Comparative study of efficiency and safety of using of different zinc and copper sources in broiler chicken feeding for poultry meat production

E A Sizova¹, Yu N Belyatskaya¹² and S A Miroshnikov¹

¹ Federal Research Centre of Biological Systems and Agrotechnologies RAS, Orenburg, Russia
² Orenburg State Agrarian University, Orenburg, Russia

E-mail: bel1201@mail.ru

Abstract. The paper presents the study on the environmental-biology assessment of different zinc and copper sources in broiler chicken diets. The first experiment included a comparative evaluation of copper sulfate and zinc sulfate, nanoparticles of these metal alloy (ZnCu NP), and Cu and Zn asparaginate. The second experiment implied that copper sulfate was replaced with Cu_I NP (d=55±15 nm); Cu_II NP (d=103±2 nm); Cu asparaginate. In the third experiment, the ZnSO₄·7H₂O in the chicken diet was replaced with Zn NP, with Zn asparaginate. The first experiment revealed the greatest productive effect when using ZnCu NP. The replacement of Cu and Zn sulfates with ZnCu NP was accompanied by the increase in the copper pool in the body by 58.2 % compared to the control and by 39.3 % compared to the use of asparaginate. The differences in the amount of zinc pool in the chicken body made 17.3 and 51.9 %, respectively. In the second experiment, the greatest productive effect was found in the group receiving Cu_II NP, the efficacy was lower with the use of asparaginates, and the lowest with the feeding of copper and Cu_I NP sulfates. The highest bioavailability of copper was obtained from Cu_II NP agent. Upon the conclusion of the third experiment, the highest body weight was observed in the zinc asparaginate group. The value of the zinc pool in the poultry of this group exceeded the level of the control group by 9.5 and by 15.7 % when using Zn NP. The obtained result shows the possibility of using these forms in the industrial production of animal feed.

1. Introduction

The preparation of nanoforms is considered as one of the methods to increase the bioavailability of food components [1]. This fully applies to agents of nanoparticles (NP) of metal microelements [2]. The prospects of low-frequency metal microelements – agents with particle diameter of about 100 nm – are determined both by lower toxicity [3] and higher bioavailability of microelements compared to inorganic compounds [4]; expressed productive action [5]; ability to correct microecological status of animals [6], etc.

There are many studies demonstrating the superiority of specially created NP agents compared to mineral salts and organic forms when used in animal and poultry nutrition. This has particularly been shown for selenium agents. The comparison of selenium NP with sodium selenite, selenomethionine, methylselenocysteine shows markedly lower acute, short-term and subchronic toxicity [7], higher efficiency in the ability to increase the activity of selenoenzymes [8]. Besides selenium, there is
literature on the use of other microelements in animal nutrition, including zinc [9], copper [10], antagonist metals [11], etc.

However, in an objective assessment of the problem, it should be noted that in addition to the works demonstrating the prospects of metal NP agents, there are indications of superiority in the complex of indicators of organic forms of metals as sources of microelements in animal feeding [12]. Meanwhile, most studies usually cover only certain properties of new substances, there is no uniform regulation for selection and testing of metal NP agents, science does not have a complex of knowledge about the biological effects of agents in various ways of their use in livestock breeding. Therefore, the studies aimed at analyzing the properties of various sources of microelements in animal feeding are considered relevant. One of the problems contributing to the comparative analysis of different sources is the antagonism between microelements and overcoming the latter in feeding. One known is the antagonism between Zn and Cu [13, 14]. Significant consumption of Zn is known to inhibit intestinal absorption and liver accumulation of Cu and induce clinical signs of Cu deficiency [15]. Similarly, high Cu consumption inhibits Zn uptake [16].

2. Materials and methods

2.1. Research object

Broiler chickens.

2.2. Scheme of the experiment

The study implied three similar experiments. At the initial stage, the chickens were selected into several groups at the age of one week (n=30). All animal services and experimental studies were carried out in accordance with instructions and recommendations of the Russian Regulations, 1987 (Order No. 755 of 12.08.1977 the USSR Ministry of Health) and The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996). During the studies all necessary measures to minimize animal suffering and to reduce the number of samples were taken.

