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# Efficiency and safety of using different sources of zinc in poultry nutrition

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Abstract. Full and balanced diet of poultry with high-quality feeds and availability of minerals is the key to high safety and productivity of animals. The needs of animals for minerals can be met through various forms of compounds: inorganic, organic, chelated and nanoscale. The aim of research was a comparative assessment of influence of various forms of zinc on the productivity and morpho-biochemical parameters of blood of broiler chickens. Replacing the inorganic form of zinc with nanoscale (group I) and organic form (group II) leads to a positive productive effect by the end of the experiment, by 4.6 and 11.1 %, respectively, compared to the control. At the same time, a short-term increase in the number of leukocytes at 28 days of age was observed after feeding with nanoZn (group I) by 40 % (P≤0.05) and by 12.4 % when using its organic form (group II). The concentration of hemoglobin and the number of red blood cells tended to increase in the experimental groups compared with the control at 21, 28 and 35 days of age. The use of various forms of zinc affects the state of biochemical indicators of metabolism in the body, leading to different growth rates of animals. The study showed that it is possible to improve broiler productivity by completely replacing the inorganic form of zinc with an organic or nanoscale one. The obtained result shows the possibility of using these forms in the industrial production of animal feed.

# **1. Introduction**

Poultry farming is one of the fastest growing sectors of agriculture, which holds the primacy in meeting the needs of the population in meat and its processed products. The consumer market has seen a steady upward trend in demand for poultry meat. A full and balanced diet of poultry with highquality feeds, the availability of minerals is the key to high safety and productivity of animals. The problem of correction of animal diets by mineral composition is devoted to more than one thousand works. The dependence of meat productivity of animals on mineral composition of consumed feed has been proved, which in turn affects the economic efficiency of the industry [1]. The animal needs for minerals are met through various kinds of compounds. Inorganic salts are used as classical (main) sources of essential chemical elements. A number of researchers are positioning the use of organic [2, 3] and chelate forms [4, 5].

In the last decade, the interest of researchers to nanoscale forms of metals has increased [6]. Indeed, the rapid development of nanotechnology and the synthesis of nanoscale substances with different properties allows us to solve a number of tasks [7–9]. Thus, a positive productive growth of

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chickens was shown with the use of chromium nanoparticles [10], the relationship between the physicochemical characteristics of the nanoparticles and the observed effect [11], and the potential for combined use with amino acids [12] are presented.

The aim of the research was a comparative assessment of influence of various forms of zinc on the productivity and morphological and biochemical parameters of blood of broiler chickens.

### 2. Materials and methods

The experiments were performed according to the recommendations of the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996). All of the experimental methods and techniques were approved by the Committee on Ethics of the Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences (protocol #3 of March 21, 2018).

During the study, the sources of trace elements were asparaginate of Zn from LLC V-Min+ (SergievPosad, Russia), mineral salts  $ZnSO_4 \times 7H_2O$  from Lenreactive (Saint Petersburg, Russia), and nanopowder of Zn (nanoZn) made by LLC Advanced powder technologies, (Tomsk, Russia).

Ninety 1-d-old chickens were taken for the experiment. The chickens that received unique numbers (plastic tags on the legs) were weighed and then kept in the same conditions. At the age of 2wk, based on individual daily weighing and on the feed consumption, 3 groups were formed using the method of analog pairs: one reference and 2 experimental groups (n = 24).

The chickens were fed on complete combined feed made with consideration of recommendations [13] in accordance with the age periods. The main diet of the chickens from 28 to 42 d of age included the following ingredients (g/kg, as fed basis): wheat grain, 475; barley grain, 30; maize, 80; soybean meal, 250; sunflower meal, 70; sunflower oil, 50; premix made with consideration of the existing recommendations, 20; sodium salt, 3.4; mono calcium phosphate, 13; limestone flour, 5; DL-methionine 98.5 %, 1.6; monochlorohydrin lysine 98 %, 1; wheat grain, 435; corn, 226; soybean meal, 150; sunflower meal, 100; sunflower oil, 50; premix, 20; salt, 3; mono calcium phosphate, 10.5; limestone flour, 1; DL-methionine 98.5 %, 1.2; monochlorohydrin lysine 98 %, 2.3; and baking soda, 1.

