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THE EFFECTIVENESS OF VARIOUS FORMS OF Zn AS STIMULATORS OF THE IMMUNE RESPONSE IN BROILER CHICKENS (*Gallus gallus* L.)

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Abstract

A significant problem of modern poultry farming is insufficient viability of broiler chickens due to various reasons, including immunosuppression. Various biologically active substances and trace elements are successfully used to increase the adaptive capacity and immunoreactivity of birds. The use of zinc (Zn) in feed additives which has immunotropic properties, stimulates immune and antioxidant systems, increases the productivity and safety of animals is of undoubted interest. Here, for the first time the influence of zinc from different sources on natural resistance and morphofunctional reorganization of immunocompetent organs of Smena 7 cross broiler chickens (*Gallus gallus* L.) was shown. These data also point to the advantage of organic and ultrafine dietary forms of zinc for chick feeding compared to the inorganic form. Our goal was to compare the effectiveness of using different forms of zinc in the diet as modulators of the immune system based on biochemical parameters and characterization of the microstructure of immunocompetent organs. Studies were performed on broiler chickens of Smena 7 cross (three groups, 24 animals in each group) in the vivarium of the Federal Research Center of Biological Systems and Agrotechnologies RAS (Orenburg). Sources of trace elements were asparaginate Zn (organic form, OF; LLC V-Min+, Sergiev Posad, Russia), mineral salts $ZnSO_4 \cdot 7H_2O$ (inorganic form, IF; Lenreaktiv, St. Petersburg, Russia) and powder of ultradispersed Zn particles (UDP Zn; LLC Advanced Powder Technologies, Tomsk, Russia). Chickens in the control group received a basic diet throughout the experiment, in which Zn was normalized by the introduction of $ZnSO_4 \cdot 7H_2O$. In the test groups from day 14 to day 42 Zn sulphate was replaced with UDP Zn at a dose of 49 mg/kg feed (group I) or with asparaginate Zn at the same dose (group II). Samples for analysis were collected after poultry slaughtering at 3, 4, 5, and 6 weeks of age. Biochemical studies of blood serum were performed on an automatic analyzer CS-T240 (DIRUI Industrial Co., Ltd., China) using commercial kits for veterinary research DiaVetTest (JSC Diakon-DS, Russia). Morphological composition was determined using an automatic hematological analyzer URIT-2900 Vet Plus (URIT Medial Electronic Co., Ltd., China). Iindices of natural resistance, i.e., the bactericidal activity of blood serum (BABS), lysozyme activity (AL), β-lysine activity (Aβ-L), and immunological indices, the phagocyte number (PN) and phagocytic index (PI) were evaluated. Morphological characteristics of cloacal bursa (CB), thymus and spleen were determined on 5-6 microns thick histological sections stained with hematoxylin and eosin. General structural changes were assessed on paraffin sections stained with hematoxylin and eosin using a light-optical microscope with MT 5300L software (Meiji Techno Co., Ltd., Japan). The area of the follicle and medullary region, the width of the cortical zone were determined in the CB sections; the area of red and white pulp, cell density of red and white pulp in the spleen; the area of cortical and brain substance, their ratio (cortical index), cell density of red and white pulp in the thymus. The area of structures was determined on 125,000 μm^2, density on 1 mm^2. At 3 weeks of age, chickens of group II showed a 37.5 % ($p \le 0.05$) increase in leukocytes compared to control. By 4 weeks of age, the index also increased by 40.7% ($p \le 0.05$) when UDP Zn was fed. The increase was due to lymphocytes and monocytes. In the test groups, by the end of the experiment, the number of leukocytes was lower than the control. All indicators of white blood cells were within the normal range. Introduction of UDP Zn contributed to an increase in BABS in the range from

