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MORPHOLOGICAL AND BIOCHEMICAL BLOOD PARAMETERS IN BROILERS AT CORRECTION WITH DIETARY COPPER SALTS AND NANOPARTICLES

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Abstract

Diets of modern crosses and breeds of farm animals require mandatory correction of mineral nutrition. Ionic forms commonly used for correction are characterized by low bioavailability, prooxidant effect, and high toxicity in vivo. That is why low-toxic sources of essential chemical elements are of particular interest, including nanocrystalline metals. We firstly assessed the efficient of nanosized copper on the model of broiler chickens. A comparative study of productive and biological effects of copper nanoparticles (CuNPs) and copper sulfate was carried out on Smena 7 chickens of 14-42 days of age using different administration methods. Hematologic and biochemical parameters and elemental composition of tissues were assessed. We revealed an increase in red blood cells and hemoglobin and decrease of platelets at the highest doses of Cu NPs administrated orally or intramuscularly. The biochemical blood parameters assessed indicated an enhancement of plastic processes in the body and, as a consequence, an increase in the growth rate under Cu NPs administration. The difference of the total protein compared to the control (33.6 %, $p < 0.05$) was the highest when Cu NPs injected at a dose of 2 mg/kg which increased the supply of protein synthesis. The albumin level was higher in 28-day old chicks injected with Cu NPs (2 and 0.2 mg/kg). A replacement of dietary CuSO_4 with dietary Cu NPs led to an increase in the growth rate. When Cu NPs fed, the found biochemical parameters indicated strengthening of energy and synthetic processes in the body, and therefore, an increase in the poultry growth. At 1.7 mg/kg of dietary Cu NPs the gained weight was 13.5-23.8 % ($p < 0.01$ to $p < 0.001$) more as compared to the control. Dietary Cu NPs at 0.7 mg/kg was not enough to highly stimulate the poultry growth and resulted in the weight gain which was lower than in the poultry fed with Cu NPs at 1.7 mg/kg but higher (by 8.5-18.4 %, $p < 0.01$ to $p < 0.01$) as compared to the poultry not fed with Cu NPs. Thus, the promoting effect depends on the form of copper source. The Cu NPs effectiveness under enteral administration at 1.7 mg/kg or intramuscular double injections at 0.2 mg/kg was superior to that of dietary copper sulfate.

Keywords: nanoparticles, copper, broiler chicks, growth intensity, chemical elements, blood, biochemical and morphological parameters

Diets in the modern animal and poultry farming are subject to mandatory adjustment of mineral composition, which is determined by an increase in genetic potential [1], the specific properties of biogeochemical provinces [2, 3], and impact of regional components on availability of mineral components [4-6], etc. Generally, ionic forms act as the sources of essential chemical elements. They are characterized in vivo by low bioavailability, pro-oxidant effect and high toxicity [7]. In particular, this explains controversial attitude to metallothrapy

and special interest to low-toxic sources of essential chemical elements, which may include nanocrystalline metal forms [8-10].

Unique properties of nanomaterials may ensure the possibility of their wide use in medicine and biology [11, 12] due to low toxicity and high bioavailability at a size of about 100 nm [13]. Unique properties of nanomaterials have objectively determined the emergence of new nanoparticle-based sources of microelements, with feasibility of application in feeding animals and poultry demonstrated in several studies [14, 15].

Here we first assessed effectiveness of various copper forms (CuSO_4 and nanocrystalline compound) in poultry farming, and have revealed the tendency towards increase in erythropoiesis, albumin synthesis in liver and growth intensity due to Cu nanoparticles.

Our objective was a comparative evaluation of copper nanoparticle and mineral salt product with regard to bioavailability and effect on growth, development, hematological and biochemical parameters in broiler chickens.

