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Research Article

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Influence of feed supplementation with probiotic and organic form of zinc on functional status of broiler chickens

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Abstract

Background: The increase in the intensity of agricultural production is associated with the action of various stress factors on the organism of birds, which can lead to negative consequences. Prevention of the development of stress conditions in farm birds, particularly broiler chickens, in industrial production, is the most important task facing scientists and practitioners.

Aim: The objective of this study was to investigate the effect of a combined probiotic preparation and zinc glycinate on the indicators of immunity, biochemical parameters, and antioxidant status.

Methods: The study was conducted on broilers of Arbor Acres cross: the birds in the negative control group received a balanced feed mixture, a mineral and vitamin premix without zinc; the positive control group treated with an injection of cyclophosphamide and received the balanced feed with the mineral and vitamin premix without zinc; in experimental group I, broilers was treated with the injection of cyclophosphamide and received balanced feed, probiotic and zinc glycinate; in experimental group II, broilers received balanced feed, probiotic and zinc glycinate. At the end of the experiment, some blood from the wing vein was collected for evaluation of hematological and biochemical blood parameters using automatic morphological and biochemical analyzers, evaluation of immune status by enzyme immunoassay, and antioxidant status by colorimetric method.

Results: It was found that the developed strategy nutrition leveled the effects of immunosuppression - there was an increase in the level of lymphocytes, interleukin-4, and interleukin-10; the level of cholesterol, triglycerides, glucose, and total protein (TP) tended to the control values; there was a significant increase in serum interleukin-2 and interferon gamma; increase in TP on the background of triglycerides decreased in broilers of experimental group II. An increase in the activity of superoxide dismutase and catalase against the background of a decrease in the level of malonic dialdehyde was revealed in the experimental groups.

Conclusion: The developed strategy of broiler chicken nutrition can be used for the successful protection of birds from immunodeficiency states, improvement of antioxidant status, and maintenance of complete protein and lipid metabolism.

Keywords: Poultry, Antioxidant status, Immune status, Immunodeficiency, Metabolism.

Introduction

Modern poultry farming is one of the leading and intensively developing branches of the agro-industrial complex (Bohrer, 2017). The intensification of poultry production implies further more complete use of genetic resources for poultry productivity, which are steadily increasing under the constant influence of selection.

Increasing the intensity of agricultural production is associated with the action of various stress factors on the bodies of birds (Bulent and Niyazi, 2018; Fisinin *et al*., 2023; Korver, 2023). The immune system of farm birds is the most sensitive to stress factors. In the last decade, the relationship between the body's response to stress and the state of the gut microbiota has been increasingly studied. Studies show a relationship between stress, immunity, and the gut microbiota (Komarova and Khavkin, 2020). The development of distress syndrome leads to the suppression of cellular and humoral immunity. It leads to the development of serious disorders and mass pathologies of non-infectious etiology (Komarova and Khavkin, 2020; Wlaźlak *et al*., 2023). This makes it impossible to achieve high productivity and obtain biologically complete products of high sanitary quality (Kulappu Arachchige *et al*., 2021). Thus, prevention of the development of stress conditions in farm birds in industrial production is the most important task facing scientists and practitioners.

Supplements with immunomodulatory properties are used along with vaccines in order to increase

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resistance to stress, increase nonspecific resistance and adaptability, prevent mass morbidity, and ensure high productivity (Phillips *et al*., 2023). As a rule, specialists give preference to those adds that can not only normalize the immune system but can stimulate growth and development, increase productivity, and improve meat quality at the same time (Alagawany *et al*., 2020; Krysiak *et al*., 2021). One of the promising trends in farm animal and poultry breeding technology is the use of probiotic products (Jha *et al*., 2020). It is well known that the state of intestinal microflora plays an important role in the immune status of animals, metabolism, and absorption of nutrients (Belkaid and Hand, 2014; Rowland *et al*., 2018; Zheng *et al*., 2020). Bacteria of the genus Bifidobacterium and Lactobacillus are the most promising group of microorganisms used in agricultural practice (Nam *et al*., 2022; Sun *et al*., 2022; Lokapirnasari *et al*., 2022). Supplementation of the diet with probiotics promotes a change in the composition of the intestinal microbiota towards a more balanced structure, thereby achieving an increase in intestinal barrier function and the formation of optimal immune interactions.

