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The effect of a bone marrow-derived immunostimulatory preparation on the immunity of broiler chickens vaccinated against salmonellosis

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Abstract: The use of bone marrow-derived immunostimulants is a promising direction in poultry production. The objective of this research was to study the effect of introducing a bone marrow cell protein formulation on the immunity of chickens vaccinated against salmonellosis. According to the principle of analogues, a control and two experimental groups of chickens were formed with 20 heads each (in total 60 individuals). To Group 1 birds, a protein preparation from bovine bone marrow cells was administered with feed by irrigation with 10% suspension in physiological saline at a rate of 0.2 ml per head once per day from the first day of life for three days. In Group 2, the drug was administered once, on day 1, at a rate of 0.2 ml per head. Control chickens were injected with saline in the same volumes. All chickens were vaccinated against salmonellosis. Blood for analysis of cellular, biochemical and humoral indicators was taken on days 7 and 14 of bird life. The use of the bone marrow cell-derived protein preparation resulted in higher values in the blood of chickens of Groups 1 and 2, respectively, by day 14 of age in comparison with controls as follows: erythrocytes (15.51% and 22.28%) and leukocytes (3.93% and 3.70%), T- and B- lymphocytes (67.5% and 69.16%; 23.24% and 23.75%), neutrophil phagocytic activity (10.14% and 25.36%) and phagocytic index (17.25% and 18.74%), bactericidal (13.32% and 20.25%) and lysozyme activity (23.88% and 24.41%), total protein (13.23% and 14.21%), immunoglobulins (19.59% and 20.76%), specific antibody titre (47.50% and 51.25%). Our study confirms the suitability of using bone marrow-derived protein preparations in poultry production. In practical terms, our study has particular importance for the development and implementation of preparations based on proteins of bone marrow cells.

Keywords: poultry; bone marrow protein preparations; prevention; cellular, biochemical and humoral blood counts

The main strategy for increasing the economic efficiency of industrial poultry production is the intensification of production. This involves the introduction of advanced technologies, highly productive poultry crosses and the use of modern equipment (Glinskiy et al. 2018).

When poultry is raised under conditions of intensive technologies immunity indicators can be impaired (Castellini et al. 2012; Korver 2012; Fontana et al. 2015; Gholami et al. 2017; Huber et al. 2017; Farag and Alagawany 2018). In this regard, it is advisable to use drugs with immunomodulatory properties

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(Chuammitri et al. 2011; Kurmanaeva 2013; Amadori and Zanotti 2016; Amiranashvili et al. 2017).

Recently, research studies in the field of poultry production have been directed at finding new means of activating broiler chicken immunity and increasing the effectiveness of specific prophylaxis (Wang et al. 2015; King et al. 2018; Yang et al. 2018). Preparations based on viruses and bacteria, blood, colostral milk, thymus, spleen, cartilage, etc. are abundantly used (Petrov et al. 1997; Dzik et al. 2017).

A promising direction is the use of bone marrow-derived immunostimulants. These preparations stimulate antibody formation and have an immunomodulatory effect (Chereshnev et al. 2011; Mandro and Fedorenko 2016).

According to literature sources, bone marrow-derived preparations surpass many other immunomodulatory drugs, but development methods and administration routes for protein preparations from bone marrow cells are understudied.

Our hypothesis was that the use of a protein preparation from bone marrow cells would stimulate immunity in chickens, including vaccination against salmonellosis.

The purpose of our study was to study and compare the effect of two methods of administering a bone marrow-derived protein preparation (BPCM) on the immunity of broiler chickens vaccinated against salmonellosis.

MATERIAL AND METHODS

The study was conducted in accordance with ethical principles approved by the Animal Experiments Ethics Committee, Far Eastern State Agrarian University (Protocol No. 5 of May 25, 2018).

To obtain the drug, the bone marrow of Holstein-Friesian cattle was used. The whole herd was free of tuberculosis and brucellosis. Animals were kept in accordance with zootechnical and veterinary-sanitary rules. Bone marrow was obtained immediately after slaughter from tubular bones. The bones were thoroughly cleaned of muscle and connective tissue. On both sides, the bones were cut and the bone marrow removed. Production of protein preparation from bone marrow cells was carried out in accordance with the recommendations of Mandro and Fedorenko (2016).

