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COMPARATIVE TESTS OF VARIOUS SOURCES OF MICROELEMENTS IN FEEDING CHICKEN-BROILERS

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Abstract

Animals of modern breeds and crosses need more dietary minerals to realize more of their genetic potential but that leads to an increase in the ecological load. So the development of new sources of essential chemical elements with relatively less toxicity and higher bioavailability of the components are of relevance. Ultra-dispersed particles (UDP) are among prospective preparations. This is the first report on a comparative study of the effects of dietary Cu and Zn additives as UDP of the alloy, asparaginates and sulfates on performance and productivity of Smena 7 broiler chicks. The study showed greater availability, a more pronounced positive effect of Cu/Zn-UDP and the various impact of the forms studied on mineral metabolism. Dietary Cu/Zn-UDP accelerated bird growth by 3.9 % ($P \le 0.05$) compared to Cu and Zn mineral salts and by 4.7 % ($P \le 0.01$) compared to Cu and Zn asparaginates. Administration of Cu/Zn-UDP led to an increase in blood NO metabolites by 9.8 % ($P \le 0.05$), 21.0 % ($P \le 0.01$), 13.0 % ($P \le 0.05$), and 11.0 % ($P \le 0.05$) compared to the control on days 7, 14, 21 and 28, respectively. By the end of the study, blood erythrocytes and hemoglobin was 6.27 % higher ($P \le 0.05$) and 19.40 % higher ($P \le 0.001$) compared to the control and also 5.21 % higher and 12.60 % higher when compared to Cu and Zn asparaginates used. Replacement of copper mineral salt with dietary Cu/Zn-UDP and Cu asparaginate was accompanied by an increase in this element pool in the body of 42-day old broiler chickens by 51.6 % ($P \le 0.01$) and 13.2 %, respectively. By the end of the study, the zinc pool, on the contrary, decreased by 22.9 % compared to the control when Zn asparaginate was fed but exceeded the control by 12.5 % ($P \le 0.05$) when using Cu/Zn-UDP. Copper and zinc preparations used in various ways influenced on the exchange of a number of chemical elements in the body. Feeding with Cu/Zn-UDP and Cu and Zn asparaginates resulted in lower pools of Ni, Al, Sn and a significant increase in iodine and cobalt pools compared to control. A distinctive feature of Cu/Zn-UDP action from that of the asparaginates was an increase in Pb and Cd pools which could result from a change of the load on transport systems in the intestine when using Cu/Zn-UDP.

Keywords: ultra-dispersed particles of Cu and Zn alloy, Cu and Zn asparaginates, broiler chicks, productivity, chemical element composition, biochemical and morphological blood parameters

Some estimates suggest that development of nanotechnologies by 2020 will result in establishment of industrial and agricultural productions with turnover from $3.0 tln [1] to $3.4 tln [2]. Yet today actual production of nanomaterials exceeds 100 ths. t per annum [3]. Along with wider use in medicine and biology [4-6], nanomaterials become prevalent in agriculture [7, 8], food and pro-

cessing industry [9, 10]. Only in USA, annual growth rate in such sector comprises 25 % ($1.08 bln.) [11]. Use of nanomaterials in agriculture as microelement medications is characterized by their less toxicity (12, 13) and higher biological availability [14, 15]. The later, in particular in the context of PCR, allows decreasing pollution of environment at production and use of feeds [16].

Opportunities for use of nanosized microelement forms were demonstrated in medicine. In particular, it allowed creating medicines for treatment of anemia. Thus, Ferumoxytol (Feraheme®, AMAG Pharmaceuticals, Inc., USA) containing superparamagnetic iron oxide nanoparticles (SPION) was approved by US Food and Drug Administration (FDA) for iron replacement therapy primarily in patients with chronic renal disease [17] and got widespread use at MRT tests [18].

Today, literature sources suggest using various nanostructural microelement sources in animal breeding industry, including selenium [19], iron [20], chrome [21] zinc [22], copper (23), etc. Usually, these are medicines containing one microelement in form of nanoparticles. However, with development of the concept of synthesis and use of such substances prospects for microelement complexes, including antagonists, become evident [24].

