AGRICULTURAL BIOLOGY,
2016, V. 51, № 4, pp. 553-562
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.52/.58:636.087.72:546.72/.73-022.532

doi: 10.15389/agrobiology.2016.4.553rus
doi: 10.15389/agrobiology.2016.4.553eng

TO THE DEVELOPMENT OF INNOVATIVE MINERAL ADDITIVES BASED ON ALLOY OF Fe AND Co ANTAGONISTS AS AN EXAMPLE

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Acknowledgements:
Hematological studies were performed using standard techniques in the Laboratory of Agroecology of Nanomaterials and Test Center of All-Russian Research Institute of Beef Cattle Breeding (accreditation certificate RA. RU.21PF59 from 12/02/15). Analysis of chemical elements was performed in the laboratory of ANO Center for Biotic Medicine, Moscow (accreditation certificate GSEN.RU.TSAO.311, registration number in the State Register ROSS RU. 0001.513118).
Supported by Russian Scientific Foundation (project № 14-36-00023)
Received May 25, 2016

Abstract

The problem of joint use of antagonist elements in the nutrition of farm animals is solved through a separate feeding with such trace elements and through an increase in the dosage of substances. The unique properties of nanomaterials allow us to suggest the promising alternative solutions by combining antagonists in a single drug, i.e. ultra-fine powders of metal alloys. In this paper, we for the first time compared the growth, haematological and biochemical parameters of broiler chickens (Russian cross Smena 7) after feeding them with individual salts of two microelements or their alloy in the form of nanoparticles. The pair of antagonists (iron and cobalt) was chosen due to the same mechanism of their absorption in intestine. Salts $FeSO_4 \cdot 7H_2O$ and $CoCl_2$ (group I) or the derived nanoparticles (d = 62.5 ± 0.6 nm) of the metal alloy (group II) were used as sources of iron and cobalt and mineral supplement. After calculation of the proportion of common microelement pool to the value of its entry with fodder expressed in percentage, the excess of iron up to 50.0 % was registered in the group when alloy nanoparticles applied (comparing to the pure iron), and the excess of cobalt was 34.7 %. Biological significance of the obtained values is the amount of the element deposited in the body expressed in grams per 100 grams of element entering with the incoming feed. In this case, growth rate increased and metabolism changes in chicken were registered. During the experiment, weight gain exceeded the control in group I by 6 % ($p \leq 0.05$), and in group II by 11 % ($p \leq 0.001$). Feed costs for growing chickens in the control group was 2.48 kg that is by 9.3 and 13.7 % more than in groups I and II, respectively. Using Co-Fe alloy increased weight gain by 4.1 % ($p \leq 0.05$) compared with group I while the food consumption reduced by 4.8 %. Creatinine content in groups I and II was 63.9 % ($p \leq 0.01$) and 38.3 % ($p \leq 0.05$) higher than in the control, respectively. At the same time, the blood urea concentration in group I, and blood glucose level in group II increased compared to control by 38.5 % ($p \leq 0.05$) and 36.5 % ($p \leq 0.05$), respectively. However, the revealed increase of iron pool in group II was not associated with a significant increase in iron concentration in blood serum in relation to that in group I (it was possibly due to the homeostatic regulation, as an excess of iron may lead to the generation of reactive oxygen species) with a significant reduction in its concentration (by 74.3-78.3 %, $p < 0.01$) in control at dietary iron deficiency. Using nanoparticle preparations was accompanied by an increase in fraction of arginine in liver of experimental chickens up to 8.10±0.105 % as compared with the control value of 5.05±0.075 % (note, the growth-promoting effects of L-arginine were described in literature).

Keywords: nanoparticles of iron and cobalt, broiler chicks, growth intensity, chemical elements

In humans and animals, chemical elements interact with other elements [1, 2], amino acids [3], ferments [4], etc., in metabolism. The expediency of investigating such interactions is determined by the necessity of prenosological diagnostics and elementosis treatment, diet adjustments [5], and evaluation of true diet densities (6, 7). Historically, special attention has been paid to the dosing of

a small group of microelements (iron, zinc, copper, etc.) the absorption of which depends on their antagonists. Methods appeared to level the negative effect of some substances on the absorption of microelements, in particular, based on separate inclusion of antagonist substances in the diet [9]. Such intake of zinc and iron supplements has a positive effect on the growth and development of children in their first year of life [10]. The dosing schedules for a number of vitamin-mineral complexes (VMC) are based on the principle of separate use of antagonist microelements. The most famous Russian representative of this line of preparations is VMC Alphabet. Its efficiency is confirmed by a number of research demonstrating the facts of increased biological availability of microelements, including zinc, iron, etc. [11].