The main accounting period of experiments was the period from 14 to 42 days of bird life. In each experiment, the control group received a balanced diet, and the control diet of broiler chickens of experimental groups was replaced with separate mineral salts. In the first experiment, CuSO₄×5H₂O and ZnSO₄×7H₂O in the I experimental group were replaced with NP alloy of these metals (ZnCu) at a dose of 2.84 mg/kg of feed; II experimental group – with Cu and Zn asparaginates at a dose of 2.84 mg/kg of feed. In the second experiment, for the chickens of the I experimental group CuSO₄×5H₂O was replaced with Cu NP, II experimental group – with Cu asparaginate, III experimental group – with Cu NP at a rate of 1.7 mg/kg of feed. In the third experiment, for the chickens of the I experimental group ZnSO₄×7H₂O was replaced with Zn NP, II experimental group – with Zn asparaginate at a rate of 5 mg/kg of feed. In the course of the studies, individual weighing of poultry was performed daily and fodder consumption was estimated.

The compared microelement agents were introduced into the main diet of broiler chickens starting from the 14th day of age and fed throughout the experiment. Broiler chickens were kept in accordance with the recommendations [17].

CuSO₄×5H₂O and ZnSO₄×7H₂O (Lenreactive, Russia) were used as mineral salts. Copper and zinc asparaginates (B-Min+ LLC, Sergiyev Posad) were used as organic sources of microelements.

Four NP metal agents and their alloys were used in the study. The Cu NP agent was synthesized by plasma chemical synthesis, d=55±15 nm; composition: Cu⁹ 99.7±2.5 % O₂ 0.3±0.03 %, Z-potential=31±0.1 mV; Srel=9±0.8 m²/g. The Cu NP agent was synthesized by high temperature condensation with oxygen modification; d=103±2 nm; composition: Cu⁹ 96.0±4.5 %, Cu⁴ 4.0±0.4 %; Z-potential=25±0.5 mV; Srel=8.0±0.5 m²/g. Zinc and copper alloy NP agent was synthesized by electric explosion of conductor in argon atmosphere d=65±15 nm; composition: Cu⁹ 60±3.5 %, Zn⁹ 40±2.9 %; Z-potential=12±0.4 mV; Srel=5±1.6 m²/g. Zn NP agent was synthesized by electric explosion of conductor in argon atmosphere d=90.0±2.0 nm; composition: Zn⁹ 91±3.1 %, Zn⁹ 8±0.3 %, sorbed gases 1±0.07; Z-potential=5.3±0.2 mV; Srel=6±0.8 m²/g.
The NP agents were prepared by dispersing aqueous mixtures of particles with ultrasound (f – 35 kHz, N – 300 (450) W, A – 10 μm) for 30 minutes before introduced into the complete feed.

In the course of the study, the hematological characteristics of poultry were evaluated at the Research Centre of Biological Systems and Agrotechnologies, RAS with URIT-2900 Vet Plus automatic hematological analyzer (URIT Medial Electronic Co., China) and CS-T240 biochemical analyzer (Dirui Industrial Co., Ltd., China) using commercial biochemical kits for veterinary medicine (DEACON-DS, Russia; Randox Laboratories Ltd, UK).

The value of the pool of chemical elements in the body of chickens was calculated as the sum of their content in individual tissues and organs. The content of elements in the tissues was determined in the laboratory of the Center of Biological Medicine (Registration Certificate of ISO 9001:2000, No. 4017-5.04.06) by means of atomic emission and mass spectrometry (AES-ICP and MS-ICP) on Elan 9000 equipment (Perkin Elmer, USA) and Optima 2000 V (Perkin Elmer, USA).

The results were statistically processed during the study. The statistical analysis was performed by comparing the experimental groups with the control group using SPSS 19.0 software (IBM Corporation) and Statistica 10. The value equal to P≤0.05 was considered statistically significant.

3. Research results

The first experiment did not reveal statistically significant differences in the body weight between the experimental groups treated with ZnCu NP and the asparaginates of these metals. The body weight of broilers at the age of 42 days when using ZnCu NP was 2 576.0±14.0 g, which exceeded the level of the control group by 3.9 % and the II experimental group – by 3.3 % (P≤0.05).

The replacement of Cu and Zn sulfates with ZnCu NP in a diet was followed by the increase in the copper pool up to 3.457±0.191 mg, which exceeded the level of the control group by 58.2 % (P≤0.001) and the II experimental group – by 39.3 % (P≤0.001). The zinc pool in the chickens of the I experimental group made 46.509±2.491 mg, which exceeded the level of the control group by 17.3 % (P≤0.001) and the II experimental group – by 51.9 % (P≤0.001).

The replacement of copper and zinc sulfates with the mixture of metal asparaginate in the diet of broiler chickens was accompanied by the decrease in the concentration of copper in heart, kidneys, muscles, and feather by 39.4 % (P≤0.05), 5.9 %, 32.0 % (P≤0.05) and 32.5 % respectively in relation to the control group. At the end of the pilot studies against the background of normalization of the copper level in heart, brain and liver compared to the previous period, it decreased in the spleen by 26 %, muscles – by 48.5 % (P≤0.05) and thymus – by 27.6 % (P≤0.05) in comparison with the control values.

