in the structure of the erythrocyte membrane were evidenced by an increase in lipid peroxidation products in the blood serum of laying hens of group 1. During the experiment, an increase in the concentration of MDA was noted. The maximum increase in the concentration of MDA (by 62.8%) compared with the background value was recorded on the 60th day of the experiment (Table 3). The results obtained indirectly

indicated the presence of tissue and cellular respiration disorder and the processes of excretion of metabolic products.

On the 30th day of the experiment, an increase in the number of leukocytes by 15.9% was registered in the white blood cells. An increase in the proportion of eosinophils by 29.6% was recorded in the blood. Immunological indicators, such as T- and B-lymphocytes, phagocytic activity of leukocytes, were generally stable. In the blood serum of laying hens, an increase in the level of CIC by an average of 26.9% was shown.

In the same period, a decrease in the absolute number of immunocompetent cells – T-lymphocytes and B-lymphocytes – was observed. The maximum decrease in the absolute amount by 36.6% was noted in the T-cells. Some changes in the dynamics of the phagocytic activity of blood leukocytes of laying hens on the 45th and 60th days were registered. The phagocytic index was recorded within the reference of physiological parameters of these species. When comparing these data with the background values of group 1, it was found that the average number of absorbed microbial bodies per phagocyte decreased by 27.9% on day 45 and by 21.1% on day 60. At the same time, macrophage adhesion did not significantly differ from the background indicators. Macrophage adhesion characterizes the ability of macrophages to migrate through the walls of blood vessels into tissues, participate in cytotoxic reactions and phagocytosis of bacteria. One of the reasons for the decrease in the activity of phagocytosis, in our opinion, was the decrease in the bactericidal component of MPO in the phagocytic cells of laying hens compared with the background value – 34.5±2.7 ng/ml. On the 45th day, the amount of MPO decreased by 22.8% (26.9±2.8 ng/ml) and remained at this level for 16 days.

Thus, the studies showed that the antibiotic did not have a pronounced toxic effect on the body of laying hens of group 1. Some changes in immunohematological parameters were registered, but they, in general, did not significantly differ from the reference. At the same time, the changes persisted for a long time and affected the key functions of erythrocytes and immunocompetent cells.

The results of the study of the effect of the phytobiotic on the immunohematological parameters of the blood of experimental birds and their comparison with laying hens of control group 4 are presented in Table 4.

Table 4. Dynamics of immunohematological blood parameters of laying hens of group 3 and group 4.

Defined indicator	Background values	30 day	45 day	60 day
Erythrocytes, N 1.5-3.0·10^{12}/l	2.21±0.15* 2.27±0.22	1.97±0.45* 2.19±0.64	2.35±0.27* 2.28±0.32	2.31±0.54* 2.26±0.25
Hemoglobin, N 77-115g/l	87.9±5.7* 87.5±6.9	78.7±3.7* 88.7±5.3	89.9±7.6* 90.3±8.1	88.9±6.7* 89.5±4.9
OFT H (0.9%)	1.52±0.02	1.50±0.03	1.53±0.05	1.52±0.05
OFT H (0.45%)	27.04±1.31	27.3±1.22	26.69±1.17	27.24±1.23
Leukocytes, N 25-43·10^{9}/l	25.97±1.82* 24.83±2.48	26.50±3.34* 25.23±1.97	26.77±2.42* 26.14±2.31	26.93±1.82* 24.83±2.48
Heterophiles**, N 19-27%	26.80±2.52* 27.57±2.18	25.58±2.36* 26.79±2.20	26.34±1.92* 26.53±2.73	25.94±2.47* 26.64±2.46
Eosinophils, N 3-5%	2.75±0.67* 3.29±0.31	2.83±0.77* 2.57±0.61	2.45±0.66* 3.19±0.29	2.87±0.97* 2.89±0.59
Basophils, N 0-1%	0.75±0.05* 0,68±0.08	1.2±0.8* 0.91±0.07	1.05±0.05* 0.35±0.08	0.97±0.05* 0.96±0.07
Monocytes, N 2-5%	2.76±0.46* 2.54±0.23	3.50±0.94* 2.48±0.53	3.31±0.42* 2.79±0.48	2.71±0.65* 2.49±0.64
Lymphocytes, N 64-75%	65.97±5.64* 65.87±5.84	70.91±4.74* 69.89±6.25	68.72±5.64* 65.93±4.89	71.27±3.14* 66.34±5.27

