

# Antagonist metal alloy nanoparticles of iron and cobalt: impact on trace element metabolism in carp and chicken

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**Abstract.** Introduction: Substances reducing iron digestion in digestive tract of animals were described earlier. Methods for elimination of negative influence of individual substances on iron assimilation (including separated introduction of iron antagonists into the diet) were developed. However, with the beginning of nanoparticle production the similar result can be achieved with the use of nanoparticle-containing alloy of iron and antagonist metals. Objectives: The purpose of the current study is to check new solution for the optimization of feeding of agricultural animals using antagonist microelements and also to evaluate the influence of new microelement preparation containing iron and cobalt alloy on growth and metabolism of the chemical elements in carp and chicken. Materials and methods: Experimental research on broiler chicken “Smena” and carp was performed to study metabolism of 25 chemical elements using a control diet without mineral sources of iron and cobalt salts compared with diet containing them, diet with alloy nanoparticles of iron and cobalt and intramuscular injections with them. Results: The retention of iron from the medication containing the alloy nanoparticles was 73.9% and that from ferric sulfate was 20.7% or less by 53.2%. In a similar comparison, the difference for cobalt sources was 22.0%. In our research on carp the retention from nanoparticles was higher in comparison with iron salts by 16.4% and cobalt salts by 3.1%. Conclusion: The experiments suggest evidence of a potential promising future for substances containing an antagonist metal alloy of iron and cobalt that could be used in animal feed.

**Key Words:** nanoparticles of iron, cobalt, element metabolism, carp, chicken.

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## Introduction

A highly important feature of the metabolism of chemical elements in humans and animals is that such elements interact with other elements, i.e., phytate, amino acids and their salts (Oberleas, 1996; Goyer, 1997; Wu et al 2013), peptides and polyphenols from partially digested proteins (Hurrell et al 2010), calcium (Hallberg et al 1993), lead (Goyer, 1993), zinc (Kelleher et al 2006) and others, especially at the absorption stage in the digestive system. Such interactions require study to resolve issues of prenosological diagnostics, treatment of element deficits (Yudina et al 2003), and diet correction (Kudrin et al 2000), assessment of nutritional value of diet (Huang et al 2005; Fang et al 2007). Historically, special attention has been paid to a small group of microelements whose metabolism is subject to impact from antagonists, with iron being perhaps the most common of these materials.

The issue of iron deficiency prompted establishment of the International Nutritional Anemia Consultative Group (INACG, 1978) with a mission of setting criteria for assessment of iron status and detection of the biological availability of compounds in biologically active additives and foods. Following one of the

models for assessing the biological availability of non-heme iron (Reddy et al 2000), substances have been detected that have an influence on iron assimilation, and these include products of animal origin, ascorbic acid, and phytate, among other sources. Methods have been developed to eliminate the negative impact of certain individual substances on iron assimilation, and one of these methods suggests separated introduction of antagonists into the diet (Lazarev et al 2002). Of particular interest in the current work is the separated intake of zinc and iron-containing medications in children during their first year of life, which is a method that offers a positive influence on their growth and development (Hind et al 2004). Antagonism can be excluded at the level of the digestive system only in the case of intramuscular injection. However, because nanomaterial production is now available, a similar result can be obtained through the use of nanoparticles of metals, which are absorbed differently from ions. With a size of approximately 100 nm, the substances pertaining to this class reveal lower toxicities (Bogoslovskaya et al 2007; Bogoslovskaya et al 2009). Medications containing metal nanoparticles (and iron in particular) perform much better than other similar substances in terms of their bioavailability (Nikonov et al 2011). In addition to the high penetrating power

of nanoparticles, these features suggest the potential advantage of an alternative solution that joins the antagonists in one substance: ultra-dispersed powders of metal alloys.

The purpose of the current study is to check new solution for the optimization of feeding of agricultural animals using antagonist microelements and also to evaluate the influence of new microelement preparation containing iron and cobalt alloy on growth and metabolism of the chemical elements in carp and chicken. The choice of the iron and cobalt antagonists can be explained by the fact the two materials share a similar absorption mechanism in the intestines (Underwood, 1977; Smith *et al* 1987). We assumed that *in vivo* bioavailability of Fe and Co nanoparticles from their alloy would be higher in comparison with mineral salts. It was assumed that alloy nanoparticles of Fe and Co salts would influence on the size of pools of some elements in body of animal in different ways.