The use of ZnCu NP in the chicken feed on the 21st day resulted in the increase of zinc content in heart by 16.4 %, brain – by 71 % (P≤0.05), liver – by 7.2 %, spleen – by 49.5 % (P≤0.05), kidneys – by 42.1 % (P≤0.05), bursa fabricii – by 15 %, thymus – by 15.3 % and feather – by 49.9 (P≤0.05).

At the end of an experiment, the use of additives of metal microelements in the form of ZnCu NP was followed by the increase in the content of erythrocytes by 6.27 % (P≤0.05), hemoglobin – by 5.21 %, hematocrit – by 8.66 % (P≤0.05). Besides, the mixture of metal asparaginate reduced hemoglobin content by 6.9 % and hemoglobin – by 12.3 % (P≤0.05).

The highest number of leukocytes was observed in the blood of broiler chickens of the I experimental group two weeks after the beginning of the experiment (28 days of age) at a difference with the control group making 14.7 % (P≤0.05). A similar result (12.6 % difference with the control group) was recorded on the 35th day of age only when the asparaginate mixture (II experimental group) was added. The increase of leukocytes in the I and II experimental groups was mainly due to lymphocytes (67 % and 15.2 % difference with the control group). On the 35th day of age the blood of broiler chickens of the I and II experimental groups was characterized by the increase in the amount of monocytes by 11.9 (P≤0.05) and 18 % (P≤0.01) respectively in comparison with the control group.

The results of the second experiment recorded the largest body weight in the III experimental group – 2573.0±35.8 g, which exceeded the control level by 4.2 % (P≤0.05), I experimental group – by 17.3 % (P≤0.01) and II experimental group – by 4.7 %.
The greatest bioavailability of copper was caused by CuII NP, which use was followed by the increase of the pool of this element in the body up to $3.769\pm0.259$ mg, which exceeded the control level by $72.4\%$ ($R\leq0.001$). I experimental group – by $28.2$ ($P\leq0.01$) and II experimental group – by $93.7\%$ ($R\leq0.001$). There were no differences between the groups in zinc content.

At the end of the third experiment the largest body weight was recorded for the II experimental group – $2.522\pm41.1$, which exceeded the control level by $11.1\%$ ($P\leq0.01$) and the I experimental group by $6.1\%$ ($P\leq0.05$). At the same time, in the II experimental group the maximum efficiency of feed use was recorded on average by $6.2\%$ higher than the control level and by $2.8\%$ in the I experimental group. The zinc pool in the body of the II experimental group was the highest – $43.39\pm2.98$ mg. This was $9.5\%$ higher than in the control group and $15.7\%$ higher than in the I experimental group. On the 28th day of age the content of lymphocytes in the blood of the II experimental group was higher by $5.02\%$ ($P\leq0.05$) and by $2.5\%$ in comparison with the control and the I experimental group. So, the study of monocytes on the 28th day of age revealed that their content in the II experimental group was less in comparison with the I experimental group by $18.4\%$, and in comparison with control group by $19.1\%$ ($P\leq0.05$). It is found that the volume of platelets in the blood of broiler chickens of the I experimental and the control group was higher by $13.0$ and $15.7\%$ ($P\leq0.05$), respectively, in comparison with the II experimental group. The magnesium content on the 28th day of age in the blood serum of the II experimental group exceeded the level of the I experimental group by $8\%$; control group – by $10.5\%$ ($P\leq0.05$). On the 35th day of age similar differences made $2.9\%$ and $7.2\%$ ($P\leq0.05$), respectively. In the experiment we described the fact of statistically significant increase of calcium concentration in the blood serum of chickens of the II experimental group in comparison with the I experimental group and the control group.

4. Discussion

Mineral salts as sources of microelements in animal diets have a number of disadvantages: soil contamination with large amount of chemical elements due to their low bioavailability from salts [18]; negative effects on animal microbiomes [19]; relatively low poultry productivity and economic efficiency of production using salts [20]. One of the reasons for the replacement of mineral salts is the antagonism of chemical elements from mineral salts in the digestive tract of poultry due to competition for mineral-binding ligands and mineral absorption sites in the intestinal lining [21]. Both organic compounds of microelements and agents of nanoparticles are proposed as candidates to replace mineral salts in poultry diets [22, 23]. In this regard, the purpose of the study was to compare the productive action and bioavailability of microelements from various sources. Copper and zinc as antagonists were selected as microelements [13, 14].