Science had acquired a great deal of knowledge on the nature and mechanisms of antagonist relations between chemical elements and other elements [25-27], phytate [28], amino acids and their salts [29], polyphenols and peptides [30], etc. in human and animal body, especially at absorption stage in gastrointestinal tract. Necessity for studying of such relations is determined by the need for tackling the challenges of prenosological diagnostics and treatment of elementosis, correction of diets [31], and estimation of actual nutritional value of diets [32]. Antagonism may reduce availability of some elements which requires increasing their input norms and may negatively affect the environment. Earlier, separate feeding of antagonist substances was proposed to exclude antagonist relations at soaking (Patent of Invention RUS 2195269 14.02.2001).

In this paper, we had for the first time shown that productive and biological action of various forms of two essential microelements — zinc and copper (alloy in form of ultrafine particles, asparaginates and sulphates) and their effect on mineral metabolism in broiler chickens cross Smena 7 is different; herewith, ultrafine particles were more available in general and had more expressed positive effect.

Purpose of this paper was to study the effectiveness of ultrafine particles of copper and zinc alloy as a mineral additive in feeding of broiler chickens as compared to mineral salts and organic forms of such elements.

Techniques. Copper and zinc asparaginates (V-Min+ LLC, Sergiev Possad, Russia), mineral salts $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$ (Lenreactiv, Saint Petersburg, Russia) and powder from ultrafine particles of Cu-Zn alloy (UFP Cu-Zn) made by Peredovie Poroshkovie Tehnologii LLC (Tomsk, Russia) were used as microelement sources. Cu-Zn UFPs were produced by method of electric explosion of conductor in argon atmosphere. Material attestation of UFPs involved scanning and transmission microscopy at JSM 7401F and JEM-2000FX (JEOL, Japan). X-ray phase analysis was carried out at a diffractometer DRON-7 (Scientific Industrial Enterprise Burevestnik, Russia). Based on attestation results, size of particles (d) 65±15 μm with ratio Cu^o 60±3.5 %, Zn^o 40±2.9 %; Z-potential 12±0.4 mV, specific surface 5±1.6 m^2/g. At production of lyosols, water suspensions of Cu-Zn UFPs were treated by ultrasound at dispergator UZDN-2T (Scientific Industrial Enterprise Academpribor, Russia) at 35 KHz, 300/450 W, 10 mcA during 30 minutes. Lyosol was introduced into the combined feed by gradual mixture method.

Studies were carried out on broiler chicken (cross Smena 7) in vivarium conditions of the Institute of Bioelementology of Orenburg State University. Poultry keeping and procedures followed during tests were in line with instructions and recommendations of the Russian Regulations (Decree of the Ministry of Health of USSR Nr. 755 dated 12.08.1977) and The Guide for Care and Use of Laboratory Animals (National Academy Press, Washington, 1996). All endeavors were taken to minimize the agony in animals and to reduce the number of used specimen. 90 chickens aged 1 day were selected for tests. Chickens assigned with individual numbers (leg plastic labels), were weighted and in furtherance were kept in equal conditions. Three groups aged 2 weeks were formed based on individual daily weighting data and estimated feed costs by paranalogues: one control and two trial (n = 24 each). Chickens were fed full-value combined feeds formed accounting for the recommendations [33] according to age periods. Composition of the main diet (MD) (g/kg) during the period from 7 to 28 days: wheat grain — 475, barley grain — 30, maize — 80, soybean meal — 250, sunflower meal— 70, sunflower oil — 50, premix (made according to the effective recommendations) — 20, cooking salt — 3.4, moncalcium phosphate — 13, limestone meal — 5, DL-methionine 98.5 % — 1,6, lysine monochlohydrate 98 % — 1, cooking soda — 1; aged 28-42 days: wheat grain — 435, maize — 226, soybean meal — 150, sunflower meal — 100, sunflower oil — 50, premix — 20, cooking salt — 3, moncalcium phosphate 10.5, limestone meal — 1, DL- methionine 98.5 % — 1.2, lysine monochlohydrate 98 % — 2.3, baking soda — 1. During the tests chickens were on the Main Diet, in which copper and zinc were introduced in form of $CuSO_4 \cdot 5H_2O$ and $ZnSO_4 \cdot 7H_2O$ as part of premix including all standardized microelements. Copper and zinc sulphates in the premix for chickens from trial groups during the period from 14 to 42 days were replaced by Cu-Zn UFPs in dosage of 2.84 mg/kg of feed (group I) or by Cu and Zn asparaginates in the same dosage (group II). Chickens from all groups were supplied with distilled water.