The properties of using nanomaterials for magnetic resonance (MR) system (Pouponneau *et al* 2010) also need to be studied in details.

Alloys of Fe and Co are considered to be the most promising probes for theranostics with the required sensitivity of imaging and therapeutic effect (McCarthy *et al* 2008; Kim *et al* 2009; Xie *et al* 2009; Lacroix *et al* 2010) among different magnetic nanoparticles.

## Material and methods

### Ethics statement

The experimental research on animals was conducted according to instructions, recommended by the Russian Regulations, 1987 (Order No. 755 on 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)”.

Animals were housed in a vivarium of the Trace Element Institute of the Orenburg State University. The vivarium is equipped with all necessary equipment and has qualified personnel. Veterinary requirements are met.

### Synthesis and assessment of nanoparticles

The iron-cobalt alloy nanoparticles were obtained by high temperature condensation using Migen-3 equipment at the Institute for Energy Problems of Chemical Physics (Russian Academy of Sciences), Moscow. Synthesis strategy was described earlier (Zhigach *et al* 2000). Materials were assessed by electron scanning and transmission microscopy using the following equipment—JSM 7401F and JEM-2000FX (“JEOL”, Japan). X-ray phase analysis was performed with diffractometer DRON-7. According to the results of particle assessment it was established that iron nanoparticles are sized  $62.5 \pm 0.6$  nm. Fe and Co ratio is 7:3. There is approximately  $96.0 \pm 4.5\%$  of crystalline metal in the core of these particles,  $4.0 \pm 0.4\%$  of oxide  $\text{Fe}_3\text{O}_4$ ,  $\alpha$ - $\text{Fe}_2\text{O}_3$  and  $\gamma$ - $\text{Fe}_2\text{O}_3$  thickness of the oxide film on the surface is 6 nm.

### Mineral salts and feed

Salts  $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ ,  $\text{CoCl}_2$  were bought from “Vekton”, Russia.

### Study Design

The experimental study was performed using chicken and carp whitebait models. Set of eggs ( $n=500$ ) was prepared in hatching house. The eggs for experiment were produced in the poultry

farm “Orenburgskaya” by the cross parent flock “Smena-7”. Day-old chicks were separated according to sex. For experiment 150 chickens were selected (selection traits: good maturity, differences in live weight less than 5%). All selected chicks were tagged (plastic tags fixed on feet), weighed and housed in the same conditions. According to the data after individual weighing performed on a daily basis and assessment of feed costs all poultry were divided into four groups by paired analog method,  $n=30$ : one control group (I) and three experimental groups (II, III, IV).

Experimental animals were housed in similar conditions according to the existing standards on density, humidity, etc. Cages were equipped with automatic two-nipple drinkers and feeding system (length – 90 cm).

Poultry were fed with complete feed made with due regard to the recommendations of the All-Russian Poultry Research and Technology Institute (Fisinin *et al* 2000).

The research methodology supposed to feed poultry with basic diet that included the following: Day 14 through Day 28: wheat = 320 g/kg, barley = 10 g/kg, sunflower cake = 184 g/kg, soy ground oil-cake = 200 g/kg, fish flour = 40 g/kg, vegetable oil = 60 g/kg, maize = 163 g/kg, wheat bran = 10 g/kg, limestone = 10 g/kg, and salt = 3 g/kg; Day 28 – Day 42: wheat = 182 g/kg, barley = 50 g/kg, sunflower cake = 180 g/kg, soy ground oil-cake = 75 g/kg, fish flour = 45 g/kg, vegetable oil = 45 g/kg, maize = 400 g/kg, wheat bran = 10 g/kg, limestone = 10 g/kg, and salt = 3 g/kg. Mineral and vitamin nutrition was ensured via introduction of premixes with no iron- and cobalt-containing substances.

The elemental composition of feed and combined feed was studied by atomic emission spectrometry and mass spectrometry with inductively coupled plasma using Optima 2000 DV and Elan 9000 equipment.

Chicken of Group I (control) were fed with basic diet for the whole period of experiment. Chickens of experimental groups were also fed with iron and cobalt medication during the period from 14 to 42 days. Iron (7 mg/kg) and cobalt (3 mg/kg) with salts of  $\text{FeSO}_4 \times 7\text{H}_2\text{O}$  and  $\text{CoCl}_2$  were added to the diet of Group II.