5.8 % to 16.7 % and AL by 8.2 % in the later stages of the experiment compared to control. The tendency to an increase in Aβ-L was recorded at 5 weeks of age with a subsequent decrease by the end of the experiment. Application of OF Zn led to a statistically significant ($p \le 0.01$) increase in the activity of BABS by 13.4 % at 3-weeks of age and AL at the end of the experiment (by 8.8 %). The OF Zn provided a smooth progressive increase in Aβ-L throughout the experiment. Histological evaluation revealed that dietary OF Zn increased CB activity with an increase in lymphoid follicle area by 64.5 % ($p \le 0.01$) due to expansion of medulla zones, as well as increased cellular density of the cortical zone with a large number of macrophages and mitoses. In the thymus, together with a 20.9 % expansion of the medullary zone, there was a greater number of Hassall's corpuscles, thickening of cells in the cortical layer and proliferative activity of lymphocytes with activation of macrophages. In the spleen after the introduction of UDP Zn, enlargement of follicles by increasing the area of white pulp 2.2-fold ($p \le 0.05$) was noted. Thus, Smena 7 broiler chickens fed organic and ultradisperse Zn additives exhibit higher blood bactericidal activity and activity of lysozyme and β-lysines, which indicates the stimulation of natural resistance. The response of the immune system to Zn was also more pronounced for dietary UDP Zn and OF Zn compared to inorganic form.

Keywords: ultrafine particles, chickens, feeding, zinc, immune system, thymus, spleen, cloacal bursa, microstructure

In modern poultry farming, mortality among broiler chickens remains a problem which is due, in particular, to immunosuppression. The reasons may be imbalances in diets, physical inactivity, overcrowding, technological stress, as well as high antigen load during immunization. To increase adaptive capacity and immunoreactivity, in recent years biologically active substances have been successfully used, namely, vitamins [1], amino acids and trace elements [2]. Of undoubted interest is zinc as feed additive, which has immunotropic properties, stimulates the activity of the immune and antioxidant systems, and increases the productivity and safety of animals [3].

Feed additives contain Zn of different digestibility, i.e., inorganic, organic and chelated Zn sourses [4). Because elements in inorganic compounds can form insoluble forms that are not absorbed [5], the bioavailability of Zn is key to meeting the body's requirement for this element. Due to its low bioavailability, the inorganic form (IF) of Zn can cause a deficiency of the element in the body, which leads to a decrease in the number of T-cells, the production of interleukins and, as a consequence, inhibition of cellular and humoral immunity [6].

A promising approach may be the use of nanoforms of microelements [7, 8] which have unique properties that allow them to be competitive sources of chemical elements for mineral additives [9, 10]. A number of studies confirmes the high efficiency of metal nanoforms compared to inorganic salts and other sources [11-13].

The feasibility of using Zn in potentiating cell-mediated immune responces [14] as an inducer of phagocytosis and stimulator of a humoral response [15) has been shown. Zn is critical for the development and maintenance of immune function. The amount of Zn is a critical factor that influences antiviral immunity [16]. Therefore, Zn may be useful in immunotherapy as an additional immunoadjuvant. A search query using the keywords "zinc, immunity" in PubMed (https://pubmed.ncbi.nlm.nih.gov/) over the past 20 years yields more than 4900 references, which clearly demonstrates the diverse role of Zn in the functioning of the immune system. However, the effectiveness of Zn depends on many factors, including the form of the substance.

Here, we demonstrate for the first time the influence of different zinc sources on natural resistance and morphofunctional reorganization of immunocompetent organs of broiler chickens (*Gallus gallus* L.) Smena 7 cross. The sourses of organic and ultrafine zinc are superiior to the inorganic zinc.

This work aimes to compare the blood serum biochemical parameters and the microstructure of immunocompetent organs of Smena 7 broiler chickens in order to assess the effectiveness of various dietary zinc supplements as immunomodulators.

Materials and methods. Experiments were carried out in accordance with the recommendations of the Guide for the care and use of laboratory animals of National Research Council (US) Institute for Laboratory Animal Research (Guide for the care and use of laboratory animals. National Academies Press, Washington DC, 1996). All methods were approved by the Bioethics Commission of the Federal Scientific Center for Biological Systems and Agricultural Technologies RAS (protocol No. 3 of March 21, 2018).