The immune system of poultry is significantly influenced by diet, especially the use of feed additives in the form of micronutrients. One of the most important essential bioelements that influence immunologic resistance is zinc (Maares and Haase, 2016). Zinc is one of the necessary regulators of the development and functioning of the immune system as it ensures the proliferation and maturation of T- and B-lymphocytes (Weyh *et al*., 2022). Generally, zinc is added to farm bird feed as inorganic forms (sulfates or oxides) because of its relatively low cost and availability (Liu *et al*., 2020). However, such forms have low bioavailability and may irritate the gastrointestinal mucosa, reduce the absorption of other nutrients, and have negative environmental impacts (Barszcz *et al*., 2023). Scientists have found that organic complexes, in particular chelated zinc with methionine or lysine, have a higher assimilability (Saleh *et al*., 2018). However, the search for new organic complexes that improve the absorption of this essential chemical element continues. The zinc chelate complex with glycine (Zn-Gly) is currently a new alternative because it is characterized by greater stability, chemical and physical homogeneity, and provides better absorption of zinc through the intestinal wall (Tomaszewska *et al*., 2017; Zhu *et al*., 2022).

Thus, the use of probiotic supplements and chelate forms of zinc can be considered as a possible tool for the prevention of immunodeficiency states in birds. Despite the positive effects of probiotics and zinc glycinate on the functional status of poultry demonstrated in numerous studies, the mechanisms responsible for the effects of these supplements on immune functions are not fully understood. In this regard, the objective of this study was to investigate the effect of a combined probiotic and zinc glycinate on the indicators of cellular and humoral immunity, biochemical parameters, and antioxidant status of broilers.

Materials and Methods

Experimental design

The study was conducted on 28 daily broilers of Arbor Acres Cross (CJSC "Poultry Farm Orenburgskaya"). The following 4 treatment groups were established: the birds in the negative control group received a balanced feed mixture (BF) in accordance with requirements for chickens, as well as a mineral and vitamin premix without zinc; the positive control group treated with an injection of cyclophosphamide (cyclophosphamide-induced immunosuppressed animal model) and received also BF with the mineral and vitamin premix without zinc; in experimental group I, broilers were treated with the injection of cyclophosphamide and received BF, probiotic (0.7 g/kg of feed) was added at the age of 7–14 days birds, zinc was added in organic form in combination with glycine (230 mg of chelate/kg or 70 mg Zn/kg, respectively) at the age of 28–42 days birds; in experimental group II, broilers received BF, probiotic (0.7 g/kg of feed) and Zn-Gly (230 mg of chelate/kg or 70 mg Zn/kg, respectively) at the same periods of development.

The birds were fed with mixtures appropriate for each period of rearing: starter—days 1–14, grower—days 15–28, finisher—days 29–42. Feeding and drinking water (free from antibiotics) were provided ad libitum. The composition and nutrient levels of the basal diet are shown in Table 1. Bird feed was provided by CJSC "Poultry Farm Orenburgskaya."

The method of modeling the secondary immunodeficiency state in farm poultry is carried out by intraperitoneal injection of cyclophosphane on the 5th, 6th, and 7th day of their physiological development at a dose of 40 μ/kg of body weight.

Probiotic "Lactobifadol Forte" ("Component", Buguruslan, Russia) contains 1×10^6 colony forming units (CFUs) of Lactobacillus acidophilus and 8.0 $\times 10^7$ CFU of Bifidobacterium adolescentis in 1 g of nutrient medium with supporting component. Adding probiotics into the feed was carried out according to the instructions.

Zinc was introduced into the mineral and vitamin premix, which contained no zinc. The amount of Zn added to the feed mixtures, based on the dietary recommendations for Arbor Acres chickens, was 70 mg Zn/kg. The feed additive "Plexomin Zn 29" (Phytobiotics Futterzusatzstoffe GmbH, Germany) used contained 29% Zn.

The temperature regime and relative humidity corresponded to the norms recommended for growing broilers. The photoperiod program complied with the European Social Security Regulation 43/2007 (Council Directive 2007/43/EU laying down the minimum rules for the protection of chickens kept for meat production).

Table 1. Ingredients and chemical composition of the basal diet fed to broilers.