The quantity of the consumed elements from salts was determined in the course of the balance experiment that was performed earlier. At the same time this dosage is compared with dosage of these elements in form of nanoparticles. The chosen dosage corresponded to the digestible amount of metals from mineral salts as established experimentally for chicken at the age of 10-14 and 28-32 days (Georgievsky *et al* 1979). In Group III, the salts of iron and cobalt were replaced with nanoparticles of iron and cobalt alloy, and the total content was similar to that in the experimental groups. The chickens in Group IV consumed a diet that contained no salts or iron/cobalt. Chicken of group IV were injected (thigh) with medication containing Fe and Co alloy nanoparticles. The lyosols were prepared via ultrasonic treatment ( $f=35$  kHz;  $N=300$  (450) watts;  $A=10$  microliters) of the nanoparticle suspension (30 minutes) in distilled water. Chickens drink plenty of water from nipple drinkers.

The size of pool of 25 chemical elements in organism of 14 and 42-day chicken was assessed in the course of experiment. Animals were sacrificed before the main experimental period. 14-day chickens were slaughtered to determine background

content of microelements). In the end of the experiment 42-day chicken were slaughtered according to the technology of rearing broiler chicks (Imanguilov *et al* 2004).

Method of euthanasia was decapitation under Nembutal anesthesia (inhalation).

Feather, skin, flesh of carcass, viscera, gastrointestinal tract, visceral fat, blood, etc. of each chicken was weighed. Then elemental composition of tissue homogenate was determined. The size of pool of chemical elements in body was calculated summing up weight of elements in individual organs and tissues. Difference between an absolute value (mean - X) of some element in the experimental group and the control group was expressed in % in order to compare the pools of chemical elements.

It was subsequently divided by the average amount of this element in the compared group.

The bioavailability of cobalt and iron from salts and nanoparticles was calculated as difference between pools of these elements in body of 42-day and 14-day old chicken. Availability of these elements from basic diet was taken into account. The productive effects from the medications were assessed according to individual weighing performed on a daily basis.

The experiment involving the carp population was performed following a similar pattern. Carp underyearlings (*Cyprinus carpio*) (n=600) with weights of 3-5 g were bought from "Iriklyarba" Company. Live fish were brought to vivarium of the Orenburg State University and were kept in fish tanks with life support system within 30 days of preparatory period. Fish were separated according to the live weight before putting the fish into the tank. After the preparatory period it allowed us to form three similar experimental groups by paired analog method (n=50): one control (I) and two experimental groups (II, III).

Experimental fish were housed in similar conditions according to the existing standards on density, humidity, etc. The duration of experiment was 63 days, including preparatory period of 28 days.

Carp underyearlings were kept in fish tanks with capacity of 300 liters (125x70x40 cm), made of metal frame and silica glass. Every tank is equipped with filtration and air-intake system. Water temperature is maintained 25±1.1°C.

The feed used for the carp was control diet (CD) containing fish flour – 20%, soy ground oil-cake – 41%, sunflower ground oil-cake – 25%, vegetable oil – 5%, and wheat flour – 9%. The methodology called for maintaining the Group I fish on the control diet (CD). In addition to the CD, the Group II fish were also given a mixture of iron and cobalt salts ( $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ ,  $\text{CoCl}_2$ ), and the Group III fish were fed nanoparticles of Fe and Co alloy (dosage = 7 and 3 mg/kg of feed, respectively, calculated per element).

The feed production was based on stage-by-stage mixing of the feed components with the nanoparticles, and the feed was further subjected to extrusion (mixture humidity = 25-30%, temperature = 60/80°C). Daily weighing was performed throughout the study. The size of pool of chemical elements in body of fish was determined summing up the content of elements in individual tissues and organs.

Elemental composition of bio-substrate of chicken and carp was analyzed in the test laboratory of ANO "Centre for Biotic Medicine, Moscow, Russia (accreditation certificate ROOS. RU 0001.513118 by the Russian Federal Agency for Technique

Regulation and Metrology, ISO 9001:2008 certificate 54Q10077 by Global Certification Ltd). Sample tissues were analyzed by methods of an atomic emission and mass spectrometry with inductively-coupled argon plasma using Optima 2000 DV and ELAN 9000 equipment (<http://en.microelements.ru/laboratory/accreditaion-area/>).

This study was performed in strict accordance with the recommendations of the Committee on Ethics and the Trace Element Institute of the Orenburg State University (established according to the Order No. 89 of the Rector of the Orenburg State University as of March 21, 2008). Protocol #5 was approved by the Committee on Ethics of the Trace Element Institute. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were designed to minimize suffering. All sections of this report adhere to the ARRIVE Guidelines for reporting animal research (Kilkenny *et al* 2010). A completed ARRIVE guidelines checklist is included in Checklist. All surgery was performed under anesthesia, and all efforts were made to minimize suffering.