The analysis of the obtained results showed that the used microelement agents had different productivity. In two of the three experiments, the efficacy of the agents as measured by the body weight of chicken was the highest when NP (ZnCu, CuII) and Zn asparaginate were used. It should be noted that earlier similar experiments produced far from unambiguous results. Some researchers [12] state the superiority of organic forms of microelements over ultradisperse ones, the others [23], on the contrary, highlight the efficiency of nanoforms. Obviously, not everything is so unambiguous. Due to a large number of factors, it is impossible to compare such diverse classes of substances in general, in which we agree with the opinion of J. Jankowski et al [24] pointing to a wide variety within individual classes of mineral additives. The task is seen in the development and use of the most pronounced qualities of agents.

According to the obtained data, NP agents are generally relatively more bioavailable. Besides, the data shows that the value of the pool introduced from the NP microelement was reliably higher to its analogues. The only exception was the experiment using zinc asparaginate, where the latter exceeded the bioavailability of the NP agent. The increased bioavailability of microelements from organic complexes can be explained by the data in [25] on the absence of undesirable effects of organic forms on digestive duct mucosa; various absorption of chelates [26, 27], etc. Thus, the conclusion [28] that the use of organic forms of metal antagonists in poultry feeding excludes the antagonism of these
elements during digestion and absorption seems quite relevant. In turn, high bioavailability of microelements [29] from NP agents is determined by unique properties of ultradisperse particles to penetrate tissues [30, 31], including outside the antagonism phenomenon between metals. At the same time, the use of smaller doses of NP allows achieving the same effect as when using mineral sources.

In summary, commercially available agents of NP of metal microelements differ in size, production methods, and other properties, just as organic microelement compounds vary in their properties. This generally does not allow the results to be summarized by the superiority of one agent over the other.

Acknowledgements
The studies were performed in accordance with the plan of research works Federal Research Centre of Biological Systems and Agro-technologies of the Russian Academy of Sciences No. 0761-2019-0005.