Ingredients	Starter feed (1–14 days)	Grower feed (15–28 days)	Finisher feed (29–42 days)
Ingredients, %			
Wheat	42.00	41.50	71.88
Corn	20.50	20.00	-
Soybean meal, 46 % CP	25.34	20.89	11.45
Soybean oil	2.35	6.23	4.55
Meat meal	2.00	3.85	3.25
Sunflower oil	4.00	4.00	5.50
Salt	0.2	0.21	0.25
Monocalcium phosphate	0.9	0.56	0.35
Limestone meal	0.71	0.46	0.65
Premix*	2.0	2.25	1.95
Total	100	100	100
Calculated composition			
Metabolizable energy, kcal/100g	310	298	303
Crude protein, %	23.00	21.99	19.32
Crude fiber, %	4.49	4.06	4.68
Lysine, %	1.23	1.36	1.11
Methionine, %	0.54	0.50	0.50
Methionine+cysteine, %	0.84	0.79	0.76
Calcium, %	1.05	0.90	0.86
Total phosphorus, %	0.60	0.60	0.60
Sodium, %	0.16	0.16	0.16

* – Vitamin and mineral premix provided per kilogram of diet: Vitamin A – 12.0 KIU/kg; Vitamin D_3 – 4.0 KIU/kg; Vitamin E – 60.0 mg/kg; Vitamin K_3 – 2.0 mg/kg; Vitamin B_1 – 2.0 mg/kg; Vitamin B_2 – 8.0 mg/kg; Vitamin B_3 – 30.0 mg/kg; Vitamin B_4 – 500.0 mg/kg; Vitamin B_5 – 10.0 mg/kg; Vitamin B_6 – 3.0 mg/kg; Vitamin B_{12} – 0.025 mg/kg; Vitamin B_9 – 0.5 mg/kg; Vitamin H – 0.1 mg/kg, Fe – 25.0 mg/kg; Cu –10.0 mg/kg; Zn – 0 mg/kg; Mn – 80.0 mg/kg.

Blood collection

The material for analysis comprised samples of peripheral blood from the wing vein. The blood samples were collected in sterile vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant (Greiner Bio-One International AG, Austria) and also a blood clotting activator and a gel for separating erythrocyte mass (Greiner Bio-One International AG, Austria). Blood samples were collected on day 42. The samples were transported to the laboratory at +4°C to +8°C within 1 h. Serum was obtained by centrifuging the blood at room temperature (20°*C*–22°C) for 10 min at 1000 g. The serum was apportioned and stored at −80°C until analysis.

Hematological analysis

Hematological blood analysis was performed using a morphological automatic analyzer DF-50 Vet (Shenzhen Dymind Biotechnology Company, China) and included the determination of the following blood parameters: leukocytes, lymphocytes, eosinophils, pseudoeosinophils, basophils, hemoglobin, erythrocytes, platelets.

Biochemical analysis

Biochemical analysis was carried out on a CS-T240 automatic biochemical analyzer (Dirui Industrial Company Ltd., China) using Randox commercial biochemical kits (USA). Biochemical analysis included the determination of: glucose (GLU), total protein (TP), total cholesterol (CHOL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST).

Immune status

The immune status of broilers was assessed, including determination of serum levels of cytokines—interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10), interferon gamma (IFN-γ), immunoglobulin A (IgA), immunoglobulin Y (IgY), and lysozyme by enzyme-linked immunosorbent assay with the help of tablet spectrophotometer INNO (LTek, Republic of Korea) using the appropriate reagent kits ELISA kit, Chicken Interleukin 2 (IL2) (Cloud-Clone Corporation, USA), ELISA kit, Chicken Interleukin 4 (IL4) (Cloud-Clone Corporation, USA), ELISA kit, Chicken Interferon Gamma (IFNg) (Cloud-Clone Corporation, USA), ELISA kit, Chicken Interleukin 10 (IL10) (Cloud-Clone Corporation, USA), ELISA kit, Chicken Immunoglobulin Y (IgY) (BlueGene Biotech, China), ELISA kit, Chicken Immunoglobulin A (IgA), (BlueGene Biotech, China), respectively. The analysis steps corresponded to the kit instructions.

Antioxidant status

The antioxidant defense of broilers was evaluated using specialized total superoxide dismutase (T-SOD) activity assay kit (Elabscience, China), catalase (CAT) activity assay kit (Elabscience, China), glutathione peroxidase (GSH-Px) activity assay kit (Elabscience, China), malondialdehyde (MDA) assay kit (Elabscience, China) by colorimetric method. The analysis steps corresponded to the kit instructions.