T-lymphocytes, N 11-40%	32.61±6.14* 31.98±5.67	33.48±5.44* 30.67±6.21	33.52±6.19* 31.34±6.37	33.11±6.04* 31.98±5.67
B-lymphocytes N 15-32%	21.81±1.62* 22.40±2.91	22.33±2.11* 23.26±2.37	22.52±1.83* 22.35±2.61	21.65±2.24* 22.4±2.43
PhA N 12-25%	21.62±2.50* 22.31±2.93	22.67±2.73* 21.48±2.41	21.83±1.97* 20.68±2.73	21.95±2.75* 22.16±2.62
PhI N 2.5-6.0 c.u.	5,23±0.48* 5.41±0.97	5.45±0.59* 5.39±0.77	6.27±0.35* 5.47±0.72	5.74±0.28* 4.98±0.94
Macrophage adhesion c.u.	3.5±0.4* 3.7±0.2	3.7±0.3* 3.8±0.2	3.4±0.3* 3.6±0.2	3.6±0.2* 3.8±0.1
CIC c.u.	73.8±8.6* 87.5±2.9	75.2±8.8* 88.5±3.5	81.4±6.6* 70.6±5.9	75.6±5.2* 84.5±3.7
MDA, N1.5- 2.5 μM/l	1.92±0.21* 1.58±0.32	1.64±0.43* 1.83±0.48	1.67±0.51* 1.78±0.22	1.74±0.42* 1.67±0.29
MPO ng/ml	32.6±2.8* 34.4±2.5	37.2±2.1* 33.2±1.9	35.1±2.8* 34.4±2.5	34.7±2.6* 33.8±1.9

"*" – the results of immunohematological and biochemical studies of the blood of laying hens of group 3, who received phytobiotic with the diet.

** Heterophiles – (synonym) "Pseudo-eosinophils"

Analysis of the results of immunohematological studies of the blood of laying hens of group 3 treated with the phytobiotic showed that the drug had no direct effect on red blood cells. The increase or decrease in the concentration of erythrocytes in the blood of experimental laying hens did not exceed 12%. At the same time, the change in the concentration of erythrocytes was directly proportional to the change in the content of hemoglobin in the blood. For example, on the 30th day of the experiment, a decrease in the concentration of erythrocytes to $1.97\pm0.45\cdot10^{12}$/l was registered, which is 11.42% lower than the background value of this group of birds - $2.21\pm0.15\cdot10^{12}$/l. At the same time, a decrease in hemoglobin content by 10.2% was observed in these laying hens.

In the composition of white blood cells on the 30th day, an increase in the relative number of basophils by 1.6 times and monocytes by 1.3 times was revealed. On the 60th day of the experiment, the relative number of basophils exceeded the background value by 1.3 times. The number of monocytes did not differ from either the background value or the relative number of these cells in control group 4. In relation to other white blood cells during the experiment, no obvious quantitative and qualitative changes were detected. The hemogram and leukocyte formula in laying hens of group 3 did not significantly differ from those in birds of control group 4.

The cellular component of immune system in the third group also did not differ from those in chickens of control group 4. An increase in PhI by 19.8% (45th day of the experiment) was recorded, which may be a consequence of a physiological increase in the level of the CIC. The absence of changes in the concentration of the bactericidal component of MPO in phagocytic cells and the concentration of MDA in the blood serum testified to the physiological reasons for the increase in the CIC level.

In group 2, laying hens were given enrofloxacin with the diet in combination with a phytobiotic to assess the positive effect on chicken's organism. The results of the study of immunohematological parameters in laying hens of group 2 are shown in Figures 2-8.

In laying hens of group 2, as well as in group 1, which received only an antibiotic, changes in red blood cells were recorded: the number of erythrocytes and hemoglobin content (Fig. 2, 3). Unlike the hens of group 1, which had an increase in the number of erythrocytes in the blood from the 30th to the 60th day of the experiment, in laying hens of group 2 the number of erythrocytes was increased by 17.4% only by the end of the

experiment. The content of hemoglobin (g/l) in the blood of laying hens of group 2 did not change. Hemoglobin fluctuation amplitude did not exceed 7% compared with the background value of group 2 and in the control group.