The bone marrow was placed into a cell culture medium (TS-199) and mixed. The suspension was then passed through a filter. Protein deposition was carried out using trichloroacetic acid. The solution was centrifuged, and then the liquid was separated from the protein precipitate. The sediments were washed off with alcohol and ether, transferred to glass for drying, and then subjected to dialysis. The solution was centrifuged, the precipitates were washed off with alcohol and ether, then dried on glass. The product was heated to a temperature of 43 °C three times at intervals of 24 hours. The finished product was stored in the refrigerator.

Physico-chemical properties of the preparation and its sterility were determined. The protein content in the preparation was determined using refractometry. The concentration of immune proteins was determined by electrophoresis in agar gels. Sterility was determined by inoculation on nutrient media.

In an experiment to study the biological effects of the preparation we used broiler chickens cross IZA Habbart F-15. Day-old chickens were obtained from poultry immunised with the inactivated Nobilis® Salenvac T – Nobilis vaccine against salmonellosis at a dose of 0.1 ml.

Sixty birds were divided into three groups with 20 birds each – Control, Group 1, Group 2 – by live weight and physiological state of the organism. The chickens of each group were kept in a separate cage. All birds had food (a commercial diet) and water available *ad libitum*; veterinary and sanitary measures were similar. Group 1 birds received the protein preparation from bone marrow cells with feed by irrigation with 10% suspension in physiological saline at a rate of 0.2 ml per head per day once from day 1 of life for three days. In Group 2, the drug was administered once, on day 1, at a rate of 0.2 ml per head. This division into groups was based on preliminary studies of various methods in experimental laboratory animals in which a high level of immune response was noted. Control chickens were injected with saline in the same volumes. After the experiment, chickens were observed for a month and used in further studies.

Blood samples were taken via wing vein from chickens on days 7 and 14 after hatching. Ery-throcyte and leukocyte counts were determined using the MEC-6 400 haematology analyser. T- and B-lymphocyte counts were determined using the method of Novikov (1979) based on the ability of lymphocytes to fix red blood cells on their surfaces. Lymphocytes were mixed with a 30–100-fold

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higher number of red blood cells; the mixture was incubated at 37 °C for 5 min, centrifuged and kept at 4 °C, and then gently shaken and the percentage of lymphocytes containing red blood cells on their surfaces counted in a Goryaev chamber.

Total protein in blood serum was determined using refractometry. For protein fractionation, electrophoretic analysis of blood serum was performed in 1% agarose gels. Immunoglobulins were determined by reaction with zinc sulphate, followed by determination of the turbidity of the solution using a photometric device.

To detect the phagocytic activity of neutrophils, the method of Smirnov et al. (1989) was used. The functional activity of leukocytes was assessed using a microbial culture test. The phagocytic activity of leukocytes was determined by calculating the percentage of active leukocytes involved in phagocytosis relative to the total number of counted leukocytes. Bactericidal activity was determined by the photonephelometric method and blood serum lysozyme activity according to the method of Dorofeychuk (1968). Specific antibodies in the blood serum of chickens vaccinated against salmonellosis were determined using the agglutination reaction. Digital material was processed using descriptive statistics using the applications "Statistica 10.0" and "Microsoft Excel". Student's *t*-test was used to determine the statistical significance of differences.

RESULTS

The use of the protein preparation contributed to an increase in levels of blood parameters in the blood of chickens (Table 1). On day 7 of the experiment, the red blood cell count in Group 1 exceeded the value of the control group by 12.65%, in Group 2 by 10.12%. By day 14, the number of red blood cells in the blood of Group 1 chickens was higher than in the control group by 15.51%; in Group 2 by 22.28%. The white blood cell count in the poultry blood of the experimental groups was slightly higher than the control but was within the physiological norm. The increase in the numbers of erythrocytes and leukocytes in the blood of birds was probably caused by the process of general stimulation of the hematopoietic function of the body.

The use of the preparation resulted in increased cellular evidence of immunity. When the bone marrow cell-derived protein preparation was included in the diet, an increase in the T- and B-lymphocyte counts was observed in the blood of chickens in comparison with controls by the end of the experiment – by 67.50% and 23.24%, respectively. After administration of the preparation, the counts of T- and B-lymphocytes on day 7 were above background by 21.22% and 2.34%; by day 14 the increase was 69.16% and 23.75%. We uggest that the increase in cellular immunity may be due to the presence in the drug of certain groups of biologically active substances that stimulate and activate cellular differentiation of lymphocytes in the bone marrow.