Technique. Cu nanoparticles (Cu NPs) were synthesized by high-temperature condensation method (Migen-3, Institute for Energy Problems of Chemical Physics, RAS, Moscow) in accordance with the description [16]. The material attestation of the preparations included electronic scanning and transmission microscopy (JSM 7401F and JEM-2000FX, JEOL, Japan). The X-ray phase analysis was conducted using a diffractometer DRON-7 (Burevestnik, Russia). The size (d) of obtained nanoparticles is 103 ± 2 nm. Nanoparticle lysozoles in saline solution were prepared in an ultrasonic disperser UZDN-2T (NPP Akadempribor, Russia) (35 kHz, 300/450 W, 10 μA), with UV sterilization.

The studies were performed on Smena 7 broiler chickens (Orenburg State University vivarium). The keeping conditions and test procedures met the recommendations provided for by the national regulations (Order of the USSR Ministry of Health No. 755 d/d August 12, 1977) and The Guide for Care and Use of Laboratory Animals (National Academy Press, Washington, D.C., 1996). The day-old chickens ($n = 270$) were assigned individual tags and placed in the same conditions. At the age of 2 weeks, based on individual daily weighing and food consumption data, three control and five test groups were formed ($n = 30$ in each group). Chickens received a complete compound feed recommended by the All-Russian Scientific Research and Technology Poultry Institute (VNITIP) [17, 18]. Copper content in starting basic diet (BD) amounted to 11.79 mg/kg, in growth BD — to 9.51 mg/kg, including that due to introduction of copper sulphate (1.7 mg/kg) [18]. Basic diet only (BD, K_1), BD without $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (K_2) and BD with double intramuscular injections of saline solution at the age of 14 and 28 days (K_3) were the controls. The difference between the groups was as follows: group I — BD without $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ plus Cu nanoparticles fed at a dose of 1.7 mg/kg, group II — BD without $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ plus Cu nanoparticles fed at a dose of 0.7 mg/kg (oral consumption of nanoparticles); group III — BD without $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ plus Cu nanoparticles (2 mg/kg of live weight) injected into femoral muscles, group IV — BD without $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ plus Cu nanoparticles (0.2 mg/kg) injected into femoral muscles, group V — BD without $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ plus Cu nanoparticles (0.02 mg/kg of live weight) injected into femoral muscles (double parenteral input at the age of 14 and 28 days). All chickens received distilled water. From day 14 to day 28 the BD formulation contained 320 g/kg wheat, 10 g/kg barley, 184 g/kg sunflower cake, 200 g/kg soy bean meal, 40 g/kg fish flour, 60 g/kg vegetable oil, 163 g/kg corn grain, 10 g/kg wheat bran, 10 g/kg limestone, and 3 g/kg common salt. From day 28 to day 42 the BD contained 182 g/kg wheat, 50 g/kg barley, 180 g/kg sunflower cake, 75 g/kg soy bean meal, 45 g/kg fish flour, 45 g/kg vegetable oil, 400 g/kg corn

grain, 10 g/kg wheat bran, 10 g/kg limestone, and 3 g/kg common salt.

The tested chickens were daily weighed individually. Blood was sampled from the axillary vein, before slaughter in the morning in a fasting state, at the age of 28 and 42 days. Samples for examination of morphological parameters were placed in vacuum tubes with anticoagulant (EDTA), for biochemical studies — into vacuum tubes with a coagulation activator (thrombin). Morphological parameters were determined using an automatic hematology analyzer URIT-2900 Vet Plus (URIT Medial Electronic Co., Ltd, China). The biochemical blood serum test was made using an automatic biochemical analyzer CS-T240 (DIRUI Industrial Co., Ltd, China) and commercial veterinary kits (DiaVetTest by ZAO DIAKON-DS, Russia; Randox Laboratories Ltd., Great Britain). The blood serum was analyzed within 2 hours after blood was sampled.

