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Original Research Article

Use of nanoscale metals in poultry diet as a mineral feed additive

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ABSTRACT

The research was aimed at studying the efficiency of a nanoscale alloy of copper (Cu) and zinc (Zn) to be used as a mineral additive for feeding broiler chickens, compared to inorganic and organic forms of these elements. Biochemical studies of the blood serum were performed using an automated analyzer. The mineral composition was determined by atomic emission and mass spectrometry (MS-ISP). The study was performed on broiler chickens of cross Smena 7 ($n = 72$) in the conditions of a vivarium. There were 3 treatment groups with 24 chickens in each. Replacing the inorganic form of mineral supplements with the nanosized alloy resulted in a positive productive effect, with a tendency to increasing the content of serum protein. The nanoscale form of metals improved ($P \leq 0.05$) the activity of aminotransferases. At the same time, the liver microstructure of experimental groups is similar to that of the control. There was a moderate plethora and poor polymorphoncellular infiltration around the interlobular triads with a clear morphological organization of the stromal and parenchymal components of the liver. However, the lack of oxidative stress was confirmed by the dynamics of catalase (CT), total superoxide dismutase (T-SOD) and malondialdehyde (MDA) levels, and the concentrations of which did not exceed the reference level. Replacing Cu and Zn sulfates with the nanoscale alloy (group 1) and organic form (group 2) of these elements in the diet of broiler chickens was accompanied by the increasing pool of these elements in the organisms at the end of the experiment. Copper was accumulated throughout the experiment in experimental group 1, compared to the reference, with the maximum difference in the liver of 36.5% ($P \leq 0.05$), in the feathers 2.5 times ($P \leq 0.01$). Assessment of the Zn level dynamics in the feathers revealed a well noticeable tendency to reducing its concentrations during the experiment in all groups. Against the background of feeding a nanoscale alloy, Zn concentration in the liver exceeded the reference by 66.8% ($P \leq 0.01$) only at the end of the experiment. Thus, nanoscale forms of Cu and Zn have a cumulative effect, and may become an alternative to inorganic and organic forms of these elements in poultry nutrition.

1. Introduction

The main purpose of using nanoscale materials in agriculture is their use as preparations with trace elements. This is determined by the relatively lower toxicity of nanoparticles of micro-element metals (Mishra et al., 2010; Canoğullari et al., 2010; Zhang and Spallholz, 2011), and higher bioavailability (Sizova et al., 2016; Scott et al., 2018). Possessing the stated characteristics, nanoparticles can compete with the existing mineral substances contained in the premixes for agricultural livestock. At the same time, the high efficiency of nanocrystal forms of metals, compared to

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inorganic salts and other sources is confirmed by a number of studies (Miroshnikov et al., 2018; Mroczek-Sosnowska et al., 2016; Ognik et al., 2018; Yausheva et al., 2015; Yausheva et al., 2018).

The use of various nanoscale sources of trace elements (Mohammadi et al., 2015; Ognik et al., 2016; Shirsat et al., 2016; Siddiqi et al., 2016; Hajializadeh et al., 2017; Farag et al., 2018) in livestock breeding has been proven to be efficient in the world practice. In turn, new technologies of synthesis result in an opportunity of joint feeding antagonistic micronutrients (Miroshnikova et al., 2015; Sizova et al., 2016). This is not advisable with the use of non-organic forms due to the antagonistic relationship, since the antagonism between the elements reduces digestibility of some of them, which results in increasing the dosage of injection, which is environmentally and economically inadvisable. In this regard, it seems promising to develop alternative forms of trace elements that remove the antagonistic relationship at the stage of absorption and have high bioavailability.

As far as we know, the article shows for the first time various biological and productive effects of various forms (nanoscale, organic and inorganic) of 2 essential trace elements, zinc (Zn) and copper (Cu) on 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 Research Council US Institute for Laboratory Animal Research, 2016). 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 Cu and Zn from LLC V-Min+ (Sergiev Posad, Russia), mineral salts $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$ from Lenreactive (Saint Petersburg, Russia), and powder of ultrafine particles of the Cu–Zn alloy (UFP CuZn) made by LLC Advanced powder technologies (Tomsk, Russia).