It seems that with the beginning of nanomaterials production, a similar result may be achieved by using metal nanoparticles. The substances of this class with the size of particles of about 10 nm are characterized by low toxicity [12]. It is reported that the preparations of metal nanoparticles (in particular, iron) are substantially superior to the respective mineral salts by their bio-availability [13, 14]. A number of works demonstrate the expediency of using nanosized microelements in diets for animals and poultry [15-18].

The properties of nanoparticles (including their high penetration power) allowed us to assume the high potential of the alternative solution — a combination of antagonist microelements into one preparation in the form of ultradisperse nano-powders of their alloys.

In this work, we have for the first time compared the growth, hematological and biochemical indicators in broiler chickens of domestic cross when feeding individual salts of two microelements or their nanosized alloy. The pair of antagonists (iron and cobalt) was selected due to the same mechanism of their intestinal absorption [19, 20]. According to our hypothesis, it should be expected that the preparation of Fe and Co alloy nanoparticles will have an advantage over their mineral salts by bioavailability of these elements in vivo and their production effect.

The purpose of the experiment was to test the approach for optimizing the mineral feeding of broiler chickens by introducing the nanoparticles of the alloy of two antagonist microelements into the diet.

Technique. The nanoparticles of the iron and cobalt alloy were obtained by high-temperature condensation (Migen-3, Institute for Energy Problems of Chemical Physics, RAS, Moscow) in accordance with a described methodology [21]. 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). According to the attestation results, the size of nanoparticles was 62.5±0.6 nm with a Fe:Co ratio of 7:3. To obtain lyosols for their further feeding to chickens, the water suspensions of nanoparticles were for 30 minutes exposed to ultrasound using a disperser UZDN-2T (Akadempribor, Russia) (35 kHz, 300/450 W, 10 mcA).

The tests were conducted on Smena 7 broiler chickens in a vivarium (Orenburg State University). The keeping conditions and test procedures met the instructions and 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). All efforts were taken to minimize the suffering of animals and to reduce the number of samples used.

A total of 100 hens of 1 day old were selected for the experiment. The chickens with assigned individual numbers (leg plastic tags) were weighed and

further kept in the same conditions. At the age of 2 weeks, based on individual daily weighing data and food consumption, three groups (one control and two test groups) were formed, each including 30 hens, by analogue pairs.

The complete feeds used were made with regard to the recommendations [22]. The poultry basic diet (BD) included the following ingredients: at the age of 14-28 days — wheat (320 g/kg) and barley (10 g/kg), sunflower cake (184 g/kg), soy bean meal (200 g/kg), fish flour (40 g/kg), vegetable oil (60 g/kg), corn grain (163 g/kg), wheat bran (10 g/kg), limestone (10 g/kg), and common salt (3 g/kg); at the age of 29-42 days — wheat (182 g/kg) and barley (50 g/kg), sunflower cake (180 g/kg), soy bean meal (75 g/kg), fish flour (45 g/kg), vegetable oil (45 g/kg), corn grains (400 g/kg), wheat bran (10 g/kg), limestone (10 g/kg), and common salt (3 g/kg). The content of vitamins and mineral salts was dosed using the premixes P5 and P6 (for the chickens of up to 28 days and older, respectively) (Koudijs MKorma, Russia) including vitamins A, D, E, K_3, B_1, B_2, B_3, B_4, B_5, B_6, B_{12}, B_c and H, and microelements Fe, Mn, Cu, Zn, I, Se and Co (with a diet dose of 2 %).

For the whole test period, the chickens from the control group received BD, while for the other chickens from days 14 to 42 the diet was supplemented with iron (7 mg/kg of food) and cobalt (3 mg/kg of food) with salts $FeSO_4 \cdot 7H_2O$ and $CoCl_2$ (group I) or nanoparticles of iron and cobalt alloy (group II) in the total amount identical to that in group I. Distilled water was used for watering.