When determining Cu pool in the body in the beginning and in the end of the reference period, average samples ($n = 15$) were formed after slaughtering at the age of 28 and 42 days for muscle tissue, skin, internal organs, i.e. gastrointestinal tract (GIT), heart, lung, liver, kidney, spleen, genital tissues, bone tissue, central nervous system (CNS) and visceral fat. The samples were frozen and stored at -18°C . The overall pool of a chemical element was determined by summarizing organ and tissue parameters. Elementary content of biosubstrates and compound feed was analyzed by inductively coupled plasma atomic emission spectrometry (Optima 2000 V, Perkin Elmer, USA) and mass spectrometry (Elan 9000, Perkin Elmer, USA). The biosubstrates were ashed using a microwave decomposition system Multiwave-3000 (Anton Paar, Austria).

When calculating the bioavailability of copper from various sources (nanoparticles, salt), it was assumed that transformation coefficient of copper from BD (without regard to copper sulphate) remains unchanged in all test and control groups. Transformation coefficient of copper [18] from BD (without regard to copper sulphate) was calculated based on the total intake of Cu with feeds and the growth of Cu pool in the body in groups K_1 (BD) and K_2 (BD without $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$).

The data are presented as the arithmetic mean (M) with the standard error of the mean (m). Statistical processing was performed using Statistica 10.0 software package (StatSoft Inc., USA). The differences were considered statistically significant at $p < 0.05$.

Results. Productivity of broilers was related to copper content in the diet (Fig. 1). Thus, exclusion of copper sulphate from the premix (group K_2) led to a decrease in body weight by 8 %, as compared to the complete diet (K_1). At the same time, intramuscular injection of saline solution (K_3) during normalization of the diet with regard to Cu had no negative consequences, and live weight in this group was similar to that in K_1 (differences within the error value).

Replacement of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ with dietary Cu NPs was followed by more intensive growth of chickens. By the end of the experiment their weight in group I exceeded the weight in K_1 by 13.5 % ($p < 0.01$) and in K_2 by 23.8 % ($p < 0.001$). Cu NPs dose of 0.7 mg/kg was insufficient to meet the poultry demand in copper and ensure the maximum growth promoting effect, so live weight was lower than that in group I, but significantly higher than that in K_1 (by 8.5 %, $p < 0.01$) and K_2 (by 18.4 %, $p < 0.01$).

Similar growth promoting effect resulted from an increase of parenteral dose of Cu NPs (from 0.02 mg/kg to 2 mg/kg). Presumably, the dose of 0.02 mg/kg was insufficient to provide the poultry with this element that was confirmed by weighing results. By the end of the experiment, the difference in live weight amounted to 13.5 % as compared to K_1 in case of dietary Cu NPs at 1.7 mg/kg, and to 9.5 % as compared to K_3 in case of Cu NPs intramuscularly injected at 2 mg/kg.

Fig. 1. Growth (A, B) and relative live weight gain (C, D) in Smena 7 broiler chickens fed (A, B) or intramuscularly injected (C, D) with Cu NPs: 1, 2, 3, 4, 5 — groups I, II, III, IV and V (see description in section *Technique*); K₁, K₂ and K₃ (controls) — basic diet (BD), BD without CuSO₄ · 5H₂O and BD with intramuscular injections of saline solution. Gain values are given with regard to K₁ (C, intake of Cu NPs with the feed) and K₃ (D, intramuscular injection). By the end of the observation period, the differences between groups I and II vs. K₁, group I vs. K₂, as well as groups III and IV vs. K₃ are significant at $p < 0.05$, between group II and K₂ — significant at $p < 0.01$ ($M \pm m$, $n = 30$, vivarium).

Blood morphological parameter under a replacement of copper sulphate with Cu NPs in the diet and in intramuscular injections had a number of specific features (Table 1).