The UFP CuZn alloy was obtained by the method of wire electrical explosion in an argon atmosphere. Its material certification included electronic scanning and transilluminating microscopy on JSM 7401F and JEM-2000FX (JEOL, Japan). The X-ray phase analysis was performed on diffractometer DRON-7 (SPA Burevestnik, Russia). By the results of certification, the particle size was 65 ± 15 nm with the shares of Cu (60 ± 3.5)%, Zn (40 ± 2.9)%; the Z-potential of 12 ± 0.4 mV, specific surface of 5 ± 1.6 m^2/g. With the aim of obtaining lyosols for their subsequent introduction into combined fodders for poultry, water suspensions of UFP CuZn were processed in ultrasound disperser UZDN-2T (NPP Akadempribor, Russia) in the conditions of 35 kHz, 300/450 W, 10 μA for 30 min. After that, the obtained lyosolwas introduced into the feed by step mixing.

The study was performed on broiler chickens of cross Smena 7 in the conditions of a vivarium.

Ninety chickens at 1 d old 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 2 wk, 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 (Fisinin et al., 2009) 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 Cu and Zn were normalized by the introduction of sulfates: $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$ 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 to 42 d of age, sulphates of Cu and Zn were replaced in group 1 with nanoscale CuZn alloy at the dosage of 2.84 mg/kg of feed, and in group 2 with asparaginates of Cu and Zn at the same dosage. Chickens in all groups were watered with distilled water. The approach to choosing the dosage was determined by recommendations (Fisinin et al., 2009), however, with regard to the information about bioavailability of elements from the test forms, the parameters were reduced by 30%. The choice of pairs of antagonists (Cu, Zn) was determined by the same mechanism of their absorption in the intestines.

The growth of the experimental chickens was monitored daily by the individual weighing in the morning before feeding.

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 3 h 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).

The exchange of chemical elements was studied by the method of comparative slaughterings. After slaughtering, the weight was accounted for, and samples of tissues and organs were taken for assessment of the elemental composition, which were immediately frozen and stored at −18 °C. After that, the content of chemical elements (Cu, Zn) was determined in the samples taken. The aggregate pool of separate elements in the organisms of chickens at the moment of slaughtering was calculated as the total of their contents in individual organs and tissues.

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 μm thick, made on the semi-automatic microtome MWP 01 (Tekhnom, Russia) and stained with hematoxylin and eosin (Semchenko et al., 2003).

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 (PerkinElmer, USA). Ashing of biological substrates was performed with the use of microwave system of decomposition Multiwave-3000 (Anton Paar, Austria) (Skalny et al., 2009). 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 ± 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, body weight

The introduction of nanoscale alloy to the diet of broiler chickens has an effect on their productivity. For instance, the preslaughter live weight of broiler chickens of this treatment group at the age of 42 d was 2.576 ± 18.6 g, which was by 3.33% ($P \le 0.05$) and 3.84% ($P \le 0.01$) greater than that in the reference group and in experimental group 2. With that, the chickens in the reference group consumed by 4.45% more feed than the chickens in experimental group 2 over the period of the experiment. In the carcasses of chickens in experimental group 1, the muscle tissue was by 5.6% more than in the carcasses of chickens in the reference group.

3.2. Biochemical indicators of blood serum

Assessing the nature of the changes in the catalytic activity of aminotransferases, it may be stated that replacement of the forms of mineral supplements increased their values with the veracious difference in alanine aminotransferase (ALT) by 2.2 times ($P \le 0.01$) and 2.4 times ($P \le 0.05$) for experimental groups 1 and 2, respectively, at the age of 28 d (Fig. 1A). Activity of aspartate aminotransferase (AST) increased ($P \le 0.05$) by the end of the growing period (Fig. 1B).