Blood for test was taken at the age of 42 days before slaughter in the morning in a fasting state from the axillary vein into vacuum tubes (for morphological test — with EDTA as an anticoagulant, for assessment of the biochemical indicators — with the coagulation activator thrombin). The blood serum was analyzed within 3 hours after blood was taken. Upon slaughtering, the animals animals were weighed, and samples of tissues and organs were collected to assess the elementary content (the samples were immediately frozen and kept at –18 °C).

The morphological indicators were determined on a hematological analyzer (URIT-2900 Vet Plus by URIT Medial Electronic Co., Ltd, China). The biochemical blood serum test was made using an automatic analyzer CS-T240 (DIRUI Industrial Co., Ltd, China) and commercial veterinary kits (DiaVetTest by DIAKON-DS, Russia; Randox Laboratories Ltd., Great Britain). The amino-acid content of liver tissues was determined by capillary electrophoresis using the Kapel system (Lumex Marketing, Russia), after the preparation of samples including drying at the temperature of up to 50 °C, decreasing with petroleum ether and grinding until they passed through the sieve with square cells of 0.5 mm.

The chemical content of biomaterials and foods after ashing was studied by atomic emission spectrometry (Optima 2000 V, Perkin Elmer, USA) and mass spectrometry (Elan 9000, Perkin Elmer, USA) according to the manufacturer's instructions. The bio-substrates were ashed using a microwave decomposition system Multiwave-3000 (Anton Paar, Austria).

A pool of chemical elements in the body was assessed. The assessed ratio between the growth of substance pool in the organism and the amount of its consumption with food for the test period was taken as a conditional indicator of the chemical element bioavailability from food.

The statistical processing of the obtained data was done using the software package Statistica 10.0 (StatSoft Inc., USA) and Microsoft Excel. The results are provided in the form of average (M) and standard average error (m). The validity of differences in the indicators compared was determined by Student's t-test. The indicators with $p \leq 0.05$ were considered valid.

Results. As a confirmation for the working hypothesis on higher bioavailability of iron and cobalt from the nanoparticles of their alloy as compared to mineral salts, we consider the fact that by the end of the experiment the iron pool in chickens from test group II exceeded the control group by 72.4 % ($p \leq 0{,}001$) and was higher than Group I by 21.6 % ($p \leq 0.01$) (Fig. 1, A). The similar difference for cobalt was 59.5 % ($p \leq 0.001$) and 24.8% ($p \leq 0.05$), respectively. The difference in the content of iron and cobalt in liver and muscles varied from 20 % to 140 % (see Fig. 1, B).

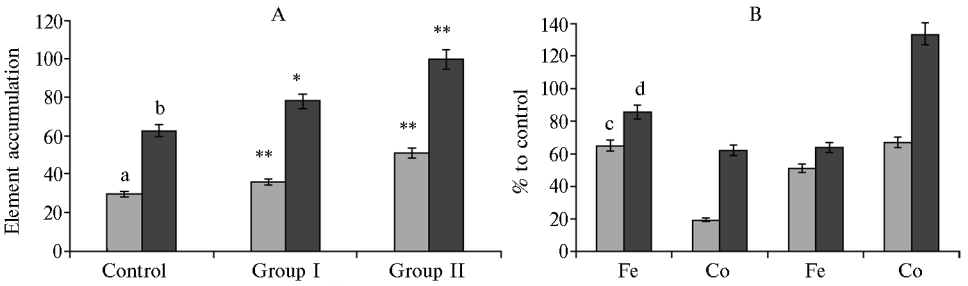

Fig. 1. The Fe and Co pool in Smena 7 cross broiler chickens of 42 days old (A) **and the difference in the accumulation of these elements in tissues as compared to the control group (without supplements)** (B) **when Fe and Co salts** (group I, $n = 15$) **or Fe and Co alloy nanoparticles** (group II, $n = 15$) **used in the diet:** a — Fe (mg), b — Co (mkg); c — group I, d — group II (see the description of groups in the *Technique* section; experiment in vivarium conditions).
*, ** Differences with control are statistically significant with $p \leq 0.05$ and $p \leq 0.01$.