1. Blood morphological parameters in Smena 7 broiler chickens at various doses and ways of Cu NPs and Cu salts intake ($M \pm m$, $n = 15$, vivarium)

Groups	Hematocrit, %	Erythrocytes, $\times 10^{12}/l$	Hemoglobin, g/l
Day 28			
<i>Dietary consumption</i>			
I (BD without CuSO ₄ · 5H ₂ O, Cu NPs 1.7 mg/kg)	25.0 ± 0.41 ^a	2.05 ± 0.02 ^a	110.0 ± 2.62 ^a
II (BD without CuSO ₄ · 5H ₂ O, Cu NPs 0.7 mg/kg)	23.4 ± 1.10 ^a	2.93 ± 0.09 ^a	102.7 ± 4.71 ^a
<i>Parenteral injections</i>			
III (BD without CuSO ₄ · 5H ₂ O, Cu NPs 2 mg/kg)	25.9 ± 1.20 ^{ac}	3.04 ± 0.12 ^a	102.3 ± 5.82 ^a
IV (BD without CuSO ₄ · 5H ₂ O, Cu NPs 0.2 mg/kg)	25.5 ± 0.27 ^{ac}	3.01 ± 0.03 ^{ac}	103.2 ± 1.90 ^a
V (BD without CuSO ₄ · 5H ₂ O, Cu NPs 0.02 mg/kg)	23.7 ± 0.81 ^a	2.89 ± 0.09 ^{ab}	94.5 ± 4.75
K ₁ (BD)	18.5 ± 1.03	2.83 ± 0.14	96.0 ± 4.84
K ₂ (BD without CuSO ₄ · 5H ₂ O)	22.3 ± 1.68	2.50 ± 0.09	86.2 ± 8.80
K ₃ (BD with double administration of saline solution)	21.3 ± 0.88	2.75 ± 0.08	93.0 ± 4.76
Day 42			
<i>Dietary consumption</i>			
I (BD without CuSO ₄ · 5H ₂ O, Cu NPs 1.7 mg/kg)	28.4 ± 0.43 ^{ab}	3.29 ± 0.02 ^{ab}	125.5 ± 4.18 ^{ab}
II (BD without CuSO ₄ · 5H ₂ O, Cu NPs 0.7 mg/kg)	21.9 ± 2.47	2.91 ± 0.02	102.0 ± 9.80
<i>Parenteral injections</i>			
III (BD without CuSO ₄ · 5H ₂ O, Cu NPs 2 mg/kg)	27.55 ± 1.43	3.23 ± 0.12	118.2 ± 7.30
IV (BD without CuSO ₄ · 5H ₂ O, Cu NPs 0.2 mg/kg)	26.6 ± 0.49	3.22 ± 0.06	117.0 ± 2.62
V (BD without CuSO ₄ · 5H ₂ O, Cu NPs 0.02 mg/kg)	28.4 ± 0.69 ^b	3.41 ± 0.06 ^b	128.2 ± 2.44 ^b
K ₁ (BD)	23.9 ± 0.98	3.98 ± 0.12	119.3 ± 3.36
K ₂ (BD without CuSO ₄ · 5H ₂ O)	22.5 ± 0.62	3.13 ± 0.06	110.3 ± 2.49
K ₃ (BD with double administration of saline solution)	24.6 ± 0.57	3.68 ± 0.11	120.3 ± 3.01

Note. BD — basic diet; ^a — differences with K₁ are significant at $p < 0.05$, ^b — differences with K₂ are significant at $p < 0.05$, ^c — differences with K₃ are significant at $p < 0.05$.