In the experimental groups, the characteristic tendency to changes in the activity of gamma glutamyl transferase (GGT) and lactate dehydrogenase (LDH) was not found (Fig. 1C and D). Veracious reduction in the level of GGT was noted at the age of 21 d by 53.3% ($P \le 0.01$) and 33.3% ($P \le 0.05$) for groups 1 and 2, respectively, and at the age of 35 d, it reduced by 6.65% ($P \le 0.05$) in group 1. Lactate dehydrogenase activity during the experiment varied in waves. By the end of the experiment, LDH activity in experimental groups 1 and 2 was lower than in the reference group by 38.2% ($P \le 0.01$) and 13.8% ($P \le 0.05$), respectively.

3.3. Antioxidant activity of the blood

The absence of oxidative stress was also indicated by the activity of catalase (CT) and total superoxide dismutase (T-SOD). The authors did not detect their peak concentrations. Due to the introduction of the mixture of CuZnasparaginate (group 2) during the first 2 wk of the experiment, activity of catalase increased by 55.3% on d 21 ($P \le 0.05$) and by 122.2% on d 28 compared to the reference, with the subsequent decrease to the values below reference samples by 18% on d 35 and by 41.7% on d 42. The introduction of the nanoscale alloy resulted in increased activity of CT only on d 28, followed by decline of values below the reference (Table 1).

Along with T-SOD and CT, an important role in the antioxidant defense system is played by caeruloplasmin. Its activity in the blood serum increased from 1.5 to 2 times, compared to the reference, at the age of 21 and 28 d ($P \le 0.05$).

The level of MDA being an indicator of lipid peroxidation in the organisms of the chickens had reduced by the age of 42 d on the background of using the nanoscale alloy by 33.3% ($P \le 0.05$), which indicated the presence of antioxidant properties, therefore, there were no facts confirming the prooxidant effects of the preparations used.

3.4. Characteristics of liver microstructure

In the liver, the control group showed moderate plethora and polymorphoncellular infiltration around the interlobular triads with clear morphological organization of liver stromal and parenchymal components. Minor vacuoles were observed in cytoplasm of individual hepatocytes. No changes in the bile ducts.

In group 1, liver structure was preserved. Cell proliferates are often observed in the visual field around triads, mainly from cells of the lymphoid series (Fig. 2A). The lumen of central veins is slightly widened. The beam structure of lobules is preserved. Hepatocytes with clear boundaries are located orderly. Small vacuoles are seen in cytoplasm of individual hepatocytes. The nuclei are not visually enlarged with clear boundaries. There are cells in a state of mitosis.

In liver histological picture of group 2, periportal cell proliferates are observed, mainly from cells of lymphoid series. In contrast to group 1, cellular infiltration is detected intralobularly. There is a plethora of the entire vascular bed. The beam structure of lobules is preserved, with the exception of foci of expansion of sinusoids in the form of lacunae, where moderate hepatocyte discomplexion is observed (Fig. 2B). The nuclei are not enlarged, their boundaries are clear.

3.5. Microelement composition

The use of nanoscale alloy (group 1) and organic forms (group 2) of Cu and Zn in the diet of broiler chickens was accompanied by an increased content of Cu in the organism of broiler chickens by 51.63% ($P \le 0.01$) and 13.22% by the end of the experiment, respectively (Table 2). Zinc content did not increase significantly, and its concentration in group 1 was by 1.4% higher, but in group 2, by 22.9% lower than that in the reference.

Assessment of Cu and Zn concentrations in some organs and tissues, such as the liver and feathers during the experiment has shown the dependence of element accumulation on the form of metal. For instance, compared to the reference group, Cu was accumulated throughout the experiment in the experimental groups with the maximum difference of 36.5% ($P \le 0.05$) in the liver in group 1 by the end of the experiment (d 42) (Fig. 3A). This was also manifested in Cu accumulation in feathers on the background of Cu and Zn nanoscale alloy consumption (group 1), the difference with the reference group reaching 2.5 times ($P \le 0.01$) (Fig. 3B). The Cu concentration after the introduction of its organic form was close to that in the reference group, or exceeded it.