The chickens in the reference group throughout the experiment received the main diet, in which Zn were normalized by the introduction of sulfates  $ZnSO_4 \cdot 7H_2O$  into the composition of the premix, which contained all normalized trace elements. In the premix for the chickens in the experimental groups, in the period from 14 and 42 d of age, sulphates of Zn were replaced in group 1 with nanoZn of feed, and in group 2 with asparaginates of Zn. Chickens in all groups were watered with distilled water. The approach to choosing the dosage was determined by recommendations [13], however, with regard to the information about bioavailability of elements from the test forms, the parameters were reduced by 30 %.

Chickens' blood samples were taken in the morning from fasted chickens before slaughtering at the age of 21, 28, 35, and 42 d from the axillary vein for assessing the biochemical parameters into vacuum tubes with coagulation activator (thrombin). The serum was studied no later than 3h after sampling.

Biochemical studies of the blood serum were performed using an automated analyzer CS-T240 (DIRUI Industrial Co., Ltd, China) with the use of commercial kits for veterinary studies DiaVetTest (Russia) and Randox Laboratories Limited (United Kingdom) (in accordance with the manufacturer's protocols).

For morphological studies at the light-optical level, liver pieces were fixed in 10 % neutral formalin and embedded in a HistoMix paraffin mixture (BioVitrum, Russia). The morphological characteristics of liver tissue samples were evaluated on histological sections of  $5-6 \mu m$  thick, made on the semiautomatic microtome MWP 01 (Tekhnom, Russia) and stained with hematoxylin and eosin.

The elemental composition of the organs and tissues was determined by atomic emission and mass spectrometry using mass spectrometer Elan 9000 and atomic emission spectrometer Optima 2000V (Perkin Elmer, USA). Ashing of biological substrates was performed with the use of microwave system of decomposition Multiwave-3000 (Anton Paar, Austria). Laboratory tests were performed at

the Test Center (accreditation certificate RA. RU.21PF59 dated 02.12.15) issued by the Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences.

Data are expressed as mean values  $\pm$  standard error of the mean ( $M \pm m$ ). Statistical analysis was performed using Statistica 10.0 (StatSoft Inc., USA) and Microsoft Excel (Microsoft, USA). Significance of the group differences was estimated using Student's *t*-test with  $P \leq 0.05$  considered as significant.

# 3. Results

#### 3.1. Feed consumption, bodyweight

The introduction of various forms of zinc into the diet had an impact on productivity. Therefore, against the background of taking nanoZn, animals improved their live weight by the end of the experiment by 4.6% ( $P \le 0.05$ ) compared with the control that received zinc in the form of sulfate. Feeding with organic form of zinc led to a more significant difference – 11.1 % compared with the control. Thus, broiler chickens showed a productive effect in all groups, but with different intensities (Table 1).

Age,days	Control	Group I	Group II
7	140.4±2.3	139.8±2.1	139.7±2.0
14	231.0±5.4	234.5±5.6	233.0±4.9
21	602.3±15.1	600.3±15.9	607.1±14.8
28	1 146.2±26.6	1 160.2±19.9*	1 196.6±33.7
35	1 809.0±26.4	1 803.3±22.8	1 824.3±27.2
42	2 270.0±32.6	2 376.0±46.7*	2 522.6±41.1*

Table 1. Live weight of broiler chickens, g

Significant difference in relation to control: \*  $P \le 0.05$ 

#### 3.2. Morpho-biochemical indicators of blood

Indicators of the morphological composition of blood of broiler chickens were within the physiological norm (table 2). However, we noted a short-term increase in the number of leukocytes at 28 days of age on the background of feeding with nanoZn by 40 % (P $\leq$ 0.05) and by 12.4 % after using its organic form.

**Table 2.** Indicators of morphological indicators in blood of broiler chickens in the experiment (mean values  $\pm$  standard error of the mean,  $M \pm m$ ) (n = 6).