The sources of microelements were Zn aspartate (organic form, OF; V-Min+ LLC, Sergiev Posad, Russia), mineral salts $ZnSO_4 \cdot 7H_2O$ (inorganic form, IF; Lenreaktiv, St. Petersburg, Russia) and powder ultrafine Zn particles (UFF Zn; OOO Advanced Powder Technologies, Tomsk, Russia). Zn UFFs were produced by electric explosion of a wire in an argon atmosphere. Certification of materials included electron scanning and transillumination microscopy using JSM 7401F and JEM-2000FX instruments (JEOL, Japan). X-ray phase analysis was performed on a DRON-7 diffractometer (NPO Burevestnik, Russia). According to the certification results, the particles had a hydrodynamic radius of 164±31.2 nm, Z-potential was 25±0.5 mV, specific surface area 5±1.6 m^2/g. To obtain lyosols, aqueous suspensions of Zn UFF were treated in an ultrasonic dispersant UZDN-2T (NPP Academpribor, Russia) at 35 kHz, 300/450 W, 10 μA for 30 min. The resulting lyosols were stepwise-mixed with the feed.

Broiler chickens of the Smena 7 cross (n = 72) were kept in the vivarium of the Federal Scientific Center for Biological Systems and Agricultural Technologies RAS (Orenburg). At the age of 2 weeks, birds analogues by weight were assigned to three treatments (one group for control feeding and two groups for test feeding, n = 24 each). Feeding was carried out according to recommendations for age periods [17].

Chickens of the control group throughout the experiment received a basal diet in which Zn was normalized by introducing $ZnSO_4 \cdot 7H_2O$. In the test groups, from day 14 to day 42, Zn sulfate was replaced with Zn UDP at 49 mg/kg of feed (group I) or with Zn aspartate at the same dosage (group II). The dosage was based on the recommendations [17], however, given the bioavailability of elements from organic and ultrafine sourses, the values were reduced by 30%.

At the age of 3, 4, 5 and 6 weeks, 6 chickens from each group were killed. Blood was sampled from the axillary vein. Biochemical studies of blood serum were comducted in accordance with the manufacturers' protocols (a CS-T240 automatic analyzer, DIRUI Industrial Co., Ltd, China; commercial kits for veterinary research DiaVetTest, AO Diakon-DS, Russia). The blood morphology was assessed using an automatic hematology analyzer URIT-2900 Vet Plus (URIT Medial Electronic Co., Ltd, China). Serum bactericidal activity (BABS, bactericidal activity of blood serum), lysozyme activity (AL), activity of β-lysines (Lβ-A), and immunological parameters, the phagocyte number (PN) and phagocytic index (PI) were measured as described [18].

After slaughter at the age of 42 days, tissue samples for morphological analysis of the cloacal bursa (CB), thymus, and spleen were obtained. The samples were fixed in 10% neutral formalin and embedded in the HistoMix paraffin mixture (BioVitrum, Russia). Histological sections 5-6 μm thick were prepared on a semi-automatic microtome MVP 01 (KB Technom, Russia) and stained with hematoxylin and eosin [19]. General structural changes were examined by light optical microscopy with MT 5300L software (Meiji Techno Co., Ltd, Japan).

On the CB sections, the follicle and medullary zone areas and the cortical zone width were measured, in the spleen, the ares of the red and white pulp and the density of red and white pulp cells were measured; in the thymus, the area of the cortex and medulla, their ratio (cortical index), and the density of red and white pulp cells were measured. The areas of the structures were determined using a 125,000 μm^2 counting grid, the density was assessed for a 1 mm^2 area. Laboratory tests were conducted at the Center for Collective Use of Biological Systems and Agricultural Technologies RAS (CCU BST RAS).

Statistica 10.0 software package (StatSoft, Inc., USA) and Microsoft Excel were used for statistical data processing. The mean values of the parameters (*M*) and standard errors of the means (±SEM) were calculated. Differences between groups were assessed by Student's *t*-test. If the distribution differed from normal, the Mann-Whitney U test was applied. Differences were considered significant at $p \leq 0.05$.

Results. At 3 weeks of age, when Zn was added (group II), chickens showed an increase in the number of blood leukocytes by 37.5% ($p \leq 0.05$) compared to the control. By 4 weeks of age, the number of leukocytes also increased by 40.7% ($p \leq 0.05$) in poultry fed UDP Zn. The increase occurred due to lymphocytes and monocytes. Thus, at 3 weeks of age, the difference in the number of lymphocytes was 10.3% for group I and 8.7% for group II. The counts of monocytes differed significantly ($p \leq 0.05$) only in favor of group II at 4 weeks of age (Table 1). A similar increase in the number of blood lymphocytes is observed with increased immunoreactivity of the birds, since lymphocytes serve as the main executive link in the cellular and humoral defense of the body. An increase in the number of monocytes when using OF Zn may indicate activation of the monocyte-phagocytic system and, consequently, an increase in the protective properties of broiler chickens [20]. By the end of the experiment, the counts of leukocytes in the test groups were lower than the control. All white blood cell parameters were within the normative values.