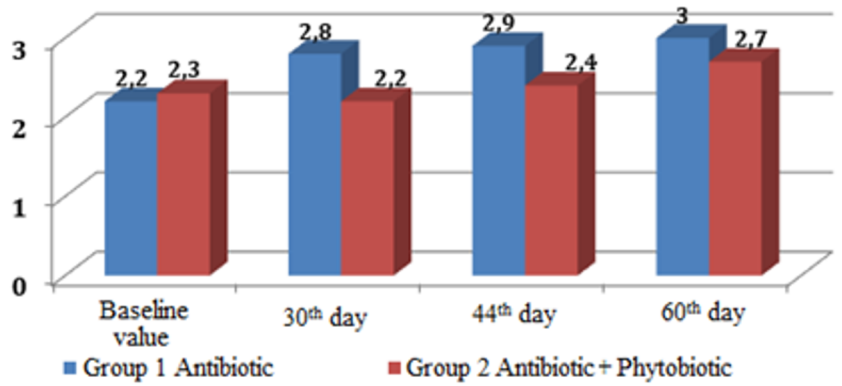

Fig. 2. The concentration of erythrocytes (10^{12}/l) in the blood of laying hens of the experimental groups.

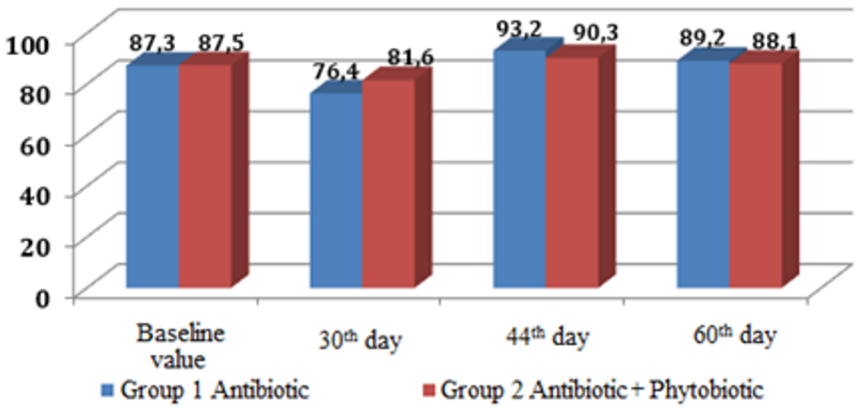

Fig. 3. The content of hemoglobin (g/l) in the blood of laying hens of the experimental groups.

The results obtained in the system "erythrocytes - hemoglobin" in laying hens of group 2 indicated the absence of tissue and cellular respiration and transport function disorders. This conclusion was confirmed by a consistent level of MDA in the blood serum, as one of the main markers of the lipid peroxidation system functioning (Fig. 4) and the dynamics of the osmotic resistance of blood erythrocytes.

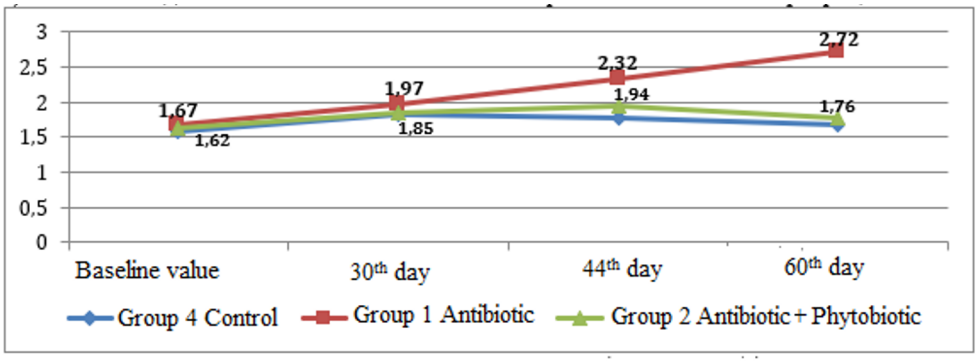

Fig. 4. The concentration of malondialdehyde (MDA, μM/l) in the blood serum of laying hens of the experimental groups.

The analysis of the data for determining the OFT showed that the values during the experiment did not differ significantly from each other: the degree of hemolysis of erythrocytes in an isotonic solution (0.9% NaCl) averaged 1.52±0.15%; in hypotonic solution (0.45% NaCl) – 27.34±1.42%.

The number of leukocytes in the blood of laying hens of group 2 during the experiment was consistent: the dynamics of the amount of leukocytes did not exceed 9.5% compared to the baseline of group 2. The revealed changes in hematological parameters in group 2 were similar to egg-cross of hens by physiological fluctuations in parameters blood [8].

Changes in the quantitative and qualitative composition of the cellular component of immune system in laying hens of group 2 during the period of the experiment were compared with group 1 (Fig. 5, 6,).