Experimental data are presented with arithmetic mean values of absolute and relative values (M), errors of mean (m) and standard deviation (s). Results were considered significant at  $P < 0.05$ . The statistical significance was determined using the Student's t test. Statistical processing of the results was performed based on generally approved methods using Excel in the software package Office XP and Statistica 6.0.

## Results

The experiments on chickens demonstrated the utility of replacing cobalt and iron salts with nanoparticles of their alloy.

The significant pool of these elements present in the control chickens at the beginning of the experiment (14 day) (Fe = 13.5 mg and Co = 12.2 mcg per chicken. The size of iron and cobalt pool was calculated on the basis of slaughter of 14-day animals. The content of these microelements was determined. Live weight differences between Groups II and III appeared at the age of 37 days only (Figure 1). In the following five days, the difference was still present, and over the entire duration of the experiment, the live weight gain in Group III was more than 74.3 g/head greater than that of Group II, representing a difference of 5.8% ( $P < 0.05$ ). Difference in live weight gain between I (control) and III group was 100.4 g or 7.9 % ( $P < 0.001$ ) in the experiment.

The experiment produced no reliable evidence for the hypothesis that the methods of introducing iron-cobalt alloy nanoparticles could be responsible for the growth intensity in the poultry. Live weight of chicken from Groups III and IV had no significant differences.

Moreover, the intramuscular introduction of nanoparticles suppressed the metabolism of most of the essential and conditionally essential elements (Table 1). It was accompanied by a significant decrease in growth intensity of chicken at the age of 24-28 days (10-14 days after the injection).

After 28 days of the experiment, the total pool of iron in Group IV was greater than that of Group III by 10.3% ( $P < 0.05$ ). The similar difference in weight of aluminum was 18.8% ( $P < 0.001$ ), cadmium (by 33.3%) ( $P < 0.001$ ), and lead (by 81.3%) ( $P < 0.001$ ). Differences in total content of iron, aluminum, cadmium were determined as a difference between groups according

Table 1. Difference in pool of chemical elements in chickens and carp, %

Chemical elements	Chicken		Carp
	Group III compared with Group IV	Group III compared with Group II	Group III compared with Group II
As	-1±0.074	-21.6±1.092*	41±0.696**
B	69.1±0.808**	41.2±0.469***	0.7±0.035
Co	52.5±0.897***	22±0.215***	5.4±0.065
Cr	32.6±0.521***	29±0.333***	-11.2±0.212
Cu	1.91±0.025***	7.01±0.078*	0.9±0.027*
Fe	-10.3±0.141*	35.4±0.52***	18.5±0.712*
I	19.2±0.585***	8.77±0.179	52.1±1.254**
Li	20±0.94	0	9.1±0.112
Mn	31.3±0.366	36.2±0.372***	1.1±0.065
Ni	4.8±0.337	-16.2±0.775	27.3±0.254**
Se	15.8±0.143**	25.6±0.207**	12.6±0.113
Si	65.4±1.451**	-15.3±0.128**	16.2±0.967*
V	16.7±0.612	-5.4±0.125	8.2±0.108*
Zn	0.05±0.0008	6.98±0.092*	23.1±0.475*
Ca	6.38±0.51	-17.4±0.966	11.9±0.032*
K	32.7±0.409**	14.1±0.117**	14±0.312
Mg	16.7±0.689	-10±0.254	19.3±0.321
Na	18.9±0.47	6.52±0.106	14.4±0.146
P	6.56±0.37	-9.01±0.343	17.3±0.245**
Al	-18.8±0.248***	73.9±0.68***	7.1±0.35
Cd	-33.3±0.745***	48.8±0.88***	41.8±0.487***
Hg	0	0	10.7±0.054
Pb	-81.3±0.65***	14.3±0.17**	-10.3±0.096
Sn	-0.52±0.042	-17±0.95	71±2.125
Sr	-0.32±0.025	-16.4±0.88	20.3±0.896

\* - results are statistically significant ( $p < 0.05$ ), \*\* - results are statistically significant ( $p < 0.01$ ) \*\*\* - results are statistically significant ( $p < 0.001$ )