Statistical analysis

All data were analyzed using Statistica version 10 (StatSoft Inc., USA). The normality of the obtained data was checked using the Shapiro-Wilk test. This test has the best power for a given significance. The hypothesis that the data belonged to a normal distribution was rejected in all cases with a probability of 95%, which justified the use of non-parametric procedures for downstream statistical analyses; we, therefore, examined differences among group means using Mann-Whitney U-tests. This method of nonparametric statistics is convenient when comparing small samples and is used by many authors when describing data with non-normal distribution. The data obtained were presented as a median (Me) and as 25th–75th centiles (Q_{25}–Q_{75}). Differences were considered statistically significant when $p < 0.05$ (Whitley and Ball, 2002).

Ethical approval

The experimental studies were conducted in accordance with the instructions and recommendations of the Russian regulations (Order of the Ministry of Health of the USSR No.755 of August 12, 1977 "On measures to further improve the organizational forms of work using experimental animals"), the protocols of the Geneva Convention and the principles of good laboratory practice (National Standard of the Russian Federation GOST R 53434-2009). All procedures on animals were performed in accordance with the rules of the Animal Ethics Committee of the FSSI FRC BST RAS. The design of the experiment was approved by the local Ethical Committee of the FSSI FRC BST RAS (No. 7 dated 06/04/2023).

Results

Hematological profile

Hematologic blood parameters provide important information about the health of farm animals. It was found that the relative content of lymphocytes and monocytes was statistically significantly lower in the model group of birds (positive control); the level of erythrocytes and hemoglobin was lower than in the negative control by 14% (p = 0.03) and 9% (p = 0.05), respectively; there was a pronounced tendency to decrease the level of leukocytes. It should be noted that the introduction of probiotic products and zinc chelate complexes into the diet of birds at critical stages of ontogenetic development of broiler chickens with modeled immunodeficiency (experimental group I) contributed to an increase in the percentage of lymphocytes in the blood. Healthy birds of experimental group II, receiving probiotic and zinc supplementation, also had a tendency to increase the relative content of lymphocytes. In addition, a 12% (p = 0.04) increase in erythrocyte level was found when compared to the negative control (Table 2).

Biochemical profile

It was found that broiler chickens with immunodeficiency (positive control) had an increase in ALT activity by 198% (p = 0.002), AST activity by 123% (p = 0.04), and triglyceride levels by 204% (p = 0.002). In addition, there was a tendency to increase the level of cholesterol and glucose in the blood against the background of a decrease in TP. The effects of cyclophosphan were leveled by immunomodulatory preparations, which was reflected in the biochemical indices of birds' blood - the level of cholesterol, triglycerides, glucose, and TP in experimental group I tends to the control values of healthy birds. However, it should be noted that the activity of ALT and AST continued to remain at rather high values and exceeded the activity of these enzymes in the negative control group by 158% (p = 0.005) and 27% (p = 0.02), respectively. In comparison with the positive control, the activity of ALT and AST in birds of experimental group I was significantly lower. The developed feeding protocol for broiler chickens had a favorable effect on the biochemical parameters of birds of experimental group II, which was characterized by a decrease in triglycerides level by 29% (p = 0.05), as well as a tendency to decrease glucose and increase the content of TP in the blood of birds in comparison with the negative control (Table 3).

Immune status

It was found that the level of IL-2, IL-4, and IL-10 in broiler chickens with modeled immunodeficiency (positive control) was lower than in the negative control group by 11.5% (p = 0.04), 23% (p = 0.05),w and 27% (p = 0.02), respectively; there was a persistent tendency to decrease lysozyme (Table 4).In experimental group I, it was found that IL-2 and IL-10 levels also remained below the values of the negative control group by 22% (p = 0.05) and 25% (p = 0.01), respectively. However, it should be noted that the serum levels of IFN-γ, IgA, and lysozyme were within control limits. At the same time, the level of IL-4 was higher by 11% (p = 0.02) when compared to the group of immunodeficient birds (positive control). Experimental group II had the

Table 2. Morphological blood parameters of broiler chickens.