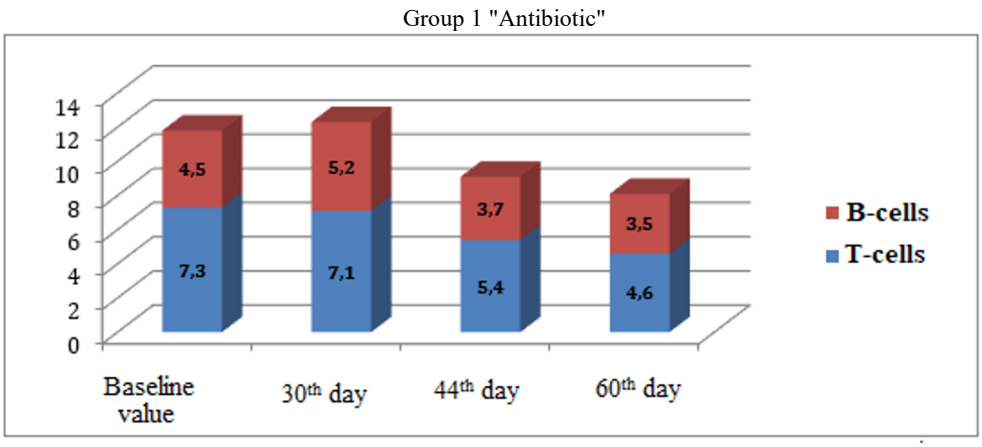

Fig. 5. Dynamics of the cellular component of immune system in laying hens of group 1 (T-cells · 10^9/l; B-cells · 10^9/l).

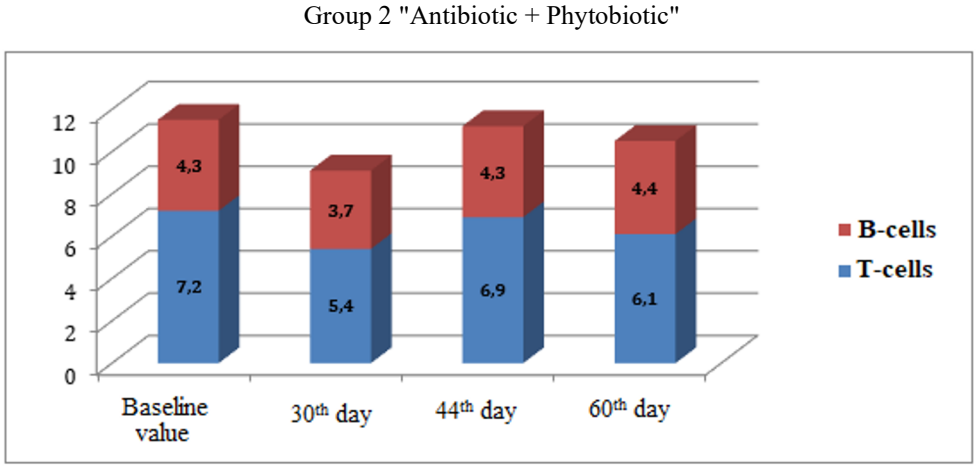

Fig. 6. Dynamics of the cellular component of immune system in laying hens of group 2 (T-cells · 10^9/l; B-cells · 10^9/l).

A decrease in the number of immunocompetent cells in laying hens of group 2 by 20.8% compared with background values was recorded on the 30th day of the experiment. At the same time, in hens from group 1, the decrease in the level of immunocompetent cells was more significant – 31.3%, it means, the phytobiotic had a positive effect on the immune system.

Similar dynamics was observed in terms of the level of the phagocytic index of heterophils: on day 45, a decrease in PhI in group 1 by 24.5% was noted, while in group 2 it was 3 times less (6.2%).

This dynamic is presumably associated with a cyclic physiological change in the immune system of egg-cross hens and is compliant with the state of the immune system during this period (Fig. 7).

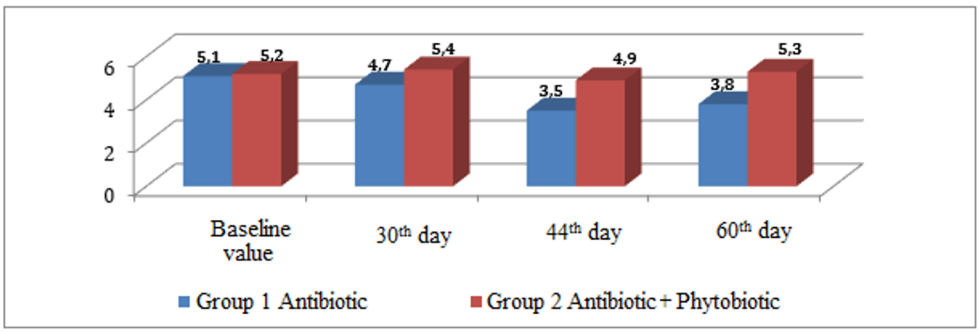

Fig. 7. Dynamics of the phagocytic index (PhI, c.u.) of blood heterophils of laying hens of experimental groups.