1. Counts of the blood white cells in broiler chicken (*Gallus gallus* L.) cross Smena 7 of different ages when fed various forms of Zn (*n* = 6, *M*±SEM, the vivarium of the Federal Scientific Center for Biological Systems and Agrotechnologies RAS, Orenburg, 2020)

Group	Age, weeks			
	3	4	5	6
Leukocytes, ×10^9/l (norm 20.0-40.0)				
Control (IF)	18.45±1.850	24.30±1.921	26.33±2.998	35.73±1.307
I test (UDP)	22.30±1.188	34.20±2.112*	25.83±0.581	27.27±1.946*
II test (OF)	25.37±1.159*	27.33±1.700	28.60±1.517	30.90±1.361
Lymphocytes, % (norm 52-70)				
Control (IF)	79.55±4.950	85.70±1.343	89.17±1.281	88.70±1.102
I test (UDP)	87.80±1.686*	87.93±2.331	87.37±1.235	89.47±1.674
II test (OF)	86.53±1.317*	90.23±1.441*	88.27±1.906	89.07±1.667
Monocytes, % (norm 4,0-10,0)				
Control (IF)	5.30±0.900	6.67±0.176	6.20±0.529	6.33±0.491
I test (UDP)	6.80±0.436	6.63±0.996	6.70±0.436	6.03±0.203
II test (OF)	6.20±0.721	7.60±0.173*	6.33±0.393	6.23±0.120

Note. IF — inorganic form, UDP — ultra-dispedsed partiles, OF — organic form. For a description of the groups, see the Materials and methods section.
* Differences from control are statistically significant at $p \leq 0.05$.

Administration of the organic Zn (group II) led to a statistically significant ($p \leq 0.05$) increase in BABS at 3 weeks of age by 13.4% and at the end of the experiment by 10.1% compared to the control. The introduction of UFF Zn (group I) contributed to an increase in BABS in the later stages of the experiment: at 4 weeks of age by 16.7% ($p \leq 0.01$), at 5 weeks of age by 14.6% ($p \leq 0.01$), by the end of the experiment the difference was 5.8%.

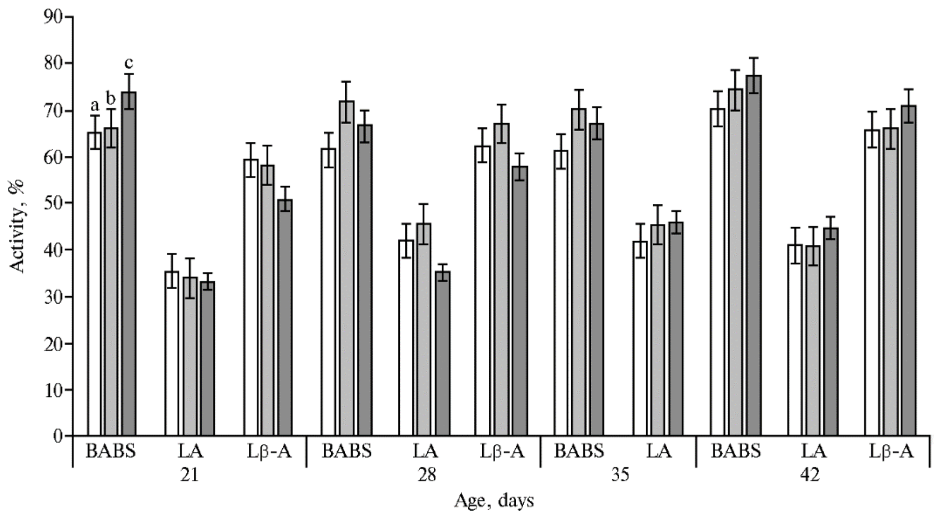

Fig. 1. Age-dependent parameters of nonspecific resistance of broiler chickens (*Gallus gallus* L.) cross Smena 7 fed different forms of Zn: BABS — bactericidal activity of blood serum, LA — lysozyme activity, Lβ-A — activity of β-lysines; a — control, b — test group I, c — test group II (n = 6, M±SEM, the vivarium of the Federal Scientific Center for Biological Systems and Agrotechnologies RAS, Orenburg, 2020). For a description of the groups, see the Materials and methods section.
*, ** Differences from control are statistically significant at p ≤ 0.05 and p ≤ 0.01.