Thus, the number of erythrocytes amounted to the maximum value in the

2. Blood biochemical parameters in Smena 7 broiler chickens fed with Cu NPs ($M \pm m$, $n = 15$, vivarium)

Parameter	Group I	Group II	K ₁
D a y 2 8			
Total protein, g/l	25.8±1.85	20.8±1.57	20.1±1.99
Albumin, g/l	12.3±0.99*	8.8±0.54	10.2±0.81
Bilirubin, μM/l	3.29±0.070*	3.20±0.070*	2.13±0.080
ALT, U/l	10.3±0.84	7.5±0.57	8.8±0.62
AST, U/l	277.5±6.06**	196.8±8.12	179.1±5.11
Urea, mmol/l	1.10±0.011	1.13±0.070	1.25±0.020
Creatinine, mmol/l	74.0±3.63	67.7±2.11	61.9±2.94
Cholesterol, mmol/l	3.28±0.042	2.57±0.027	2.98±0.011
Glucose, mmol/l	11.13±0.120**	8.90±0.840*	6.11±0.500
Magnesium, mmol/l	0.87±0.031	1.13±0.027	1.05±0.090
Calcium, mmol/l	0.64±0.009	0.54±0.001*	1.62±0.091
Phosphorus, mmol/l	1.14±0.020	0.59±0.030	1.44±0.070
Iron, mmol/l	43.20±2.690	33.19±0.880*	60.20±3.140
D a y 4 2			
Total protein, g/l	37.8±2.61	38.8±2.81	32.3±2.12
Albumin, g/l	15.8±0.45	15.7±0.15	13.2±0.13
Bilirubin, μM/l	3.94±0.092	4.74±0.080	3.8±0.091
ALT, U/l	9.50±0.620*	10.50±0.160*	7.9±0.320
AST, U/l	310.7±13.73	238.7±18.35	265.1±10.21
Urea, mmol/l	1.34±0.090	1.49±0.020	1.12±0.020
Creatinine, mmol/l	54.3±1.11*	55.8±2.60*	63.3±4.12
Cholesterol, mmol/l	3.30±0.016	2.90±0.029	3.13±0.015
Glucose, mmol/l	8.95±0.440	9.09±0.110	11.09±0.970
Magnesium, mmol/l	0.32±0.009	0.68±0.005	0.91±0.006
Calcium, mmol/l	1.13±0.050	1.68±0.040	1.84±0.086
Phosphorus, mmol/l	1.03±0.030	1.70±0.070	1.62±0.090
Iron, mmol/l	21.7±1.64	28.5±1.16	23.1±1.15

Note. Groups I and II — basic diet (BD) without $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ with Cu NPs added (1.7 and 0.7 mg/kg feed, respectively); K₁ — BD; ALT — alanine aminotransferase, AST — aspartate aminotransferase.

*, ** Differences with K₁ are significant at $p < 0.05$ and $p < 0.01$, respectively.

3. Blood biochemical parameters in Smena 7 broiler chickens intramuscularly injected with Cu NPs ($M \pm m$, $n = 15$, vivarium)

Parameter	Group III	Group IV	Group V	K ₃
D a y 2 8				
Total protein, g/l	25.2±1.76	30.5±1.14*	38.5±1.70	19.5±0.18
Albumin, g/l	14.8±0.76*	14.2±0.47*	15.2±0.83	9.2±0.11
Bilirubin, μM/l	1.13±0.010	2.78±0.190	1.46±0.080	1.91±0.050
ALT, U/l	8.0±0.48	4.0±0.51**	8.5±0.62	9.8±0.72
AST, U/l	228.3±6.62	277.5±8.17**	191.2±4.40	183.6±9.36
Urea, mmol/l	1.47±0.011	1.25±0.021	1.47±0.035	1.27±0.020
Creatinine, mmol/l	71.3±4.14	69.5±3.11	72.5±3.49	62.8±3.54
Cholesterol, mmol/l	3.13±0.025	3.67±0.024**	3.87±0.034**	2.78±0.021
Glucose, mmol/l	4.79±0.480	3.88±0.190	3.47±0.170	5.19±0.500
Magnesium, mmol/l	0.50±0.060	0.78±0.020	0.94±0.025	1.02±0.018
Calcium, mmol/l	1.20±0.032	0.92±0.03*	0.65±0.03	1.42±0.018
Phosphorus, mmol/l	1.88±0.090	1.14±0.050	2.47±0.080	1.34±0.017
Iron, mmol/l	96.49±1.65*	49.31±1.92	72.73±3.88	58.0±4.94
D a y 4 2				
Total protein, g/l	44.5±1.33**	43.8±1.54**	40.3±1.99**	33.3±1.16
Albumin, g/l	15.7±0.95	16.2±0.31	16.5±0.67	14.2±0.25
Bilirubin, μM/l	4.37±0.081	2.79±0.070	4.77±0.077	3.5±0.028
ALT, U/l	10.83±0.35	15.33±0.60	10.33±0.61	6.9±0.27
AST, U/l	298.0±9.46	354.0±12.17	310.3±11.84	273.1±10.25
Urea, mmol/l	1.46±0.090	0.81±0.080	1.09±0.090	1.32±0.020
Creatinine, mmol/l	59.3±4.01	66.0±2.24	81.7±3.84	61.3±3.66
Cholesterol, mmol/l	3.33±0.037	2.95±0.046	2.53±0.027	2.93±0.018
Glucose, mmol/l	10.10±0.140	14.79±0.150*	8.48±0.520	10.08±0.270
Magnesium, mmol/l	0.65±0.012	0.78±0.020	0.81±0.020	0.86±0.076
Calcium, mmol/l	1.73±0.040	1.81±0.020	1.77±0.040	1.74±0.015
Phosphorus, mmol/l	1.38±0.080	1.44±0.320	0.90±0.010	1.51±0.050
Iron, mmol/l	56.1±2.13	35.9±1.09	29.8±2.08	25.2±1.04