The assessment of the total intake of chemical elements with food and the increase of their total pool allowed us to calculate the specific share accumulated in chickens for the test period. It was ascertained that with the use of nanoparticles the iron retention from food reached 50.0 % and that of cobalt was 34.7 %. It is in line with the results of M.F. Aslam et al. [23] obtained when using the preparations of iron nanoparticles and is naturally related to the specifics of cellular uptake of nano- and soluble forms of metals [23]. It is well known that iron can be intestinally absorbed by means of endocytosis as part of complex aggregates, for example, vegetable-derived ferritin. Ferritin may contain several thousand iron atoms [24, 25]. Metal nanoparticles are transported, in particular, by endocytosis [23]. This phenomenon is well described in the literature. The intensity of metal release from nanoforms is lower as compared to ionic forms, which suggests that nanoparticles of metals are a better alternative to mineral salts (for example, as a source of microelements). The relatively slow release of iron from nanoforms and the preserved content of this element in blood make sure there is no homeostatic pressure on the iron exchange as a reason for the oxidative stress. It may be an advantage in case of substantial iron consumption in the digestive tract, which is observed after the absorption of therapeutic doses of soluble iron [26, 27]. The lower capability of enterocytes to retain metals in the form of nanoparticles as compared to the ionic form also plays a certain role [28].

There are no grounds to believe that the regulation of Fe exchange released after the degradation of Fe+Co nanoparticles and from $FeSo_4$ will be based on various mechanisms. Given this, the increase in the total iron pool in the organism when using the preparation of nanoparticles should be considered as a result of relatively slow release of this element from the nanoform.

The identified increase in the iron pool in group II was not associated with the significant growth of blood iron concentration as compared to group I (Table 1). It may be related to homeostatic reactions regulating the amount of iron in biological fluids (it is known that excessive iron may lead to the gen-

eration of reactive oxygen species) [29]. At the same time the iron deficient diet fed to control chickens was accompanied by reduced iron concentration in blood as compared to group I and group II by 78.3 % ($p < 0.01$) and 74.3 % ($p < 0.01$), respectively.

Slight changes of hemoglobin content in the blood of chickens from group I and group II correspond to the research on Swiss mice (CD1) (30) showing that nano-Fe (III) by its ability to efficiently recover the amount of hemoglobin is very similar to iron sulfate.

1. Blood morphology and biochemical indicators of 42-day old Smena 7 broiler chickens with the diet including Fe and Co salts (group I) **or nanoparticles of Fe and Co alloy** (group II) ($M \pm m$, $n = 15$, experiment in vivarium)

Parameter	Control (without supplements)	Group I	Group II
Iron, mmol/l	15.2±0.40	27.1±0.25*	26.5±0.75*
Hemoglobin, g/l	95.6±1.01	98.0±1.63	101.0±1.01*
Erythrocytes, $\times 10^{12}$/l	2.1±0.13	2.6±0.13	2.6±0.12
Leucocytes, $\times 10^{9}$/l	16.3±1.76	19.5±1.13	21.0±1.09*
Glucose, mmol/l	6.3±0.27	6.4±0.23	8.6±0.15*
Cholesterol, mmol/l	3.6±0.05	4.4±0.27	4.3±0.37
Urea, mmol/l	1.3±0.02	1.8±0.06*	1.5±0.10
Total protein, g/l	33.3±1.20	33.0±2.51	37.3±1.28*
Alkaline phosphatase, $nmol \cdot sec^{-1} \cdot l^{-1}$	343.6±24.86	336.6±17.39	423.3±16.36
Creatinine, μmol/l	31.3±1.66	51.3±1.49*	43.3±1.73*
Bilirubin, μmol/l	3.5±0.27	3.6±0.02	1.1±0.05

* Differences with control are statistically significant with $p \le 0.05$.

We have found the increase of creatinine in blood by 63.9 % ($p \le 0.01$) in group I and by 38.3% ($p \le 0.05$) in group II. Moreover, the chickens from group I had a higher concentration of urea by 38.5 % ($p \le 0.05$), and in group II the amount of glucose exceeded that in the control group by 36.5 % ($p \le 0.05$).

2. Growth and food consumption in Smena 7 broiler chickens with the diet including Fe and Co salts (group I) **or nanoparticles of Fe and Co alloy** (group II) ($M \pm m$, $n = 15$, experiment in vivarium)

Group	Live weight				Food consumption per 1 kg of weight gain	
	total, g		gain for the experiment (days 14-42)			
	day 14	day 42	kg	to control, %	kg	to control, %
Control	262.3±7.5	1523.3±10.4	1.26±0.08		2.48	
I	267.7±9.1	1606.7±19.7	1.34±0.11	106	2.25	90.7
II	266.7±12.7	1661.3±10.9*	1.40±0.09	111	2.14	86.3

N o t e. Weight gain and food consumption in the control group are taken as 100 %.
* Differences with control are statistically significant with $p \le 0.05$.