Parameters	NC	PC	Ex(I)	Ex(II)
Leukocytes, 10^9/L	44.5 (41.6–45.62)	36.0 (35.3–36.7)	30.8 (23.9–37.2)	39.1 (31.2–42.4)
Lymphocytes, %	41.3 (29.6–50.05)	32.7 (31.4–32.9)*	52.2 (47.9–64.6)	42.4 (35.3–49.9)
Pseudoeosinophils, %	57.8 (53.7–58.0)	51.5 (41.2–54.7)	39.3 (28.2–39.9)	49.0 (41.8–55.6)
Monocytes, %	1.8 (1.3–5.8)	0.5 (0.3–0.55)*	0.7 (0.4–1.2)	0.6 (0.5–1.2)
Eosinophils, %	7.3 (7.1–8.8)	9.5 (7.9–16.1)	6.5 (6.0–11.5)	7.7 (5.6–8.6)
Basophils, %	0.5 (0.4–0.6)	0.7 (0.5–0.8)	0.5 (0.3–0.8)	0.3 (0.2–0.6)
Erythrocytes, 10^{12}/l	2.5 (1.6–2.5)	2.15 (2.11–2.2)*	2.05 (1.43–2.07)	2.81 (2.55–3.04)#
Hemoglobin, g/l	134.0 (87.5–136.0)	122.0 (121.5–122.5)*	110.0 (79.0–120.0)	135.0 (104.0–138.0)

NC: negative control (BF), $n = 7$; *PC*: positive control (cyclophosphamide-induced immunosuppressed animal model, BF), $n = 7$; Ex(I): experimental group I (cyclophosphamide-induced immunosuppressed animal model, BF + probiotic + Zn-Gly), = 7; Ex(II): experimental group II (BF + probiotic + Zn-Gly), $n = 7$; *– ($p \leq 0.05$) – *p*level comparing broiler chickens of positive control with negative; # – ($p \leq 0.05$) – *p*level comparing broiler chickens of the experimental group II with negative control.

highest content of a number of circulating cytokines. IL-2 and IFN-γ levels exceeded those of negative controls by 7% ($p = 0.05$) and 52% ($p = 0.02$).

Antioxidant status

It was found that against the background of modeling immunodeficiency state the activity of antioxidant enzymes decreased. The activity of superoxide dismutase, CAT, and glutathione peroxidase was lower than in negative control birds by 37% ($p = 0.002$), 31% ($p = 0.05$), and 11% ($p = 0.02$), respectively. It should be noted that the level of malonic dialdehyde increased by 30% ($p = 0.002$). There was an increase in the activity of enzymes at introduction to the diet of birds with immunodeficiency probiotic supplements and chelate forms of zinc. The activity of superoxide dismutase was higher than in birds with immunodeficiency by 20% ($p = 0.05$), there was a tendency to increase the activity of CAT on the background of a decrease in the content of malonic dialdehyde in the blood. There was an increase in the activity of antioxidant enzymes against the background of reduction of peroxidation products in experimental group II. The activity of superoxide dismutase was higher by 37 % ($p = 0.04$), and CAT by 10% ($p = 0.007$) than in birds of the negative control group; the content of malonic dialdehyde was lower than in the negative control by 8 % ($p = 0.04$) (Table 5).

Discussion

The current research shows that the combined use of probiotic preparation "Lactobifadol Forte" ("Component," Buguruslan, Russia) and Zn-Gly chelates "Plexomin Zn 29" (Phytobiotics Futterzusatzstoffe GmbH, Germany) as feed additives during critical periods of broiler chick development contributes to the improvement of protein and lipid metabolism; guarantees the maintenance of the Type 1 T helper cells and Type 2 T helper cells (Th1/Th2) balance, shows the participation of zinc in the activation of cellular immune mechanisms that provide a functional response; provides improvement of the state of the antioxidant system of birds.

In the conducted study, a model of secondary immunodeficiency was used to confirm the effectiveness of a combined probiotic preparation based on Bifidobacterium and Lactobacillus bacteria and a chelate complex of zinc with glycine added to the diet of broiler chickens during critical periods of development. According to the literature analysis, the most convenient model for studying immune system disorders and approbation of various immunomodulatory drugs is the model of immunodeficiency induced by administration of cyclophosphan (Feng *et al*., 2016; Liu *et al*., 2020; Chang *et al*., 2021). The results of the study showed

Table 3. Biochemical blood parameters of broiler chickens.

Parameters	NC	PC	Ex(I)	Ex(II)
Glucose, mmol/l	11.27 (10.4–12.46)	12.21 (8.59–12.83)	9.03 (8.83–10.06)	9.14 (8.13–10.72)
TP protein, g/l	30.63 (28.86–33.75)	23.28 (20.88–23.95)	27.66 (25.61–29.66)	34.34 (29.86–34.89)
Cholesterol, mmol/l	2.83 (2.59–2.92)	3.28 (2.85–3.64)	2.66 (2.42–2.73)	2.47 (2.29–2.56)
Triglycerides, mmol/l	0.78 (0.55–0.95)	2.37 (2.36–2.41)**	2.41 (2.34–2.52)	0.55 (0.03–0.88)#
ALT, U/l	17.9 (17.21–19.37)	53.3 (36.11–70.1)**	46.26 (45.04–48.25)^^	17.0 (12.9–19.8)
AST, U/l	135.2 (127.83–165.78)	300.9 (266.12–656.0)*	171.63 (161.34–193.7)^	160.1 (110.4–115.6)