Note. Groups III-V — basic diet (BD) without $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ with Cu NPs administration (injections at 2; 0.2 and 0.02 mg/kg live weight, respectively); K₃ — with double administration of saline solution; ALT — alanine aminotransferase, AST — aspartate aminotransferase.

*, ** Differences with K₃ are significant at $p < 0.05$ and $p < 0.01$, respectively.

groups with the maximum dose of Cu NPs, both oral and intramuscular. For example, in group I the deference, as compared to the control, was 12.6 % ($p < 0.001$) at

the age of 28 days, and 75.0 % ($p < 0.05$) at the age of 42 days. Hemoglobin content (regardless of the Cu NPs administration route) exceeded the same of the control (with maximum difference of 8.9 % in group I). Similar effect of nanoparticles on hematological parameters was described earlier [19].

The highest level of total protein was found in chickens which received Cu NPs intramuscularly (Table 2, 3). At that, maximum difference with the control (33.6 %, $p < 0.05$) was at a dose of 2 mg/kg, which improved protein synthesis. Significant differences in concentrations of albumins in groups received Cu NPs at the doses of 2 and 0.2 mg/kg, were observed in chickens aged 28 days. Intake of 0.7 mg/kg Cu NPs with the feed promoted albumin synthesis in liver to a lesser extent. Dynamics of urea and creatinine concentrations also confirmed high activity of metabolic processes in broiler chickens. Content of creatinine (a dehydrated form participating in energy metabolism in muscles) [20] has decreased by 8.96-11.41 % ($p < 0.05$), as compared to the control, in case of oral intake of Cu NPs, which is quite natural due to active transformation of creatinine to creatine phosphate and delivery to muscles in the form of energy during activation of biochemical processes. It was reported that the amount of creatinine decreases by 12-27 % under arabinogalactan added to the broilers diet, which is followed by intensification of metabolic processes [21].

Dietary Cu NPs at 0.7 mg/kg was not sufficient to compensate for complete absence of copper sulphate in the diet and did not meet the requirements of the body. An increase in blood glucose level under Cu intake with feed is indicative of intensification of synthesis and energy processes, which leads to more intensive growth (especially at the age of 28 days).

The amount of bilirubin formed in liver reticuloendothelial system as a result of catabolism of heme containing proteins, tended towards increase in case of Cu NPs intake with an increase in control groups as the chickens were growing. The amount of bilirubin in chickens aged 42 days, which received Cu NPs, slightly exceeded the control values.