The Zn concentration in the liver of broiler chickens during the experiment changed in waves in all groups. At the end of the first week of the experiment, the Zn concentration in the liver exceeded the reference values by 7.5%, when organic forms were fed (group 2). The Zn accumulation in the liver reached the significant difference of 66.8% ($P \le 0.01$) only by the end of the experiment (d 42) (Fig. 3C) on the background of feeding a nanoscale alloy (group 1). Assessment of the Zn level dynamics in the feathers revealed the well noticeable tendency to reducing its concentrations during the experiment in all groups (Fig. 3D).

The Zn concentration that was close to the values in the reference group, or slightly exceeded these values, was found in the feathers on the background of introducing the nanoscale alloy (group 1).

4. Discussion

The obtained data about the growth stimulating effect of the nanoscale form of microelements are consistent with the results of other researchers that describe the growth stimulating effects of preparations of various metal nanoparticles, also compared to the traditional sources of microelements (Kwong and Niyogi, 2009; Mroczek-Sosnowska et al., 2016; Sizova et al., 2016). With that, it should be noted that significant difference between the groups in terms of the growth intensity in the experiment was obtained on the background of high growth intensity (from 80.8 to 83.9 g/d) of the

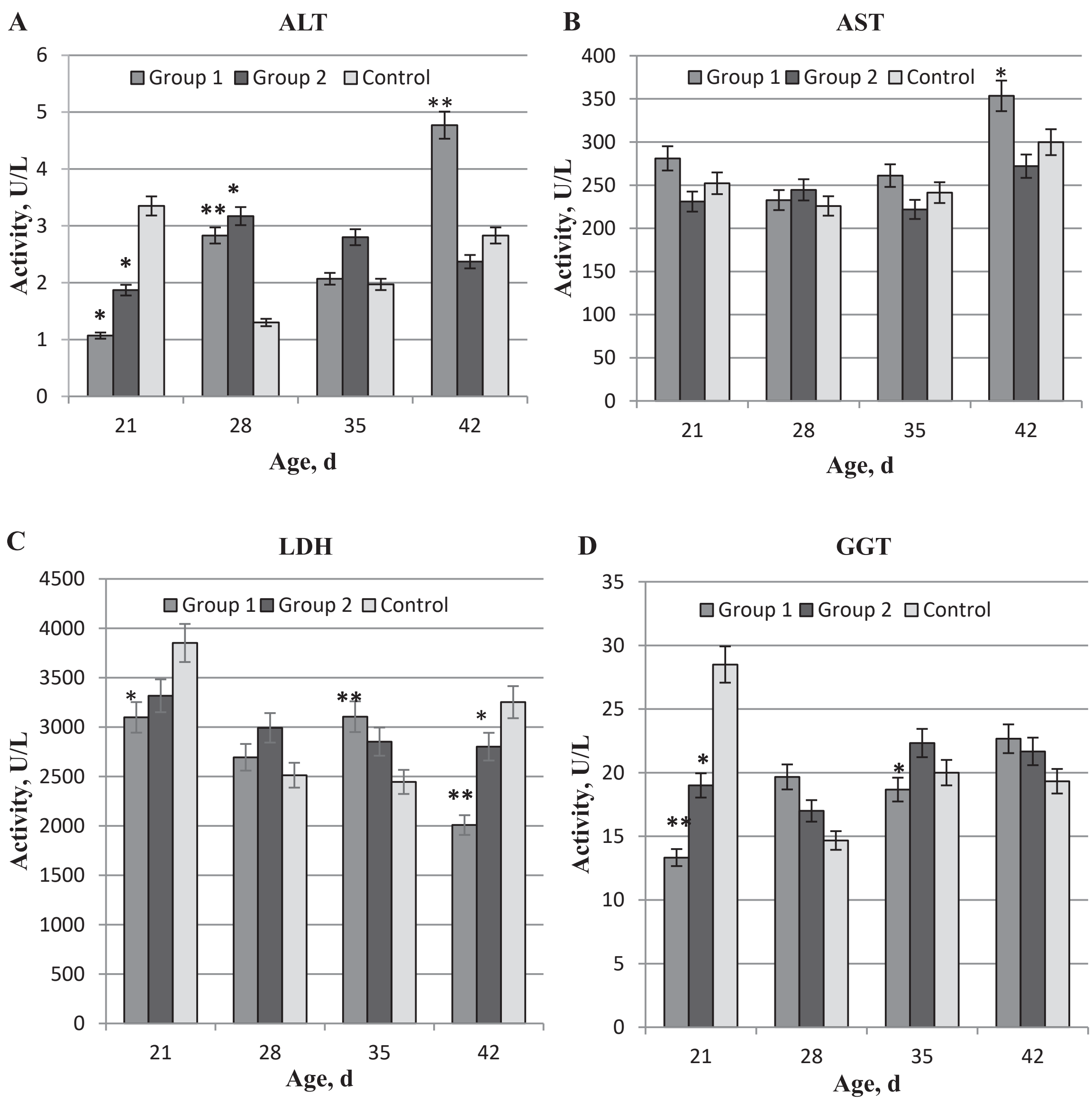

Fig. 1. Dynamics of ALT (A), AST (B), LDH (C), and GGT (D) of broiler chickens' blood serum in the experiment (mean values ± standard error of the mean, $M \pm m$) ($n = 6$). ALT = alanine aminotransferase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; GGT = gamma glutamyl transferase. Group 1, nanoscale CuZn; group 2, CuZnasparaginate; control, inorganic CuZn. Significant difference in relation to control: $^{*}P \le 0.05$, $^{**}P \le 0.01$.