The positive effect of the phytobiotic on the immune system was confirmed by the content of the bactericidal component of MPO in phagocytic blood cells of laying hens of group 2 for the entire experiment, in contrast to similar indicators in group 1 (Fig. 8).

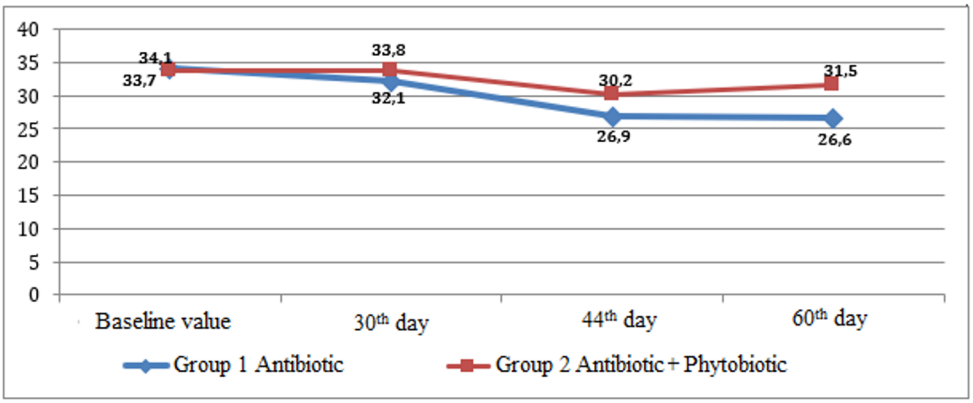

Fig. 8. The concentration of the bactericidal component (MPO, ng/ml) in the leukocyte fraction of blood cells of laying hens of the experimental groups.

The results of the study of the adhesive ability of phagocytic blood cells of laying hens of experimental groups 1, 2 and 4 are presented in table 5.

Table 5. Dynamics of the index of adhesive ability of phagocytic blood cells of experimental laying hens (c.u.).

Group of experimental birds	Background values	30th day	45th day	60th day
Group 1 "Antibiotic"	3.7±0.2	**5.3±0.3** ↑	3.8±0.2	3.1±0.1
Group 2 "Antibiotic + Phytobiotic"	3.5±0.1	3.8±0.3	3.7±0.1	3.5±0.2
Group 4 "Control"	3.7±0.2	3.8±0.2	3.6±0.3	3.8±0.1

When mononuclear phagocytes are included in any biological process, it is accompanied by their structural and functional changes [13]. As presented in table 5 the processes of functional activation were found only in group 1. It can be assumed that from 1 to 30 days of the experiment in birds of group 1 a compensatory shift occurred towards non-specific defenses, as a result of a change in the quantitative and qualitative composition of leukocyte cells. However, in the subsequent period (from 45 to 60 days) a decrease in the adhesive ability of mononuclear phagocytes was found compared to background values. In group 2, macrophage adhesion did not undergo significant changes during the experiment, which is also probably due to the positive effect of the phytobiotic.

4 Conclusions

Thus, following results were achieved:

- the antibiotic enrofloxacin had an indirect effect on the body of laying hens, which was expressed in a shift in immunohematological parameters to the lower limit of the physiological reference. A compensatory shift in the body's immunoreactivity towards nonspecific immunity was found. The identified immunohematological changes persisted for 30 days after abolition of antibiotic.

- the combined use of an antibiotic and a phytobiotic resulted in improvement of the indirect effect of enrofloxacin on the body of laying hens. Positive changes in immunohematological parameters in experimental laying hens were consistent and were

similar to physiological fluctuations in blood parameters in egg-crosses of hens. During the combined use of "Antibiotic + Phytobiotic" (1-30 days) in the system "erythrocytes - hemoglobin" there were no tissue and cellular respiration, ion transport and permeability of erythrocyte membranes disorders. Changes in the quantitative and qualitative composition of the cellular component of immune system in laying hens of this group were not significant. Immunohematological changes were recorded only in the period from 1 to 30 days. The research results showed that the combined use of an antibiotic and a phytobiotic had an immunocorrective, and, in general, a positive effect on laying hens under the conditions of the technological cycle.

Acknowledgements

The research was supported by a grant from the Russian Science Foundation, Project № 18-16-00040.

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