Poultry growth was daily controlled by individual weighting in morning hours before feeding.

Blood was collected from axillary vein on empty stomach in morning hours before killing of chickens aged 21, 28, 35 and 42 days (for morphologic studies — in vacuum vials with anticoagulant EDTA, for biochemical — in vacuum vials with thrombin as coagulating stimulant). Blood serum was analyzed within no later than 3 hours following sampling.

Morphologic indicators were determined by automated hematologic analyzer URIT-2900 Vet Plus (URIT Medial Electronic Co., Ltd, PRC). Biochemical analysis of blood serum was conducted with the use of an automated analyzer CS-T240 (DIRUI Industrial Co., Ltd, PRC) and commercial veterinary kits (DiaVetTest, DIAKON-DS CJSC, Russia; Randox Laboratories, Ltd, United Kingdom).

Metabolism of chemical elements was studied by comparative slaughter method. Mass was calculated, and tissue and organ specimen were collected upon slaughter for assessment of the elementary composition frozen and stored at temperature of –18 °C. Specimens were analyzed for 25 chemical elements (Ca, Cu, Fe, Li, Mg, Mn, Ni, As, Cr, K, Na, P, Zn, I, V, Co, Se, Ti, Al, Be, Cd, Pb, Hg, Sn, and Sr). Total element pool in vivo upon slaughter was calculated as total content in organs and tissues, retention was determined as pool difference at the end and beginning of the experiment.

Elemental composition of organs and tissues was analyzed by nuclear-emission spectrometry methods with inductively bound plasma (Optima 2000 V, PerkinElmer, USA) and mass-spectrometry (Elan 9000, PerkinElmer,

USA). Ashing of biosubstrates was performed in microwave decomposition system Multiwave-3000 (Anton Paar, Austria).

Data was statistically processed by Statistica 10.0 software (StatSoft, Inc., USA) and MS Excel 2000 software package. Mean (M) and standard errors of the mean (±SEM) values were determined. Differences assessed by Student's t-test are deemed statistically significant at $P \leq 0.05$.

Results. Control species by 4.5 % left behind the species from II group by consumption of combined feeds during tests. At that, feed consumption to surplus of 1 kg live mass in the control comprised 1.73 kg, that is by 3.40 and 4.04 % more than in I and II groups. Feeding of broiler chickens by Cu-Zn UFPs was accompanied by more intensive growth (Fig. 1).

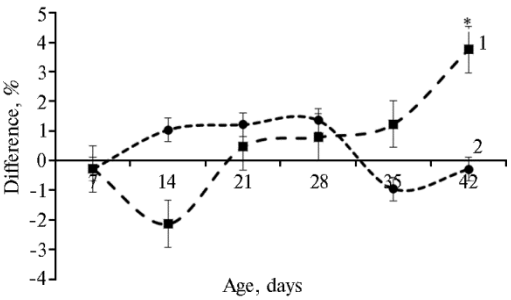

Fig. 1. Difference in live weight between cross Smena 7 broiler chickens in group I fed Cu-Zn alloy ultrafine particles (1) **and in group II fed Cu and Zn asparaginates** (2)**, as compared to control group where Cu and Zn in the ration were regulated by sulphates of the elements** (groups of n = 24 each, testing in vivarium conditions). Star means that deviations from control are statistically significant at $P \leq 0.05$.

During the main accounting period (14-42 days of life), live weight surplus of 2349.9 g was noted in group I that exceeded similar indicator in control and in group II by 3.9 ($P \leq 0.05$) and 4.7 % ($P \leq 0.01$), respectively. The results correlate to results of other studies describing growth stimulating effects of UFP metal medicines as compared to traditional microelement sources [34, 35]. It should be noted that valid intergroup difference in growth intensity in our test was noted at high growth rate in studied poultry (80.8-83.9 g/d). It is hard to explain by only greater biodiversity of microelements from UFP medicines since poultry, before commencement of the main accounting period, was kept on a balanced diet and accumulated pool of assessed microelements, which is quiet sufficient for further active growth, especially that their required quantity was supplied during the entire test.