Age, days				
	21	28	35	42
		Leukocytes, 10 <sup>9</sup> /l		
Group I	22.30±1.188	34.20±1.112*	25.83±0.581	27.27±1.946*
Group II	25.37±1.159	27.33±1.700	28.60±1.517	30.90±1.361
Control	$18.45 \pm 1.850$	24.30±1.921	26.33±2.998	35.73±1.307
Erythrocytes, 10 <sup>12</sup> /l				
Group I	1.97±0.124	2.55±0.069*	$2.14\pm0.132$	2.42±0.095
Group II	$1.94\pm0.075$	2.13±0.092	$2.25 \pm 0.085$	2.37±0.060
Control	1.41±0.635	$2.16 \pm 0.081$	$2.26 \pm 0.145$	2.55±0.030
		Hemoglobin, g/l		
Group I	122.33±5.696	136.00±7.211	133.33±2.963	140.67±4.333
Group II	120.67±2.667	125.33±2.404	133.33±4.256	139.67±2.906
Control	$97.50 \pm 28.500$	$125.00 \pm 5.000$	$132.00 \pm 6.028$	149.33±2.186
Hematocrit, %				
Group I	24.27±1.037	30.47±0.869*	25.13±1.342	28.93±0.817
Group II	24.57±0.418	25.50±0.551	26.23±1.120	28.37±0.533
Control	18.05±0.150	25.10±0.529	25.47±1.017	30.17±0.467

Significant difference in relation to control: \*  $P \le 0.05$ , \*\*  $P \le 0.01$ .

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At the end of the reference period, the number of leukocytes in blood of control chickens was higher among all groups and approached UNL. The concentration of hemoglobin and the number of red blood cells tended to increase in the experimental groups at 21, 28 and 35 days of age compared with the control.

The morphological picture of blood indicates the absence of a negative effect on health status of broiler chickens in the estimated forms of trace elements.

Age, days				
21	28	35	42	
	ALT, U/I			
2.40±0.166	1.13±0.043	$1.80\pm0.098$	3.40±0.164	
2.43±0.134	1.87±0.093	2.13±0.135*	4.13±0.167*	
3.35±0.150	1.30±0.189	$1.97\pm0.198$	2.83±0.189	
	AST, U/I			
258.8±9.81	228.8±5.83	234.5±3.51	309.0±9.36	
242.2±7.38	241.3±15.20	239.2±10.48	289.1±18.19	
252.2±10.80	225.9±6.03	241.4±10.64	299.9±13.41	
	LDG, U/I			
3183.0±189.84	3337.0±234.13	3320.3±210.65*	3697.3±153.00	
3313.0±212.43	3431.3±206.25*	3177.3±239.51*	2738.6±149.09	
3851.0±134.00	2512.0±171.19	2444.3±135.87	3252.0±273.81	
	GGT, U/I			
15.00±1.887*	24.00±1.245*	18.67±1.333	17.67±1.202	
$15.33 \pm 0.848$	14.67±1.202	20.67±1.186	20.00±0.577	
28.50±1.500	14.67±0.882	20.00±1.646	19.33±2.404	
	$\begin{array}{c} 2.40 \pm 0.166\\ 2.43 \pm 0.134\\ 3.35 \pm 0.150\\ 258.8 \pm 9.81\\ 242.2 \pm 7.38\\ 252.2 \pm 10.80\\ 3183.0 \pm 189.84\\ 3313.0 \pm 212.43\\ 3851.0 \pm 134.00\\ 15.00 \pm 1.887 \ast\\ 15.33 \pm 0.848\\ \end{array}$	$\begin{array}{c cccccc} 21 & 28 \\ & & & & \\ & & & \\ \hline & & & \\ \hline & & & \\ 2.40\pm0.166 & 1.13\pm0.043 \\ 2.43\pm0.134 & 1.87\pm0.093 \\ 3.35\pm0.150 & 1.30\pm0.189 \\ & & & \\ & & & \\ & & & \\ \hline & & & \\ 258.8\pm9.81 & 228.8\pm5.83 \\ 242.2\pm7.38 & 241.3\pm15.20 \\ 252.2\pm10.80 & 225.9\pm6.03 \\ & & & \\ \hline & & & \\ \hline & & & \\ 252.2\pm10.80 & 225.9\pm6.03 \\ & & & \\ \hline & & & \\ \hline & & & \\ 13183.0\pm189.84 & 3337.0\pm234.13 \\ 3313.0\pm212.43 & 3431.3\pm206.25* \\ 3851.0\pm134.00 & 2512.0\pm171.19 \\ & & \\ \hline & & & \\ \hline & & & \\ \hline & & & \\ 15.00\pm1.887^* & 24.00\pm1.245^* \\ 15.33\pm0.848 & 14.67\pm1.202 \\ \end{array}$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	

<b>Table 3.</b> Biochemical indicators of blood serum of the broiler chickens in the experiment (mean
values $\pm$ standard error of the mean, $M \pm m$ ) ( $n = 6$ ).