NC: negative control (BF), *n* = 7; *PC*: positive control (cyclophosphamide-induced immunosuppressed animal model, BF), *n* = 7; Ex(I): experimental group I (cyclophosphamide-induced immunosuppressed animal model, BF + probiotic + Zn-Gly), *n* = 7; Ex(II): experimental group II (BF + probiotic + Zn-Gly), *n* = 7; **– ($p \leq 0.01$) – *p*level comparing broiler chickens of positive control with negative; ^ – ($p \leq 0.05$) – *p*-level comparing broiler chickens broiler chickens of experimental group I with negative control; # – ($p \leq 0.05$) – plevel comparing broiler chickens of the experimental group II with negative control.

Table 4. Indicators of immune status of broiler chickens.

Parameters	NC	PC	Ex(I)	Ex(II)
IL-2, pg/ml	13.55 (13.02–13.63)	11.99 (11.89–12,0)*	10.63 (8.94–11.1)^	14.52 (12.68–15.06)#
IL-4, pg/ml	93.53 (84.27–103.38)	72.02 (62.11–81.55)*	80.02 (79.18–81.79)$	87.85 (84.72–89.4)
IL-10, pg/ml	45.37 (42.31–45.7)	33.18 (32.07–40.93)*	34.27 (31.39–36.67)^^	47,71 (46.16 –49.05)
IFN-γ, pg/ml	10.98 (10.42–11.7)	12.83 (10.03–17.64)	10.57 (9.82–13.22)	16.72 (12.71–23.06)#
IgA, *μg/m*l	52.1 (50.29–52.85)	54.66 (47.11–61.01)	50.14 (45.06–60.88)	56.58 (49.53–64.2)
IgY, *μg/m*l	371.01 (361.9–376.88)	334.79 (286.40–299.0)	312.31 (286.59–383.27)	328.98 (323.16–376.77)
Lysozyme, μg/ml	24.7 (23.3–25.1)	19.7 (19.1–20.1)	21.04 (19.99–21.12)	24.5 (23.7–25.89)

NC: negative control (BF), *n* = 7; *PC*: positive control (cyclophosphamide-induced immunosuppressed animal model, BF), *n* = 7; Ex(I): experimental group I (cyclophosphamide-induced immunosuppressed animal model, BF + probiotic + Zn-Gly), *n* = 7; Ex(II): experimental group II (BF + probiotic + Zn-Gly), *n* = 7; *– ($p \leq 0.05$) – *p*level comparing broiler chickens of positive control with negative; ^ – ($p \leq 0,05$) – *p* level comparing broiler chickens broiler chickens of experimental group I with negative control; ^^ – ($p \leq 0,01$) – *p*-level comparing broiler chickens broiler chickens of experimental group I with negative control; # – ($p \leq 0.05$) – *p*level comparing broiler chickens of the experimental group II with negative control; $ – *p*-level comparing broiler chickens of experimental group I with positive control.

Table 5. Indicators of antioxidant status in broiler chickens.

Parameters	NC	PC	Ex(I)	Ex(II)
T-SOD, U/ml	138.8 (127.94–185.53)	87.69 (86.39–93.87)**	104.95 (103.28–112.56)^,$	190.24 (183.62–221.55)#
CAT, U/ml	160.1 (151.8–167.8)	111.15 (104.12–116.2)*	131.51 (127.9–136.22)	175.6 (172.2–179.3)##
GSH-Px, U/ml	240.7 (233.6–244.3)	212.9 (204.4–218.3)*	222.9 (222.2–225.4)	238.6 (234.6–246.1)
MDA, nmol/ml	7.7 (7.15–8.52)	9.99 (9.86–10.81)**	9.25 (8.99–9.95)	7.1 (6.69–7.52)#