grown chickens. It can hardly be explained only by high bioavailability of micro elements from nanoscale sources, as before the main accounting period, the chickens were kept on a balanced diet, the organisms had accumulated a significant pool of assessed microelements that was quite sufficient for active growth afterwards, especially because the necessary amount of them was supplied throughout the entire experiment. The increased productivity on the background of feeding a nanoscale alloy, in the opinion of the authors, was determined by a different mechanism of nanoparticles' action on the organisms of animals described earlier (Mazhitova, 2011), including through the increased exchange of arginine and synthesis of nitric oxide. Confirmation of this hypothesis are the data obtained during the previous studies, which showed increased concentrations of NO-metabolites in the blood serum of chickens during the entire experiment (Yausheva et al., 2015).

The direction of biochemical processes in the organism is shown by the metabolic axis: the level of total protein (somatic status and tolerance)—AST activity (activation of mitochondria and reverse synthesis of glucose)—ALT activity (glucose–alanine cycle [GAC] and pyruvate amination)—activity of GGT (amino acids transport through membranes)—urea level (intensity of catabolism and utilization of proteins in gluconeogenesis via GAC) (Fokina et al., 2013). Let us consider from these positions the dynamics of each component in the metabolic axis separately.

Given the fact that AST is a marker of mitochondria activity in the cells of the organism (Sookoian et al., 2015), we can assume that nanoform increases the rate of using free amino acids in the synthesis of energy through the Krebs cycle. The obtained data, on the one hand, were the evidence of changes in the degree of amino acid residues' involvement in the biochemical processes of animal organisms due to transamination reactions, and on the other hand, of the changes in the permeability of in ternal organs' cell membranes. The increased ALT activity indicates changes in the metabolic flows in the experimental groups, compared to reference group, mostly

Table 1
Indicators of the antioxidant system in the blood of the broiler chickens in the experiment (mean values ± standard error of the mean, $M \pm m$) (n = 6).