Some white blood cells, in particular monocytes, serve as producers of nonspecific body defense factors, including lysozyme [21]. In chickens fed UDP Zn, serum AL increased at 4 weeks of age by 8.5% (p ≤ 0.05), at 5 weeks of age by 8.2% (p ≤ 0.05) compared to the control. Organic Zn added to the diet increased AL by 10.0% (p ≤ 0.05) and 8.8% (p ≤ 0.05) at 5 weeks and 6 weeks of age, respectively (Fig. 1). An increased blood lysozyme activity in birds of different ages contributes to an increase in their nonspecific resistance.

Various forms of Zn also influenced the activity of β-lysines. In birds fed UDP Zn, this parameter increased by 16.1% (p ≤ 0.05) at 5 weeks of age followed by a decrease towards the end of the experiment. OF Zn provided a smooth increase in activity throughout the experiment (see Fig. 1). Being an important serum bactericidal systems, β-lysines are thermostable and selective against gram-positive bacteria. β-Lysine is a cationic protein of platelet origin [22]. An increased activity of β-lysines in the test groups may indicate a higer bird's resistance to gram-positive microorganisms [23].

The effect of Zn on the defense response was realized through an increase in AL, activity of β-lysines and, therefore, in bactericidal activity of blood serum as an integral indicator of humoral resistance which indicates the ability of the blood to self-purify. A decrease in serum bactericidal activity is more often than its increase, which is typical mainly for various stressful situations, violations of feeding and housing conditions, and in diseases [24]. Organic and UDP Zn stimulated serum bactericidal activity to a greater extent than inorganic Zn.

Zn contributed to an increase in phagocytic activity, expressed as PN. In group I, PN increased by 10.4 (p ≤ 0.05) and 19.5% (p ≤ 0.05) vs. control at 3 and 4 weeks of age, respectively. In group II, PN exceeded control by 8.6% (p ≤ 0.05) at 4 and 5 weeks of age (Fig. 2).

The intensity of phagocytosis (PI) in test group I exceeded control values at 4 weeks of age by 19.1% (p ≤ 0.05), and decreased by the end of the experiment. In group II, the differences vs. control were 16.6% at 3 weeks of age (p ≤ 0.05), and by the end of the experiment, PI decreased by 12.2% vs. control (see Fig. 2).

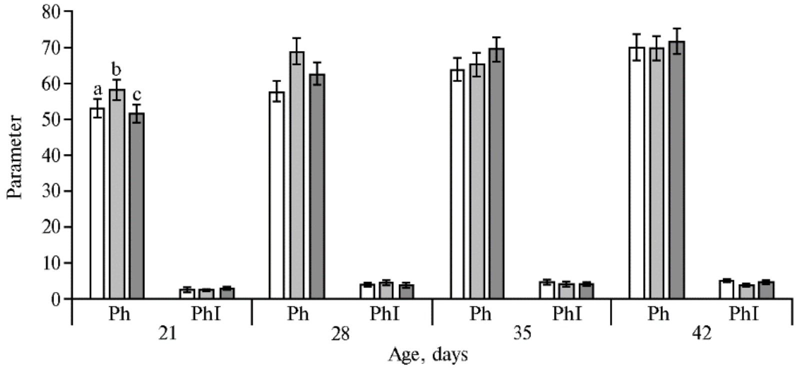

Fig. 2. Age-dependent blood phagocytic activity in broiler chickens (*Gallus gallus* L.) cross Smena 7 fed different forms of Zn: Ph — the number of phagocytes (PN) involved into phagocytosis, %, PhI — phagocytic index (PI); a — control, b — test group I, c — test group II (n = 6, M±SEM, the vivarium of the Federal Scientific Center for Biological Systems and Agrotechnologies RAS, Orenburg, 2020). For a description of the groups, see the Materials and methods section.
* Differences from control are statistically significant at $p \leq 0.05$.