Significant difference in relation to control: \*  $P \le 0.05$ , \*\*  $P \le 0.01$ .

Against the background of taking the organic form of zinc (group II), ALT activity significantly (P $\leq$ 0.05) increased on days 35 and 42 on 8.12 and 45.9 %, respectively, compared with the control. Moreover, AST activity does not significantly change.

According to the dynamics of LDH, animals of the experimental groups I and II reliably outperform the control, starting from 28 days old by 32.8 % (P  $\leq$  0.05) and 36.6 % (P  $\leq$  0.05), respectively. On day 35, a significant (P  $\leq$  0.05) difference in this indicator was 35.8 % and 29.9 %. On day 42, the value exceeding the control (by 13.6 %) is retained only when nanoZn is taken (group I). In the II experimental group, LDH activity decreases by 15.7 % compared to the control.

The dynamics of GGT activity during feeding nanoZn (group I) has a wave-like character. So, in the first week of the experiment, it decreases by 47.3 % (P  $\leq 0.05$ ). Then, during the experiment (28 days), it increases by 63.5 % (P  $\leq 0.05$ ) and approaches the control figures towards the end of the experiment. When feeding the organic form of zinc (group II), the activity of GGT is close to the control figures.

The dynamics of GGT activity during feeding with nanoZn (group I) has a wave-like character. So, in the first week of the experiment, it decreases by 47.3 % (P $\leq$  0.05). Then, during the experiment (28 days), it increases by 63.5 % (P  $\leq$  0.05) and approaches the control figures towards the end of the experiment. When feeding the organic form of zinc (group II), the activity of GGT is close to control figures.

# 4. Discussion

The good growth performance of broiler chickens receiving organic and nanoscale forms of zinc may be due to the excellent availability of Zn from these sources. In addition, a decrease or absence of the antagonistic effect between divalent metals, which can be leveled by the form of the introduced substance [14], will provide a more complete satisfaction of the body's need for zinc when feeding with nano and organic forms.

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Another reason may be increased nutrient utilization. Thus, the use of zinc-bearing zeolite clinoptilolite modulates the activity of digestive enzymes, as well as structural activity and function of intestinal mucosa, which generally leads to the efficient use of food [15]. In addition, nanoparticles can bypass the usual physiological pathways of the distribution and transport of nutrients through tissues and cell membranes, as well as protect compounds from destruction to achieve their goals [16]. The form of zinc supplementation can affect the expression of zinc transporter genes [17]. Moreover, the effect can be associated with the release of  $Zn^{2+}$  ions and the accumulation of this element in organs in ionic form, rather than in the form of particles [18]. Zinc, in the composition of nanoparticles, has antioxidant and anti-stress properties [19], affects the intestinal microbiome of broiler chickens [20].

The results of our studies showed that blood enzymes are one of the fast-responding links to the action of various forms of zinc in biochemical homeostasis.

Aminotransferases, as enzymes that catalyze the reversible transamination reactions involved in nitrogen metabolism, are an indicator of the usefulness of protein nutrition [21]. Increased ALT activity indicates a change in metabolic fluxes in the experimental groups compared to the control largely for the organic form. Apparently, the rate of gluconeogenesis (ALT marker) increased in the liver of animals treated with organic zinc; it contributes to the accumulation of glucose. And then substrates for the tricarboxylic acid cycle are formed during the oxidative decomposition of glucose. Consequently, the activity of enzymes depends on the form of mineral supplement entering the animal organism.

The specificity and peculiarities of glycolysis reactions can be judged by the activity of LDH [22-24]. An increase in LDH activity by the age of 35 days in the experimental groups leads to an acceleration of the conversion of lactic acid into pyruvic acid and an increase in the oxidative breakdown of glucose. At the same time, the enzyme characterizes the metabolic profile of tissues and organs of the body.

# 5. Conclusion

The use of various forms of zinc affects the state of biochemical indicators of metabolism in the body, leading to different growth rates of animals. The study showed that it is possible to improve broiler productivity by completely replacing the inorganic form of zinc with an organic or nanoscale one.

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