NC: negative control (BF), *n* = 7; *PC*: positive control (cyclophosphamide-induced immunosuppressed animal model, BF), *n* = 7; Ex(I): experimental group I (cyclophosphamide-induced immunosuppressed animal model, BF + probiotic + Zn-Gly), *n* = 7; Ex(II): experimental group II (BF + probiotic + Zn-Gly), *n* = 7; *– ($p\leq0.05$) – *p*level comparing broiler chickens of positive control with negative; **– ($p\leq0.01$) – *p* level comparing broiler chickens of positive control with negative; ^ – ($p\leq0,05$) – *p* level comparing broiler chickens broiler chickens of experimental group I with negative control; ^^ – ($p\leq0,01$) – *p*-level comparing broiler chickens broiler chickens of experimental group I with negative control; # – ($p\leq0.05$) – *p*level comparing broiler chickens of the experimental group II with negative control; $ – *p*-level comparing broiler chickens of experimental group I with positive control.

that in broiler chickens of the positive control group, immunosuppressive effects of cyclophosphan action were observed, which was characterized by a decrease in complex indicators of nonspecific resistance of the organism and specific immunological defense mechanisms, the main parameters of humoral and cellular immune reactions at the systemic and cellular levels. It was experimentally shown that threefold intraperitoneal administration of cyclophosphan to broiler chickens depressed lymphocytopoiesis. The birds of the model group showed leukopenia characterized by a decrease in the level of leukocytes; the relative content of lymphocytes, neutrophils, and monocytes was lower than in the control; the level of lysozyme in blood serum was also lower than control values. The mechanisms of changes in specific immune defense under cyclophosphan exposure were due to the inhibition of the function of Thl- and Th2-lymphocytes, which was accompanied by a decrease in serum levels of IL-2, IL-4, and IL-10. It should be noted that the results obtained are consistent with a number of studies in which the immune-suppressive effect of this drug was observed (Godovalov *et al*., 2013; Kumar and Venkatesh, 2016). In addition, among hematologic parameters, immunodeficient broilers also showed decreased erythrocyte and hemoglobin levels in the blood, which is mediated by the direct cytotoxic effect of cytostatics (Skverchinskaya *et al*., 2023). The evaluation of blood biochemical parameters revealed an increase in the activity of ALT and AST enzymes, the content of triglycerides, cholesterol, and glucose in the blood against the background of a decrease in the level of TP, as well as a decrease in the activity of antioxidant enzymes (T-SOD, CAT, GSH-Px). The above may be the result of side effects of cyclophosphamide mediated by the formation of reactive oxygen species that damage organs and tissues of the body, particularly liver hepatocytes (Wójcik and Dąbkowska, 2010; Subramaniam *et al*., 2013; Jeelani *et al*., 2017; Cengiz *et al*., 2020). The results indicate that cyclophosphamide has immunosuppressive effects and can be effectively used for chemical induction of immune system disorders in farm birds.

The study showed that the addition of combined probiotic preparation and Zn-Gly chelate complex to the diet of broiler chickens with modeled immunodeficiency (experimental group I) during critical periods of their development leveled out the effects of the immunosuppressive effect of cyclophosphan. There was an increase in the content of lymphocytes, serum levels of IL-4 and IL-10 were also higher than in the positive control group; the content of IFN-γ, IgA, and lysozyme in birds of this group was within the values of negative control. It should be noted that in broilers of experimental group II against the background of feed additives introduction, there was a significant increase in blood serum IL-2 and IFN-γ relative to the negative control. The results obtained from the evaluation of the immune status of farm birds in general show that the feed additives used counteract immunosuppression and enhance immunity. The role of probiotics and zinc in immune maintenance, their effects on immune cells, and underlying molecular mechanisms have been described by a number of authors (Read *et al*., 2019; Neveling and Dicks, 2021). Zinc is known to play an important role in the development of the Th1 response, which is characterized by a cascade of cytokine secretion that stimulates immunocompetent effector cells (Jarosz *et al*., 2017). Scientists have shown that stimulation of the Th1 response is characterized by the secretion of IL-2 and IFN-γ. It leads to the activation of cellular immune mechanisms, which provides a functional response. This is supported by the results of our study. The increase of these cytokines in serum was observed in broilers. It should be noted that IL-2 also stimulates the synthesis and release of other cytokines, including IFN-γ (Lillehoj *et al*., 2011). The presented data on immunobiology show the participation of zinc in the processes of induced immunogenesis as a factor providing maintenance of Th1/Th2-balance. The obtained results of the study are in agreement with a number of works that showed the participation of zinc in the activation of systemic cellular immune reactions (Medoro *et al*., 2023; Smerchek *et al*., 2023).