Along with factors of nonspecific resistance, the histostructure of immunocompetent organs is informative to evaluate cause-and-effect relationships and the functioning of the immune system. Since CB plays a key role in the formation of poultry immunity, the study of its morphofunctional characteristics in connection with the search for effective methods to optimize mineral nutrition is among the most important tasks.

In chickens of group I, the microstructure of the CB (Fig. 3, A) differed from the control (see Fig. 3, B) in the moderate enlargement of follicles by 15.4% ($p \leq 0.05$) (Table 2) with an increase in the cortical zone width. There were numerous mitoses in the lymphoid cells of the cortical zone. In the cells of the glandular epithelium, small vacuoles were visible, elongated nuclei were located basally, and moderate focal proliferation occurred. Small cysts were detected.

The general histological structure of CB in experimental group II differed from the control by an increase in the area of the follicles by 64.5% ($p \leq 0.01$), mainly due to an increase in the area of their medullary zone (up to 82.7%) (see Table 2), as well as a more dense population of cells in both zones. Small cysts were found in individual follicles. In the narrow cortical zone, a large number of eosinophilic granulocytes were noted (see Fig. 3, B). Among the lymphoid cells, many macrophages and mitotically active cells were identified. The medullary zone was wide. An increase in the area of the follicular apparatus and a decrease in the connective tissue of the CB stroma indicated the activation of the reaction and, as a consequence, an increase in the adaptive reserve. It is known that Zn can improve the productivity and histomorphology of immune organs [25].

The spleen of mammals is primarily considered as a blood depot, while the spleen of birds serves exclusively as an immunocompetent organ [26]. In spleen of chickens from group I, we revealed functional activation in the white pulp microstructure, namely, the enlargement of follicles, an increase in their number, and a 2.3 times more dense arrangement of cells vs. control ($p \leq 0.001$) with a predominance of mature lymphocytes, lymphoblasts, plasma and macrophage cells. The area of the white pulp increased by 2.2 times ($p \leq 0.05$) (see Table 2). On the contrary, the activity of the red pulp decreased, as indicated by the presence of zones with depletion of cellular contents and exposure of the organ stroma (see Fig. 3, D). Macrophages were identified in large numbers among the predominant lymphoid cells of the pulp. The content of lymphoblasts and plasma cells decreased markedly.