Item[1]	Age, d			
	21	28	35	42
T-SOD, %				
Group 1	477 ± 23.8*	363 ± 24.2	324 ± 18.8**	274.9 ± 10.7*
Group 2	565 ± 33.6*	498 ± 39.9	195 ± 14.4	475.9 ± 22.1*
Control	642 ± 42.6	323 ± 27.4	291 ± 16.6	869.4 ± 43.4
CT, μmol H_2O_2/L per min				
Group 1	712.9 ± 31.8	1,412.7 ± 65.7*	531.0 ± 47.2**	833.5 ± 25.6**
Group 2	1,491.4 ± 59.8*	1,696.9 ± 32.0*	706.3 ± 32.5	630.7 ± 39.4**
Control	960.3 ± 49.5	763.2 ± 38.0	862.8 ± 54.4	1431.4 ± 56.9
MDA, μmol/L				
Group 1	2.10 ± 0.011	2.02 ± 0.001	2.07 ± 0.003*	2.08 ± 0.009*
Group 2	2.10 ± 0.017	2.02 ± 0.001	2.02 ± 0.001	2.10 ± 0.011
Control	2.11 ± 0.068	2.01 ± 0.006	3,07 ± 0.004	3.12 ± 0.011
Ceruloplasmin, mg/L				
Group 1	0.03 ± 0.002*	0.03 ± 0.002*	0.05 ± 0.001	0.05 ± 0.001**
Group 2	0.06 ± 0.002*	0.03 ± 0.007*	0.03 ± 0.003	0.03 ± 0.003
Control	0.01 ± 0.009	0.01 ± 0.009	0.05 ± 0.012	0.02 ± 0.001

T-SOD = total superoxide dismutase; CT = catalase; MDA = malondialdehyde.
Significant difference in relation to control: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.
[1] Group 1, nanoscale CuZn; group 2, CuZnasparaginate; control, inorganic CuZn.

Table 2
Copper (Cu) and zinc (Zn) concentrations in broiler chickens during the experiment (mean values ± standard error of the mean, $M \pm m$) (n = 6).

Element	Group 1	Group 2	Control
Cu	1.002 ± 0.039	1.342 ± 0.029**	0.885 ± 0.0471
Zn	12.360 ± 0.89*	18.055 ± 0.37**	16.050 ± 0.47

Group 1, nanoscale CuZn; group 2, CuZnasparaginate; control, inorganic CuZn.
Significant difference in relation to control: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.

for the nanoscale form. Consequently, enzymes' activity depends on the form of mineral supplement entering the organism.

The GGT activity, being a marker of amino acid balance in the organism, in the experiment tends to decrease mostly with the introduction of the nanoscale form, due to the absence of using the somatic protein pool and reduced amino acids' transport through membranes.

The use of nanoscale alloy increased the T-SOD activity in the initial stages of the experiment, and reduced it, compared to the reference, by the end of the experiment. Therefore, T-SOD, being an element of the antioxidant defense system and the primary antioxidant, prevents the formation of new free radicals, converting the superoxide into hydrogen peroxide, which is less reaction-active, mostly on the background of using the nanoscale alloy, compared to the mixture of asparaginates of these elements. Some elements in the form of nanoparticles, such as Nano-Se, play a protective role, preventing the oxidative stress (Kocsis et al., 2010). These results were obtained in a number of studies. For instance, biologically active nanoparticles of Zn oxide (nano-ZnO) improve the growth performance and antioxidative capacity of broilers at the optimum concentration of 20 mg/kg (Xueting et al., 2018). The antioxidant status of broiler chickens depends on the administered dosage. For instance, the antioxidant and immune defense of chickens may be improved by adding nano-Cu to their diet in the amount of 12 mg per chicken over six weeks, i.e., up to the level not exceeding 7% of NRC (National Research Council) recommendation for growing broiler chickens (Zhao et al., 2014).

Plethora and polymorphoncellular infiltration with a tendency to the development of fatty degeneration are overall morphological changes in the liver of broiler chickens of all groups. Estimation of fat accumulation in cytoplasm of hepatocytes is ambiguous. Normally, this process can also occur in healthy birds, with intensive fattening. At the same time, it can be considered as a result of metabolic processes leading to an increased synthesis of fatty substances — hepatosis (Trifonov et al., 2009).