Due to a metabolic shift under Cu NPs intake, catalytic activity of aminotransferases differed from the control. The ALT activity increased significantly in broilers aged 42 days under oral intake of Cu NPs at various doses, e.g. by 36.9 % ($p < 0.05$) in group I and by 51.3 % ($p < 0.05$) in group II. Intramuscular administration lead to higher activity of enzymes, up to the difference of 56-120 % ($p < 0.05$), as compared to the control.

4. Chemical composition of tissues in Smena 7 broilers which received Cu NPs ($M \pm m$, $n = 15$, vivarium)

Parameter	K ₁	Group I	Group IV
Dry matter (DM)	41.6±0.53	37.3±0.45 ^{ab}	40.7±0.82 ^b
Protein	16.2±0.67	18.0±0.16 ^{ab}	16.8±0.32 ^b
Lipids	22.6±1.50	18.1±1.32 ^a	21.0±1.74
Ash	2.2±0.07	2.4±0.08 ^a	2.7±0.09
Energy, MJ/kg DM	25.7±0.92	30.9±0.67	31.4±1.52

Note. Group I — basic diet (BD) without CuSO₄ · 5H₂O with Cu NPs added at 1.7 mg/kg feed; group IV — BD without CuSO₄ · 5H₂O with Cu NPs intramuscularly injected at 0.2 mg/kg live weight; K₁ — OP; ^a — differences with K₁ are significant at $p < 0.05$, ^b — differences between groups I and IV are significant at $p < 0.05$.

Dietary Cu NPs decreased the amount of Ca more significantly than intramuscular injections. Blood Ca concentration increased with age and was close to control values. It is known that excessive Cu intake may inhibit iron absorption [22]. In our experiments iron concentration in serum reached maximum in the chickens aged 28 days, which received high doses of Cu NPs (2 mg/kg). The period from injection to slaughter (2 weeks) ensured prolonged release of copper to the bloodstream. At the age of 42 days the broilers of this group still showed higher iron level, but its amount decreased comparing to that at the age of 28 days. In

28 day-old chickens which received Cu NPs with the feed these parameters were below the control values (by 25 % and 42 % in group I and II, respectively).

We have observed a decrease in fat accumulation in chickens from test groups, e.g. by 4.5 % ($p < 0.05$) in group I, which took place with simultaneous increase in protein content (by 1.8 %, $p < 0.05$) (Table 4).

Fig. 2. Cu pool in organs and tissues of Smena 7 broiler chickens aged 42 days when fed with Cu NPs (group I, 1.7 mg/kg feed) and intramuscularly infected with Cu NPs (group IV, 0.2 mg/kg live weight): a — muscles, b — internal organs, c — skin, d — total (control — K₁, basic diet; $M \pm m$, $n = 30$, vivarium).

Examination of Cu accumulation in biosubstrates discovered inter-group dynamics for internal organs only (Fig. 2). The differences for skin and muscles were insignificant, with relatively constant copper content. The way and multiplicity of NPs intake, as well as their dose played the key role. In case of daily chronic intake copper content was stable during the whole experiment, which was not observed in case of double intramuscular administration. An increase in copper pool in the body when using nanoparticles was previously observed by other researchers [23].

Calculated Cu bioavailability for the compared additives amounted to 20.08 mg per broiler (including 4.05 mg with copper sulphate) in K₁ and 30.56 mg per broiler (including 4.13 mg with nanoparticles) in group I, respectively. Taking into account that Cu transformation coefficient in the basic diet without CuSO₄ (group K₂) was 32.5 %, apparent bioavailability of Cu from mineral additives amounted to 46.1 % for copper sulphate, and to 71.0 % for dietary Cu NPs.

Thus, the effectiveness of copper preparations as a micronutrient depends on their form. Cu NPs when added to diet at a dose of 1.7 mg/kg of feed and injected intramuscularly at 0.2 mg/kg of live weight turned out to be optimal for broiler chicken farming.

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