An increase of total protein concentration in the blood serum of chickens was detected. In Group 1, this indicator exceeded the control on day 7 by 8.01%, on day 14 – by 13.23%. In Group 2, total protein in the blood serum of chickens exceeded that of the control group by 7.96% on day 7 and by 14.21% on day 14.

The concentration of albumin in Group 1 in comparison with the same indicator in the control was lower on day 7 by 6.05%, on day 14 – by 6.65%. In Group 2, the albumin level was lower than the background on day 7 by 5.58% and on day 14 by 8.93%.

The use of the preparation resulted in larger globulin protein fractions. On day 7, the increase in α-globulins in Group 1 was 22.93%, it β-globulins it was 9.80%, while in γ-globulins the increase was 4.61%. Positive growth dynamics of these fractions in the blood of chickens was also observed on the 14th day of the study. The level of α-globulins increased by 7.09% relative to the control, those of β-globulins by 13.32%, while those of γ-globulins rose by 8.33%.

In Group 2 on day 7 of the study, the level of α-globulins was higher than the same indicator in the control group by 20.78%; levels of β-globulins were higher by 10.04% and those of γ-globulins increased by 3.98%. At the end of the study, the increases were 9.31%, 15.88% and 11.94%, respectively. The increase in γ-globulin indicates a high level of immunity.

On day 7 of the experiment, the phagocytic activity of leukocytes in chickens receiving the preparation with feed exceeded the same indicator in the control group by 22.53%. In Group 2, phagocytic activity was higher than the background by 17.60%. By day 14, the phagocytic activity of the blood leukocytes of the chickens in Group 1 had increased by 10.14%; in Group 2 this value was 25.36% higher.

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Table 1. Morphological blood values and immune variables of broiler chickens after administration of a protein preparation derived from bone marrow (M ± S.D.; n = 60)

Value	Group of chickens					
	control		experimental 1		experimental 2	
	7th day	14th day	7th day	14th day	7th day	14th day
Erythrocytes (10^{12}/l)	3.16 ± 0.05	3.48 ± 0.02	3.56 ± 0.01**	4.02 ± 0.06*	3.48 ± 0.16*	4.28 ± 0.03*
Leukocytes (10^9/l)	23.16 ± 0.45	21.08 ± 1.06	23.77 ± 1.36**	21.91 ± 0.98***	23.64 ± 0.68**	21.86 ± 1.24*
Total protein (g/l)	41.41 ± 0.26	52.68 ± 0.23	44.73 ± 0.21*	59.65 ± 0.75**	44.71 ± 2.57*	60.17 ± 1.22**
Albumin (%)	66.39 ± 0.13	57.54 ± 0.15	62.37 ± 0.21***	53.71 ± 0.16*	62.68 ± 1.49***	52.40 ± 0.13*
α-globulins (%)	11.16 ± 0.25	14.24 ± 0.17	13.72 ± 0.13*	15.25 ± 0.18*	13.48 ± 0.65*	15.64 ± 0.34*
β-globulins (%)	8.16 ± 0.18	9.38 ± 0.58	8.96 ± 0.14**	10.63 ± 0.24**	8.98 ± 0.51*	10.87 ± 0.06**
γ- globulins (%)	14.29 ± 0.10	18.84 ± 0.33	14.95 ± 0.21**	20.41 ± 0.19*	14.86 ± 1.07**	21.09 ± 1.55*
T-lymphocytes (th/μl)	4.24 ± 0.18	2.40 ± 0.15	5.12 ± 0.31*	4.02 ± 0.22**	5.14 ± 0.08**	4.06 ± 0.03*
B-lymphocytes (th/μl)	2.67 ± 0.06	2.28 ± 0.19	3.36 ± 0.13**	2.81 ± 0.24*	3.32 ± 0.05**	2.97 ± 0.08*
Phagocytic activity (%)	1.42 ± 0.05	1.38 ± 0.26	1.74 ± 0.08*	1.52 ± 0.06**	1.67 ± 0.02*	1.73 ± 0.04**
Phagocytic index (%)	6.90 ± 0.56	19.26 ± 0.41	7.11 ± 0.42*	22.68 ± 1.74*	7.09 ± 0.47***	22.87 ± 1.25*
Lysozyme activity of blood serum (%)	7.34 ± 0.21	15.28 ± 0.29	7.98 ± 0.18***	18.93 ± 5.16*	7.64 ± 0.94*	19.01 ± 0.61**
Bactericidal activity of blood serum (%)	22.18 ± 2.18	42.90 ± 3.42	26.06 ± 1.48*	51.19 ± 6.68**	25.87 ± 1.08*	51.59 ± 1.23*
Immunoglobulins (units)	1.95 ± 0.08	3.42 ± 0.23	2.42 ± 0.03**	4.09 ± 0.24**	2.26 ± 0.05**	4.13 ± 0.02*