The iron and cobalt in the diet was followed by higher intensity of chickens growth. In particular, for the test period the weight gain in group I exceeded the control indicators by 6 % ($p \le 0.05$), and in group II — by 11 % ($p \le 0.001$). Food consumption in the control group was higher by 9.3 % and 13.7%, respectively. Moreover, as compared to the effect of salts of these microelements, using their alloy in the form of nanoparticles increased the live weight gain by 4.1 % ($p \le 0.05$) with reduced food consumption by 4.8 % (Table 2).

Using nanoparticles was followed by an increase in the mass fraction of arginine in the liver of chickens from group II up to 8.1±0.105 % as compared to control value (5.05±0.075 %) (Fig. 2).

It is generally in line with the previously obtained data [31, 32] on the biological effect of iron and copper particles on broiler chickens. It was ascertained that the intake of nanoparticles and microparticles ($d = 10\text{-}40$ μm) was followed by the accumulation of arginine in the liver. It is known that arginine metabolism is closely related to the development of inflammatory

and oxidative stress [33, 34]. Arginine is one of the factors regulating the growth of young animals [35-37]. The growth stimulating effects of L-arginine are related to the changes in the balance of consumed and expended energy of fat or the reduced white fat growth. L-arginine activates the mitochondrial biogenesis and the development of brown adipose tissue [38]. A.M. Fouad et al. [39] showed the reduction of abdominal adipose tissue and the amount of circulating lipids in Cobb 500 cross broiler chickens under the influence of dietary L-arginine.

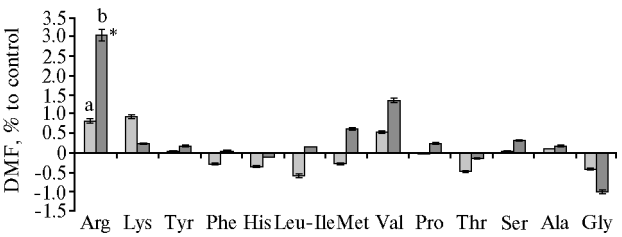

Fig. 2. The difference in the mass fractions (DMF) of aminoacids in the liver of 42-day old Smena 7 broiler chickens with the diet including Fe and Co salts (a, group I, n = 15) **or nanoparticles of Fe and Co alloy** (b, group II, n = 15) (experiment in vivarium).
* Differences with control are statistically significant with $p \leq 0.05$.

In our experiment, the use of nanoparticles of Fe and Co alloy did not result in critical accumulation of protein metabolism end products, in particular urea. At the same time, group I demonstrated a tendency towards the increase in these values with a statistically significant difference with control after administration of Fe and Co salts (38.46 %, $p < 0.05$).

The analysis of nitrogen containing compounds showed the intensified protein exchange after the intake of cobalt in various forms. The presence of cobalt in nanoparticles might become an additional factor in protein exchange activation. Earlier, similar results were reported by L.G. Kashirina et al. [40].

The use of the preparative form of nanoparticles (unlike salts) was followed by an increase in blood protein content by 12.0 % ($p \leq 0.05$) as compared to the control group. The growth rate of chickens from group II was significantly higher. The blood protein content in group I did not differ from the control group.

An essential factor of intensification was the higher food digestibility. In particular, in group II the digestibility of food protein grew by 3.1 % ($p \leq 0.01$), and that of carbohydrates — by 2.3 % ($p \leq 0.05$). The differences between the control group and group I were statistically invalid.

Thus, the research supports the proposed approach to optimizing the mineral diet of poultry by using alloys of nanosized antagonist microelements. It is known that in solutions metal salts dissociate, hydrate and become physiologically non-available. Moreover, unconjugated iron is an inductor of lipid peroxidation and peroxide protein distruction. Metals in nanoparticles, if they release gradually, will have a higher availability and their toxic effect will be reduced to the minimum. This may be the reason for the observed positive effect on growth in weight and certain increase in the hemoglobin content in chickens at administration of the nanopreparation. In our opinion, the revealed experimental facts evidence a higher availability and productive effect of the Fe and Co alloy nanoparticles as compared to the mineral salts of these elements.

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