1. Content of blood NO metabolites (μmol/l) **in cross Smena 7 broiler chickens depending on chemical formula of Cu and Zn used for microelement-based regulation of ration** (M±SEM, n = 6, testing in vivarium conditions)

Group	Age, days			
	21	28	35	42
I	28.9±0.27*	33.8±1.05**	33.5±1.27*	31.0±0.59*
II	24.7±0.70	28.6±0.77	30.5±0.49	32.4±1.96*
Control	26.4±0.20	27.9±0.44	29.6±0.78	27.8±2.36

N o t e. See description of groups in section "Methodology".
*, ** Differences from control are statistically significant at $P \leq .0.05$ and $P \leq 0.01$.

In our opinion, growth stimulating effect of UFPs was determined by specific nanoparticle action mechanism on animal organism [36], of which through strengthening of arginine metabolism and synthesis of nitrogen oxide. This hypothesis is supported by data (Table 1) demonstrating growth of NO metabolite concentrations in blood serum of chickens from group I during the entire test on days 7, 14, 21 and 28, respectively, by 9.8 ($P \leq 0.05$), 21.0 ($P \leq 0.01$), 13.0 ($P \leq 0.05$) and 11.0 % ($P \leq 0.05$) as compared to control.

Differences in action mechanisms of the studied medicines on poultry were also confirmed by assessment of hematologic parameters. Thus, use of UFP medicines and asparaginates promoted erythropoiesis. It was well demonstrated

during the first 3 weeks of additive use (Table 2).

2. Age-specific dynamics of several hematologic indicators in cross Smena 7 broiler chickens depending on chemical form of Cu and Zn used for microelement-based regulation of diet (M±SEM, n = 6, testing in vivarium conditions)

Group	Age, days			
	21	28	35	42
	Erythrocytes, $\times 10^{12}$/l			
I	2,76±0,095*	2,39±0,179*	2,27±0,083	2,71±0,139*
II	2,95±0,041**	2,40±0,182*	2,35±0,018	2,27±0,076*
Control	2,41±0,635	2,16±0,081	2,26±0,145	2,55±0,030
	Hemoglobin, g/l			
I	116,7±1,84	135,7±1,36**	134,3±2,67	146,7±1,57**
II	117,3±1,76	125,0±1,72	139,7±1,96*	130,3±5,93
Control	97,5±2,50	125,0±5,00	132,0±6,03	139,3±2,19
	Hematocrit, %			
I	21,83±0,067**	28,10±1,854*	26,40±0,529	27,53±1,780*
II	24,50±0,321*	28,40±1,629*	27,97±0,463*	26,60±1,790
Control	20,05±0,150	25,10±0,529	25,47±1,017	25,17±0,467
	Leucocytes, $\times 10^9$/l			
I	28,10±0,290	39,37±0,406	36,73±0,687	30,43±0,767
II	32,97±0,373	33,50±0,139	40,93±0,476	35,40±0,312
Control	28,45±0,850	34,30±0,921	36,33±0,998	35,73±0,307
	Lymphocytes, $\times 10^9$/l			
I	12,73±0,024	18,20±0,781*	15,73±0,210	13,13±0,820
II	11,20±0,195	12,97±0,730	18,20±0,318*	15,63±0,524
Control	11,00±0,600	10,87±0,822	15,87±0,513	15,77±0,724

Note. See description of groups in section "Methodology".
*, ** Differences from control are statistically significant at $P \leq .0.05$ and $P \leq 0.01$.

Commencement of changes in erythrocytes is reflected on hematocrit that within 7 days after commencement of tests was increased in groups I and II by 8.8 and 22.0 %, respectively, above the control group. In furtherance, the difference had changed within the range from 3.5 to 11.0 %. By the end of tests on chickens from group I, erythrocytes, hemoglobin, and hematocrit indicators were higher than in control and in group II by 6.27 ($P \leq 0.05$) and 19.4 % ($P \leq 0.001$), 5.21 and 12.6 %, 8.66 ($P \leq 0.05$) and 3.4 %. Similar effect of copper UFPs on hemoglobin and erythrocyte concentration is described earlier [37, 38].