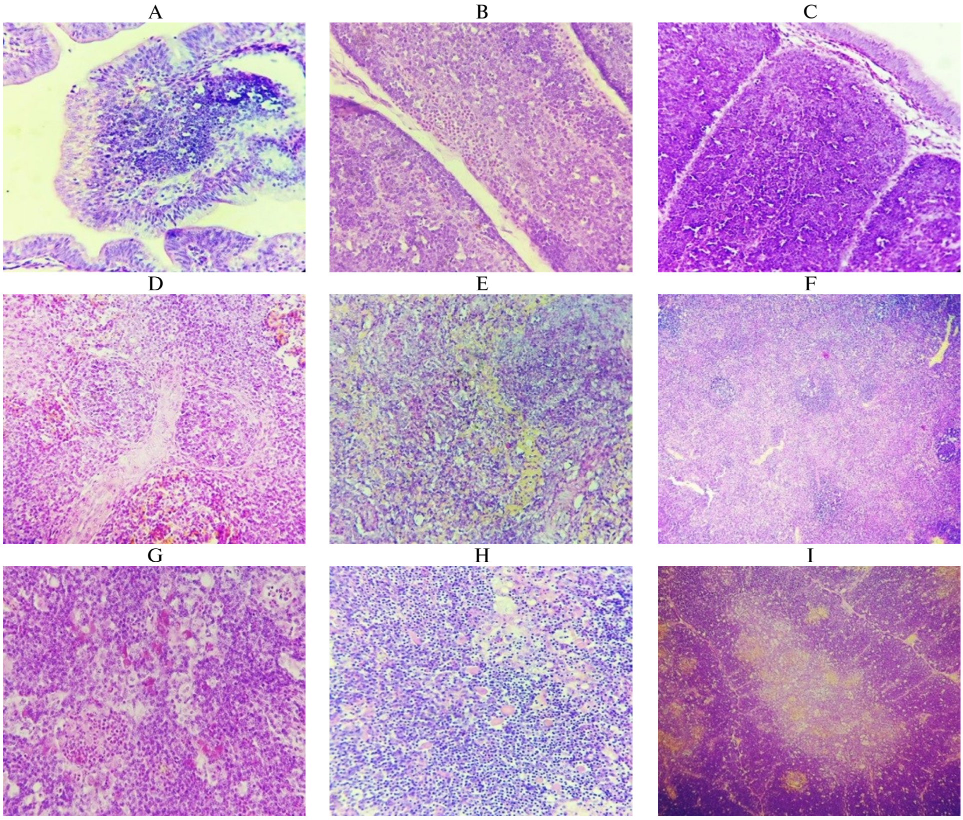

Fig. 3. Microstructure of the cloacal bursa (A, B, C)**, spleen** (D, E, F) **and thymus** (G, H, I) **in 42-day-old broiler chickens (*Gallus gallus* L.) cross Smena 7 fed different forms of Zn** (the end of the experiment)**:** A, D, G — test group I (A — increased epithelial proliferation, ×100; D — clear boundaries of the follicles, plethora of the red pulp with areas of cellular sparseness, ×400; G — cortical layer with accumulation of macrophages, eosinophilic granulocytes, ×400); B, E, H — test group II (B — wide medullary zone, a large number of eosinophilic granulocytes in a narrow cortical zone, ×400; E — hyperplasia of lymphoid tissue without clear boundaries with red pulp, ×400; H — a large number of lymphoid cells and Hassall bodies, ×400); C, F, I — control group (magnification ×100) (the vivarium of the Federal Scientific Center for Biological Systems and Agrotechnologies RAS, Orenburg, 2020). Hematoxylin and eosin staining, a MT 5300L microscope (Meiji Techno Co., Ltd, Japan).

The morphofunctional state of the spleen in chickens from test group II (see Fig. 3, E) indicated a higher activity of both zones of the parenchyma compared to the control (see Fig. 3, F). The area of the white pulp increased 1.7 times ($p \le 0.05$). The density of follicle cells increased by 70.5% ($p \le 0.001$) compared to the control; macrophage cells and immature forms of lymphocytes were present among them (see Table 2). In the red pulp, general plethora and increased cellular density were noted. Given active participation of the spleen in immune responses, reactive structural changes in this organ are natural [25]. In our experiment, similar changes occurred to a greater extent when chickens were fed organic Zn.

In the thymus histostructure of chickens from group I (see Fig. 3, G) vs. control (see Fig. 3, I), the area of the medullary zone was 20.9% larger ($p \le 0.05$) and the cell density was higher in both the cortex and medulla by 31.4 ($p \le 0.001$) and 37.5% ($p \le 0.01$), respectively. In the cortical layer, there was a significant number of elements of the macrophage system with visible minimal degenerative changes, forming small clusters. Signs of proliferative processes in the lymphatic cells of the cortical zone were noticeable. The medullary zone was characterized by a small number of small-sized Hassall bodies.

2. Morphometric parameters of the cloacal bursa, spleen and thymus microstructures in 42-day-old broiler chickens (*Gallus gallus* L.) cross Smena 7 fed different forms of Zn (n = 6, M±SEM, the vivarium of the Federal Scientific Center for Biological Systems and Agrotechnologies RAS, Orenburg, 2020)

Parameter	Group		
	control (IF)	test I (UDP)	test II (OF)
Cloacal bursa			
Follicle area, mm^2	0,039±0,0010	0,045±0,0026	0,063±0,0018**
Area of the medullary zone of the follicle, mm^2	0,029±0,0008	0,032±0,0015*	0,053±0,0019**
Proportion of the medullary zone in the follicle, %	74,7±1,32	70,9±0,99	83,6±1,69
Width of the cortical zone of the follicle, μm	34,16±1,081	57,00±0,950***	32,50±0,510
Spleen			
Red pulp area, mm2	0,175±0,0121	0,089±0,0061*	0,118±0,0081
White pulp area, mm2	0,075±0,0081	0,162±0,0080*	0,133±0,0082*
Red pulp cell density	2051,2±56,10	1856,0±49,23	2329,6±62,83
White pulp cell density	1865,6±52,33	4201,6±87,21***	3180,8±54,63***
Thymus			
Area of the cortical zone, mm^2	0,665±0,0281	0,545±0,0113*	0,626±0,0126
Brain zone area, mm^2	0,583±0,0184	0,705±0,0158*	0,624±0,0191
Cortical index	1,14±0,093	0,77±0,019	1,00±0,030
Cortical cell density	2808,0±59,21	3690,7±51,73***	4805,3±65,01***
Medulla cell density	1749,3±44,91	2405,3±50,13**	2728,0±60,63**