References
[1] Rein M J, Renouf M, Cruz-Hernandez C et al 2013 Bioavailability of bioactive food compounds: a challenging journey to bioefficacy Br J Clin Pharmacol. Mar. 75(3) 588–602 DOI: 10.1111/j.1365-2125.2012. 04425.x
[2] Fisinin V I, Miroshnikov S A, Sizova E A et al 2018 Metal particles as trace-element sources: current state and future prospects World's Poult. Sci. J. 74(3) 523–40
[3] Zhang J 2009 Biological properties of red elemental selenium at nano size (Nano-Se) in vitro and in vivo. In: Sahu S C, Casciano D (ed) Nanotoxicity: From In Vivo and In Vitro Model To Health Risks (West Sussex, UK: John Wiley and Sons)
[4] Sizova E, Miroshnikov S, Yaushsheva E and Polyakova V 2015 Assessment of morphological and functional changes in organs of rats after intramuscular introduction of iron nanoparticles and their agglomerates. Biomed. Res. Int. 2015 243173 DOI: 10.1155/2015/243173. Epub 2015 Feb 19
[5] Yaushsheva E, Miroshnikov S, Sizova E et al 2015 Comparative assessment of effect of cooper nano and microparticles in chicken Oriental J. of chem. 31(4) 2322336 Retrieved from: http://dx.doi.org/10.13005/ojc/310461
[6] Bouwmeester H, Van der Zande M and Jepson M A 2017 Effects of food-borne nanomaterials on gastrointestinal tissues and microbiota Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol DOI: 10.1002/wnan.1481
[7] Zhang J and Spallholz J 2011 Toxicity of selenium compounds and nano- selenium particles In: Casciano D, Sahu S C (ed) Handbook of Systems Toxicology (West Sussex, UK: John Wiley and Sons)
[8] Zhang J, Wang X and Xu T 2008 Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: comparison with semethylselenocysteine in mice Toxicol. Sci. 101 22–31
[9] Zhao Yong, Li Lan, Zhang Peng-Fei et al 2016 Regulation of egg quality and lipids metabolism by Zinc Oxide Nanoparticles Poult. Sci. 95(4) 920–33 DOI: https://doi.org/10.3382/ps/pev436
[10] Ognik K, Stępniowska A, Cholewińska E and Kozłowski K 2016 The effect of administration of copper nanoparticles to chickens in drinking water on estimated intestinal absorption of iron, zinc, and calcium Poult. Sci. 95(9) 2045–51 DOI: https://doi.org/10.3382/ps/pew200 Published: 15 June 2016
[11] Miroshnikova E, Arinzhanov A, Kilyakova Y et al 2015 Antagonist metal alloy nanoparticles of iron and cobalt: impact on trace element metabolism in carp and chicken. Human & Veterinary Medicine Int. J. of the Bioflux Society 7(4) 253–9
[12] Bakhshalinejad R, Akbari Moghaddam Kakhi R and Zoidis E 2018 Effects of different dietary sources and levels of selenium supplements on growth performance, antioxidant status and immune parameters in Ross 308 broiler chickens Br. Poult. Sci. 59(1) 81–91 DOI: 10.1080/00071668.2017.1380296
[13] Hall A C, Young B W and Brenner I 1979 Intestinal metallothionein and the mutual antagonism between copper and zinc in the rat J. Inorg. Biochem. 11 57–66
[14] Southern L L and Baker D H 1983 Zinc toxicity, zinc deficiency and zinc-copper interrelationship in Eimeria acervulina-infected chicks J. Nutr. 113 688–96
[15] Sheida E, Sipailova O, Kvan O et al 2014 Functional properties of antimicrobial peptides extracted from hens' platelets Life Science Journal 11(9) 25, 180-184
[16] Van Campen D R 1969 Copper interference with the intestinal absorption of Zn-65 by rat J. Nutr. 97 104–8
[17] Fisinin V I, Egorov I A, Okolelova T M and Imangulo v Sh A 2003 Kormlenie sel'skokhozyaistvennoi ptitsy (Sergiev Posad) 375 p
[18] Sheppard S C and Sanipelli B 2012 Microelements in feed, manure, and manured soils J. Environ. Qual. 41(6) 1846–56 DOI: 10.2134/jeq2012.0133
[19] Yausheva E, Miroshnikov S and Sizova E 2018 Intestinal microbiome of broiler chickens after use of nanoparticles and metal salts Environ. Sci. Pollut. Res. Int. 25(18) 18109–20 DOI: 10.1007/s11356-018-1991-5
[20] Olukosi O A and van Kuijk S Y 2018 Han Copper and zinc sources and levels of zinc inclusion influence growth performance, tissue trace mineral content, and carcass yield of broiler chickens Poult. Sci. 97(11) 3891–8 DOI: 10.3382/ps/pey247
[21] Santon A, Giannetto S, Sturniolo G C et al 2002 Interactions between Zn and Cu in LEC rats, an animal model of Wilson’s disease Histochem. Cell. Biol. 117 275–81
[22] Ao T, Pierce J L, Power R, Dawson K A et al 2006 Evaluation of Bioplex Zn® as organic zinc source for chicks Int. J. Poult. Sci. 5 808–11
[23] Sizova E A, Miroshnikov S A, Lebedev S V et al 2018 Comparative tests of various sources of microelements in feeding chicken-broilers Sel'skokhozyaistv. Biol. 53(2) 393–403
[24] Jankowski J, Ognik K, Stepniowska A, Zduńczyk Z and Kozłowski K 2019 The effect of the source and dose of manganese on the performance, digestibility and distribution of selected minerals, redox, and immune status of turkeys Poult. Sci. 1(98(3)) 1379–89 DOI: 10.3382/ps/pey467
[25] Wedekind K J, Collings G, Hancock J and Tigemeyer E 1994 The bioavailability of zinc-methionine relative to zinc sulfate is affected by calcium level Poult. Sci. 73(suppl. 1) 114
[26] Aldridge K, Saddoris L and Radcliffe J S 2007 Copper can be absorbed as a Cu-peptide chelate through the PepT1 transporter in the jejunum of weanling pigs J. Anim. Sci. 85(suppl. 1) 154
[27] Ao T, Pierce J L, Pescatore A J, Cantor A H et al 2007 Effects of organic zinc and phytase supplementation in a maize-soybean meal diet on the performance and tissue zinc content of broiler chicks Br. Poult. Sci. 48 690–5
[28] Ao T, Pierce J L, Power R et al 2009 Effects of feeding different forms of zinc and copper on the performance and tissue mineral content of chicks Poult. Sci. 88(10) 2171–5 DOI: 10.3382/ps.2009-00117
[29] Ibrahim D, Ali H A and El-Mandrawy S A 2017 Effects of different zinc sources on performance, bio distribution of minerals and expression of genes related to metabolism of broiler chickens Zag. Vet. J. 45 292–304
[30] Ruparelia, J P, Chatterjee A K, Duttagupta S P and Mukherji S 2008 Strain specificity in antimicrobial activity of silver and copper nanoparticles Acta Biomater. 4 707–16
[31] Duskaev G K, Rakhatmatullin S G, Kazachkova N M, et al 2018 Effect of the combined action of Quercus cortex extract and probiotic substances on the immunity and productivity of broiler chickens Veterinary World 11(10) 1416-1422