Studying of effects from introduction into the chicken's ration of various microelements demonstrates their effect on blood morphology and leucogram in the context of stimulation of oxidation-reduction processes, which, in its turn, promoted more intensive metabolism [39-41]. In our research, quantity of white blood cells in broiler chickens from all groups was within the physiological limits. Indicators of chickens aged 28 days from group I moved towards the upper limit norm (within 2 weeks after commencement of the research), provided 14.7 % difference from the control. Upon introduction of asparaginate mixture, similar result (12.6 % difference with control) was noted only in chickens aged 35 days, i.e. 3 weeks following commencement of the research. Growth of leucocyte population in groups I and II had occurred mainly due to 67.0 % and 15.2 % difference in lymphocytes as compared to control). Effect of growth of the number of white blood cells under the effect of ultrafine medicine is not described here for the first time. Earlier, V.B. Borisevich and V.G. Kaplunenko [42] have identified moderate leucocytosis and strengthening of phagocytic activity in leucocyte cells of broilers due to dietary copper UFPs and mixture of nano-aquachelates of Ag, Cu, Zn, Mg, Co.

Introduction of various forms of copper and zinc sources had resulted in different changes in biochemical blood indicators of broiler chickens (Tables 3).

Total blood protein tends to increase as compared to control groups from day 28 to day 35 at feeding with UFPs. By the end of test (day 42) statistically significant difference from the control is 11.2 % ($P \leq 0.05$), which presupposes positive effect of the additive on protein metabolism. The same is con-

firmed by increase of urea indicators as compared to control during the entire research. No critical changes in creatinine appear that confirms the lack of nephrotoxic action. Blood concentration of glucose in chickens of group I exceeded control values with maximum differences of 39.1 % ($P \leq 0.05$, day 28) and 21.8 % ($P \leq 0.05$, day 42). Also, triglyceride concentration has a tendency towards growth (by 11.7-53.3 %, $P \leq 0.05$).

3. Age-specific dynamics of blood biochemical indicators in cross Smena 7 broiler chickens depending on chemical form of Cu and Zn used for microelement-based regulation of diet (M±SEM, $n = 6$, testing in vivarium conditions)

Group	Age, days			
	21	28	35	42
	Alanine aminotransferase, IU/l			
I	1.07±0.064*	2.83±0.129**	2.07±0.178	4.77±0.296**
II	1.87±0.069*	3.17±0.437*	2.80±0.289	2.37±0.110
Control	3.35±0.150	1.30±0.189	1.97±0.198	2.83±0.189
	Aspartate aminotransferase, IU/l			
I	281.0±11.40	232.7±9.60	261.1±13.10	353.5±9.40
II	231.0±9.60	244.6±18.10	221.9±12.70	272.0±8.70
Control	252.2±10.80	225.9±6.00	241.4±10.60	299.9±13.40
	Lactate dehydrogenase, IU/l			
I	3098.3±36.50*	2693.3±121.40	3104.3±25.00**	2008.0±24.10**
II	3316.3±200.40	2992.3±462.30	2852.0±111.50	2801.3±11.30*
Control	3851.0±54.00	2512.0±71.10	2444.3±15.80	3252.0±33.80
	γ-Glutamyle transferase, IU/l			
I	13.33±0.882**	19.67±1.764	18.67±0.333*	22.67±1.480
II	19.00±1.155*	17.00±0.142	22.33±1.202	21.67±0.333
Control	28.50±1.500	14.67±0.882	20.00±1.646	19.33±1.404
	Creatinine, μmol/l			
I	15.9±1.34	19.7±1.34	16.1±1.12	16.9±0.79
II	16.5±0.72	17.2±0.38	15.9±1.39	17.2±1.28
Control	24.9±1.65	16.3±1.39	17.8±0.67	16.3±1.83
	Glucose, mmol/l			
I	10.9±0.77*	10.3±0.37*	9.9±0.53	10.8±0.13**
II	9.3±0.64	7.9±0.60	8.9±0.01*	5.9±0.43
Control	6.1±0.74	7.4±0.85	9.8±0.82	8.9±0.65
	Total protein, g/l			
I	30.6±2.75	33.3±0.48*	31.4±1.33	33.1±1.49*
II	33.3±1.22	32.8±0.24*	32.6±0.37	28.2±2.09
Control	30.9±1.14	29.4±0.85	30.1±1.85	29.8±1.17
	Cholesterole, mmol/l			
I	4.5±0.08*	4.3±0.07	2.9±0.01**	2.1±0.11**
II	4.3±0.39	4.1±0.25	2.0±0.01	2.2±0.19*
Control	4.3±0.52	4.3±0.18	2.1±0.01	1.2±0.06
	Triglicerides, mmol/l			
I	0.51±0.03	0.16±0.029	0.23±0.036*	0.19±0.021
II	0.36±0.08	0.18±0.069	0.27±0.028*	0.10±0.024
Control	0.65±0.07	0.19±0.023	0.15±0.038	0.17±0.007
	Urea, mmol/l			
I	1.07±0.09	1.00±0.058	1.00±0.058	1.10±0.000*
II	1.37±0.07	1.07±0.033	0.93±0.088	0.97±0.033
Control	1.55±0.35	0.93±0.033	0.87±0.176	0.93±0.033