Note. IF — inorganic form, UDP — ultra-disperded particles, OF — organic form. For a description of the groups, see the Materials and methods section.
*, **, *** Differences from control are statistically significant at p ≤ 0,05, p ≤ 0.01, and p ≤ 0.001

The histostructure of the thymus in group II (see Fig. 3, H) turned out to be generally similar to that for group I. There was a higher immunological activity of the thymus, expressed in a larger medullary zone, a higher cell density in the cortical and medullary zones, by 71.9 ($p \le 0.001$) and 55.9% ($p \le 0.001$) (see Table 2), respectively, and the number of mitotic cells was higher compared to the control.

The described effects could be the result of good absorption of Zn from OF and UDP in the intestines of broiler chickens. In addition, apparently, OF Zn better overcomes antagonistic and other inhibitory factors of the digestive system, therefore the boundaries of the absorption fund of the element expand [27].

The works of other authors have shown that dietary correction with Zn [28, 29] and other substances, pre- and probiotics [30], butyric acid [31], sodium butyrate [32] leads to changes in the microarchitecture of lymphoid organs and the intestinal wall: the length increases and the surface area of the villi, the number of goblet cells.

Since the ingredient composition of the diet in our experiment was the same in all groups, except for different Zn sourses, we assume that the immunostimulation in the test groups compared to the control resulted from structural changes in the organs of the immune system, the spleen, thymus and CB.

Dietary Zn shows good absorption in the intestines and, influencing the immune system, activates a number of mechanisms. In general, these mechanisms are similar for many trace elements and involve interactions with specific receptors, the influence on phagocytosis, apoptosis, chemotaxis, adhesion, on the activity of enzymes, hormones, on carrier proteins, and on enhanced production of immunoglobulins. Zn^{2+} cations can stimulate the proliferation of cells of immunocompetent organs, in particular the spleen [15, 33]. Zn potentiates the body's cell-mediated defense responses to bacteria and viruses [14].

Thus, dietary organic (OF) and ultradispersed (UDP) Zn contribure to higher bactericidal activity of blood serum, activity of lysozyme and β-lysines, thereby stimulating the natural resistance of the Smena 7 broiler chickens. We did not reveal pathological changes of white blood. The tendency to increased numbers of leukocytes, lymphocytes and monocytes at the age of 3-4 weeks was noted to a greater extent in broilers fed dietary organic Zn. However, UDP Zn contributed to an increase in the bactericidal activity of blood serum in the range from 5.8 to 16.7% and AL by 8.2% at later stages of the experiment compared to the control. A trend towards an increase in β-lysine activity was recorded at 5 weeks of age, followed by a decrease towards the end of the experiment. The use of the organic Zn led to a statistically significant ($p \leq 0.01$) increase in the bactericidal activity of blood serum by 13.4% at 3 weeks of age and of lysozyme by 8.8% at the end of the experiment. Organic Zn provided a smooth progressive increase in activity of β-lysine throughout the experiment. Histological evaluation revelaed that the organic Zn provided functional activation of the cloacal bursa with an increase in the area of lymphoid follicles by 64.5% ($p \leq 0.01$) due to the expansion of the medullary zones and to an increase in the cellular density of the cortical zone with a large number of macrophages and mitoses. In the thymus, along with an expansion of the medullary zone by 20.9%, there were a greater number of Hassall bodies, compaction of cells of the cortical layer and proliferative activity of lymphocytes with activation of macrophages. In the spleen, upon administration of UDP Zn, we noted enlargement of follicles due to an increase in the area of the white pulp by 2.2 times ($p \leq 0.05$). In general, it can be argued that the immune response to various sourses of dietary zinc was more pronounced for UDP Zn and organic Zn compared to inorganic Zn.

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