The supplements used had positive effects on protein, fat, and carbohydrate metabolism. In broiler chickens with modeled immunodeficiency (experimental group I) against the background of the introduction of probiotics and Zn-Gly into the diet, the content of cholesterol, triglycerides, glucose, and TP tended to the control values of healthy birds. In birds of experimental group II, there was an increase in blood TP on the background of triglycerides decrease. It is known that zinc is an essential trace element for the body. Scientists have identified more than 3,000 zinc proteins, which are essential for enzymatic and structural functions. In this regard, this chemical element plays a key role in the normal course of many biochemical processes, including protein synthesis, as well as the regulation of metabolism of substances (Skalny *et al*., 2021; Notova *et al*., 2022). It should be noted that the results obtained are consistent with a number of studies. Scientists have found that adding zinc to farm bird feed reduces blood glucose levels, which may be a result of the relationship between zinc and insulin, and also helps increase TP levels (Feng *et al*., 2010; Yalçinkaya *et al*., 2012). Researchers have also found that zinc supplements help lower cholesterol concentrations (Foster *et al*., 2010). Probiotic supplements play an equally important role in improving digestion and boosting and stimulating metabolism and energy. Probiotics are known to favorably impact the intestinal microbiota. The modulation of the amount and nature of bacteria present in the intestinal tract has an impact on mineral metabolism. One of the reported effects of probiotics in birds is to modify the intestinal morphology, having a potentially positive impact on nutrient and mineral absorption and digestibility, including zinc (Sureshkumar *et al*., 2022; Shanmugam *et al*., 2024). The increase in TP in the blood may also be mediated by the fact that bacteria of the genus Bifidobacterium and Lactobacillus, when they multiply, produce enzymes, including proteases that accelerate the breakdown of protein molecules in the gastrointestinal tract, after which the resulting amino acids are used for protein synthesis (Derakhshan *et al*., 2023). In addition, reduced triglyceride levels in probiotic-treated birds may be the result of increased hydrolysis of bile salts, which causes inadequate absorption of lipids in the small intestine. Lactobacillus is known to exhibit high hydrolytic activity against bile salts, which consequently leads to their deconjugation in the gastrointestinal tract (Reuben *et al*., 2022). Thus, by analyzing the data of blood biochemical analysis we can conclude about more complete protein and lipid metabolism in the organism of birds of experimental group II in comparison with the control group.

When considering the antioxidant status of broiler chickens of experimental groups I and II, it was found that the using of the combined probiotic and Zn-Gly during critical periods of poultry development promoted an increase in the activity of the enzymes superoxide dismutase and CAT against the background of a decrease in the content of malonic dialdehyde in blood serum. It can be hypothesized that the change in the activity of antioxidant enzymes was mediated by the biological role of zinc (Powell, 2000; Costa *et al*., 2023). It is known that this trace element is part of the structure of the key enzyme systems of the body, in particular, it participates in the functioning of the enzymatic link of antioxidant defense, being a structural component of the enzyme superoxide dismutase (Lee, 2018). In addition, Zn^{2+} ions induce the synthesis of metallothioneins, which are able to scavenge free (hydroxyl and superoxide) radicals in the body (Krężel and Maret, 2017). These results are consistent with studies by Chinese scientists, which showed that zinc supplementation in the diet of Japanese quail and broiler chickens improved the overall antioxidant capacity of birds and reduced the level of MDA compared to the control group (Mohammad Malyar *et al*., 2019). Researchers also note that probiotics have antioxidant potential and their inclusion in the diet may be a good strategy to improve the body's antioxidant status (Mishra *et al*., 2015; Yu *et al*., 2019). In experimental studies, it has been shown that probiotic microorganisms can help reduce MDA levels as well as increase overall antioxidant capacity (Ogbuewu *et al*., 2022; Elbaz *et al*., 2023; Musazadeh *et al*., 2023).

Conclusion

The study confirmed the positive effect of the combined use of probiotic preparation and chelate complex Zn-Gly in critical periods of development of broiler chickens on protein and lipid metabolism, on the formation of immune and antioxidant status of birds. More research to understand the mechanisms of action underlying these effects in broiler chickens is necessary.

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None.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contributions

All authors contributed to the study's conception and design. SL developed the original hypotheses, designed the experiments, collaborated in interpreting the results; OM collected the data for this study, conducted the statistical analyses, collaborated in the interpretation of the results; TK collaborated in interpreting the results, and finalized the manuscript. All authors have read and approved the finalized manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest related to this article.

Data availability

All data are provided in the manuscript.

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