Note. See description of groups in section "Methodology".
*, ** Differences from control are statistically significant at $P \leq .0.05$ and $P \leq 0.01$.

Replacements of copper mineral salt with UFP and asparaginate were followed by 51.6 % ($P \leq 0.01$) and 13.2 % growth in Cu pool in chickens aged 42 days (Fig. 2). Zinc pool decreased by 22.9 % in group II, but exceeded control values by 12.5 % ($P \leq 0.05$) in group I. Possibly, for whatever reasons biological availability of copper from asparaginate (as compared to zinc) was higher. In such a case, Cu and Zn antagonism will result in reduced digestion of zinc. For the same reason, use of separate copper- and zinc-based diets is successful [43].

Elemental status analysis in the tested poultry indicates different effects of UFP and asparaginates on chemical metabolism. This clearly follows from comparison of pools of metals. Total concentrations of Zn, Pb, Cd, Ni, Al, and Sn decrease in chickens of group II (see Fig. 2). The same is true with Ni, Al,

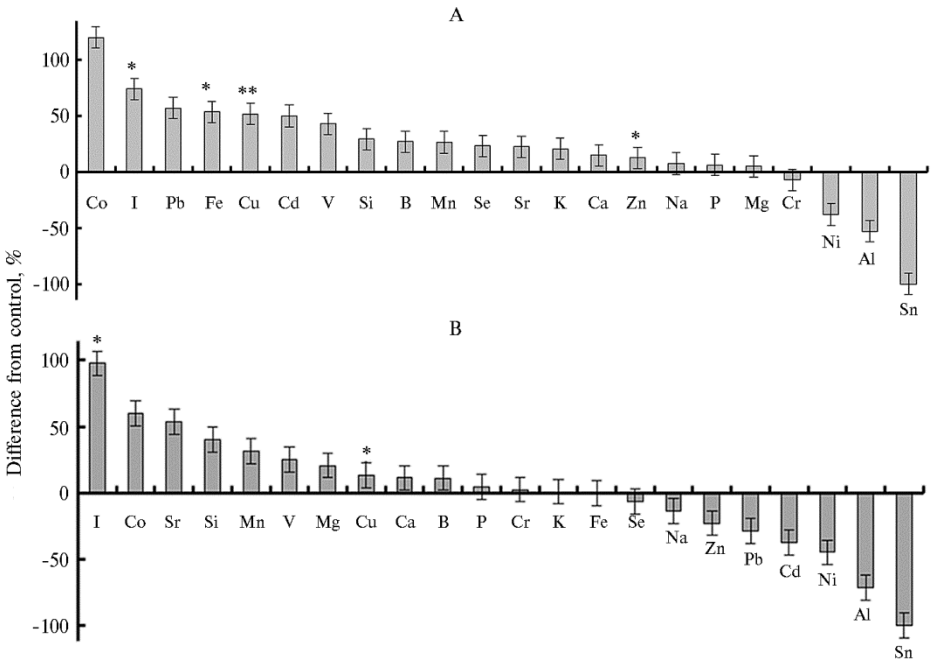

Fig. 2. Difference in pools of chemical element in cross Smena 7 broiler chickens aged 42 day from groups I (A) **and II** (B) **depending on formula of dietary Cu- and Zn-based additives.** See description of groups in section "Methodology". Star means that differences from control are statistically significant at $P \leq 0.05$.