*P < 0.05; **P < 0.01; ***P < 0.001 in comparison with control

The phagocytic index at the end of the experiment in chickens of Group 1 was 17.25% higher than the background; in Group 2, the increase was 18.74%.

On day 7 of the study, the increases in the lysozyme and bactericidal activities of the blood serum of chickens from Group 1 were 8.71% and 17.49%, respectively. On day 14, the lysozyme activity of the blood serum of chickens was higher than background by 23.88%; bactericidal activity was increased by 19.32%. The lysozyme and bactericidal activities of the blood serum of Group 2 birds on day 7 of the study exceeded the control data by 4.08% and 16.63%; on day 14 these values were higher by 24.41% and 20.25%, respectively.

The level of immunoglobulins on day 7 of the experiment in Group 1 exceeded the background by 24.10%; in Group 2, the increase was 15.89%.

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On day 14, the increases were 19.59% and 20.76%, respectively.

Analysis of the results of our experiments show that the use of a protein preparation derived from bone marrow cells has a positive effect on the specific immune response of broiler chickens in response to vaccination against salmonellosis. The titres of specific antibodies in the blood of Group 1 chickens at the beginning of the study were at the level of 1 : 210 ± 5.03, and in Group 2 came to 1 : 204 ± 3.78, which are higher than the analogous parameters in the control by 31.25% and 27.50%, respectively. By day 14 of age, antibody titres in the experimental groups were 1 : 118 ± 7.20 and 1 : 121 ± 4.13, which exceeded the background by 47.50% and 51.25%.

The use of the protein preparation facilitated a reduction in the incidence of gastrointestinal diseases of chickens. The morbidity of chickens was determined on the basis of clinical signs. In sick chickens, a depressed state, lack of appetite and diarrhoea were noted.

In the control group of chickens, the incidence was 25.00% over the entire period of the experiment. In Group 1, the incidence was determined at 5.00%; in Group 2 it was 10.00%. The safety of chickens in the experimental groups on the 14th day of the experiment was 100.00%, while in the control group it was 85.00%.

DISCUSSION AND CONCLUSIONS

In production conditions, many factors adversely affect physiological condition and avian immunity. To improve the immunity of birds, a number of authors recommend the use of immunostimulatory preparations. Positive reports on bone marrow preparations (Gein et al. 2010; Mandro and Fedorenko 2016; Grishko et al. 2017) motivated us to study these drugs as immunostimulants for broiler chickens.

The positive effect of the preparation on the immune system of the chickens was accompanied by a decrease in the incidence of gastrointestinal diseases and an increase of the livability of the chickens.

Our data confirm the suitability of using protein preparations in poultry production. The use of bone marrow-derived protein preparations contributed to an increase in erythrocytes and leukocytes, biochemical indicators of blood and cellular and humoral factors of immunity by the end of the experiment. Administration of the preparation had a positive effect on the content of specific antibodies.

Thus, a protein preparation from bone marrow cells exerts an immunostimulatory effect on the broiler chickens, increases the effectiveness of specific prevention of salmonellosis, reduces its incidence and increases broiler livability.

In conclusion, for the first time, the effect of various methods of administering a protein preparation from bone marrow cells on the immunity of poultry vaccinated against salmonellosis has been studied. Dosing and methods of administering the preparation have been determined, and the potential of its use has been proven.

Significant changes in the indicators at the beginning of the experiment were observed in Group 1 chickens who received the preparation with feed. However, in Group 2 broilers, an increase in immune indicators was only noted by the 14th day of the experiment.

In practical terms, our study has particular importance for the development of preparations based on proteins of bone marrow cells and opens new avenues for their use in the treatment and prevention of immunodeficiency states. Further, such preparations might stimulate antibody production during vaccination.

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