The deposition of Cu and Zn in the organism with the use of nanoscale, organic and inorganic sources was different. Perhaps, the bioavailability of the elements from the nanoscale alloy was higher, compared to inorganic and organic forms. In turn, the bioavailability of asparaginate from Cu was higher, compared to Zn. In this case, the antagonism of these substances could result in a reduced digestibility of Zn. For this reason, the method of separate feeding Cu and Zn is successful (Ognik et al., 2018).

This fact can be explained by the competition for common transporters for Cu, Zn and other bivalent metals in the intentines (Bressler et al., 2007; Kwong and Niyogi, 2009). Due to the high penetration ability of nanoparticles, metals in their composition can penetrate cells of the intestines, bypassing the traditional ways

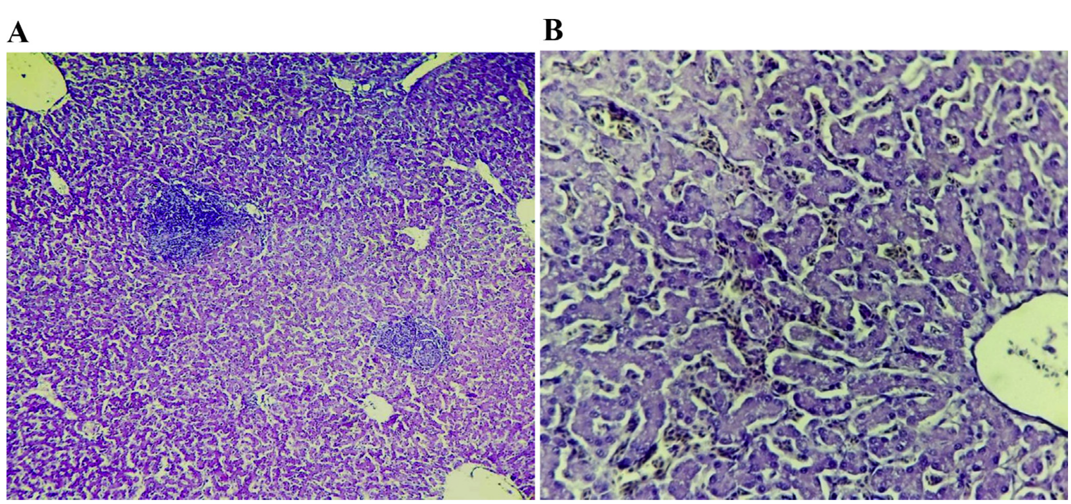

Fig. 2. Liver microstructure of broiler chickens of the experimental group 1 (A), stained with hematoxylin and eosin (liver plethora and cell proliferates in interlobular tissue), 100 × magnification, and that of the experimental group 2 (B), stained with hematoxylin and eosin (plethora, small-scale hepatocyte dystrophy), 400 × magnification.