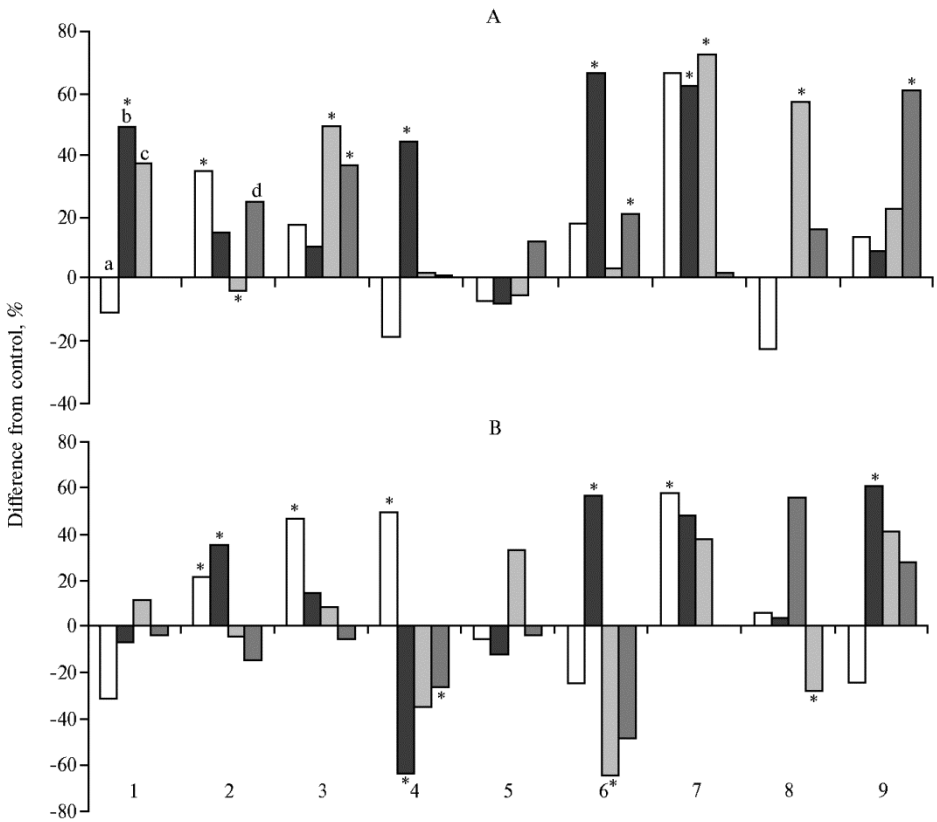

Fig. 3. Difference in accumulation of Cu as compared to control in cross Smena 7 broiler chickens from groups I (A) **and II** (B) **depending on formula of dietary Cu- and Zn-based additives:** 1 — hearth, 2 — brain, 3 — liver, 4 — spleen, 5 — kidneys, 6 — muscles, 7 — bursa of Fabricius, 8 —

thymus, 9 — feather; a, b, c, d — age of 21, 28, 35 and 42 days, respectively. See description of groups in section "Methodology". Star means that differences from control are statistically significant at $P \leq 0.05$.

and Sn in group I, while Pb and Cd levels rise. We explain this fact by competition for common transporters of copper, zinc, and other bivalent metals in intestines [44, 45]. Nanoparticles of metals, due to high penetration ability, may enter intestine cells not linking up with transportation proteins [46]. Then, transportation systems defining transfer of Zn and Cu could be used by other bivalent analogues (Pb, Cd) in group I but are more specific in group II.

Different mechanisms of entering and use of metals from UFPs and asparaginates is also supported by concentration dynamics of such elements in specific tissues and organs (Fig. 3).

Provision of Cu asparaginate during week 1 decreased Cu accumulation in feather by 24.5 % compared to control, however, afterwards, Cu excess over control reached 60.9 % after day 14, 41.4 % after day 21, and 28.1 % after day 28. In group I, this indicator during the entire research was higher than control values by 13.5, 8.8, 22.3 and 60.9 %. Feather is a marker biosubstrate to assess mineral status in poultry (Patent of the Russian Federation RU2478956C1). To our opinion, data we report in this paper show permanent change in availability of microelements in growing chickens. UFP-based diet results in more even entering copper to organism.

Therefore, comparison of Cu- and Zn-based dietary additives in the preparation forms of mineral salts, asparaginates, and ultrafine particles indicates that UFP-based forms increase biological availability of the microelements and has more expressed positive effect on productive properties of broiler chickens.

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