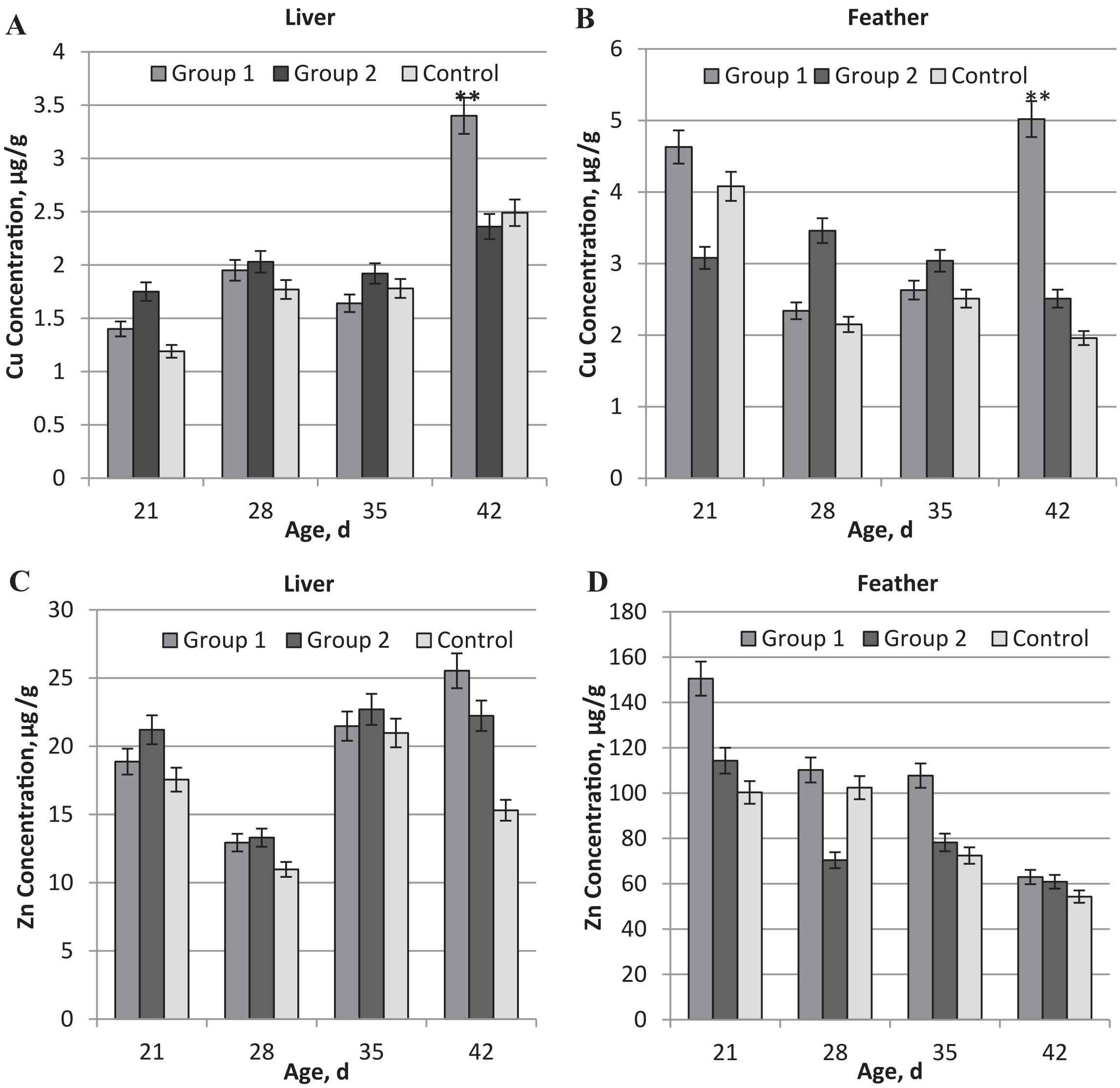

Fig. 3. Concentration of copper (Cu) in the liver (A), feathers (B) and concentration of zinc (Zn) in the liver (C) and feathers (D) ($n = 6$). Significant difference in relation to control: $^{**}P \leq 0.01$. Group 1, nanoscale CuZn; group 2, CuZnasparaginate; control, inorganic CuZn.

of binding and transport by proteins (Bárány et al., 2005). Therefore, the transport systems that determine zinc and Cu transfer in experimental group 1 can be used by other bivalent analogs. At the same time, in experimental group 2, on the contrary, they are used for the purpose. The different mechanism for receiving and using metals from nanoscale and organic forms in the organisms of the experimental chickens is confirmed by the dynamics of the concentrations of these elements in certain tissues and organs. Given the results of the studies (Nesterov et al., 2013) that determine feathers as a marker of elements' supply for the chickens, it can be assumed that the elemental status of the chickens throughout the entire experiment was continuously changing. Moreover, giving nanoscale forms of microelements determined more uniform supply of Cu to the organisms of the chickens.

5. Conclusion

Thus, the use of nanoscale forms of Cu and Zn in the diet of broiler chickens is accompanied by increased bioavailability of the assessed microelements, and more pronounced productive action, compared to the use of inorganic and organic forms.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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