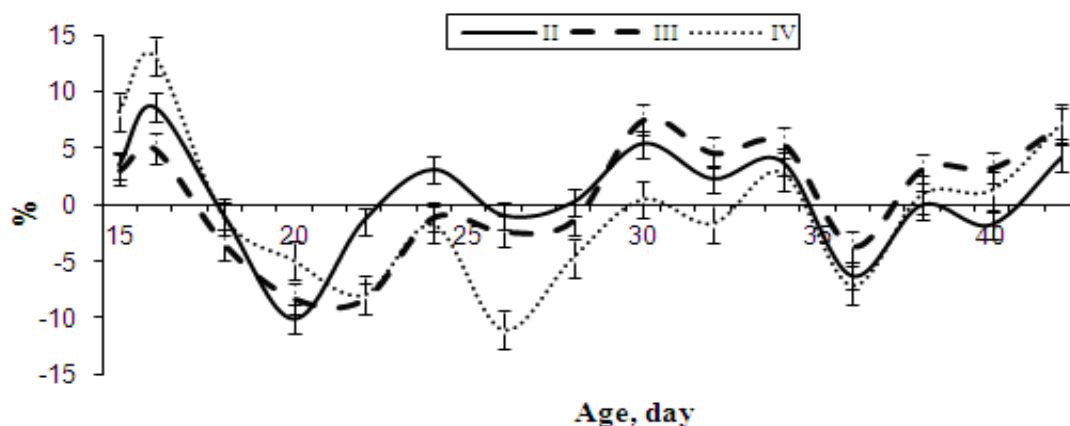


Figure 1. Dynamics of difference in live weight between Groups II(-); III(--); IV(---) and I (control group). Live weight differences between Groups II and III appeared at the age of 37 days only. It remain in the following five days.

to the content of these microelements in the organism. The total content of some element in organism was determined by the weight of this element in separate organs and tissues; this weight was calculated on the basis of weight of an organ (tissue) and its chemical composition.

The research project also demonstrated the relatively high bio-availability of elements from nanoparticles introduced per os. At the end of the experiment, the average Fe pool in body of chicken in Group II was  $35.8 \pm 1.41$  mg, Co  $77.9 \pm 8.85$  microgram, in Group III Fe  $50.7 \pm 2.89$  mg and Co  $99.5 \pm 7.42$  microgram. Fe pool in chicken from I (control) group was  $29.4 \pm 3.41$  mg, Co

62.4±5.85 microgram. Taking into account the deposits of Fe 15.9 mg, Co 5.1 microgram in the body of control chickens for 28 days, the deposition of elements from the injected products was Fe 5.9 mg, Co 14.7 microgram in Group II, in Group III - Fe 20,8 mg and Co 36,7 microgram. It makes it possible for us to calculate the efficiency of element conversion from the injected medications.

The retention of iron (endogenous losses not included) from nanoparticles (Group III) was 73.9% and that from ferric sulfate (Group II) was 20.7% or by 53.2 % less. In a similar comparison, the difference for cobalt sources was 22.0%.

The total pool of the majority of the essential microelements in the chickens of Group II was observed to decrease (Table 1). In addition, compared with Group III, the largest change in Group II chickens was observed in the pool of cobalt (22%) ( $P < 0.001$ ) and iron (35.4%) ( $P < 0.001$ ). The alloy nanoparticles of the two metals had no negative impact on the pool of iron and cobalt antagonists. In contrast, the pool of iodine and zinc in the Group III chickens was observed to increase. An important result is that the Group III chickens showed an increase of up to 1.14 millimole/kg W0.75 in the total pool of the fourteen essential and conditionally essential elements, accounting for a 24.1% ( $P < 0.001$ ) increase over the same index in Group II. At the same time, the use of alloy nanoparticles produced a significant increase in the pools of cadmium (48.8 %) ( $P < 0.001$ ) and aluminum (73.9) ( $P < 0.001$ ). Similar results were obtained from the experiments on the carp population in which introduction of a medication containing the respective nanoparticles resulted in a significant increase in the levels of cadmium (41.8%) ( $P < 0.001$ ) in the fish. Additionally, the level of toxic elements did not exceed the acceptable concentration (Table 1).

Different influence of the compared medications on metabolism of Sn, As, other elements in body of chicken and carp may be explained by different absorption and outflow of substance for fish in water environment.

When the experiment ended, the live weight of the Group III fish was 45.4 g, which was 5.9% ( $P < 0.05$ ) greater than the same index in Group II and 11.3% ( $P < 0.01$ ) above that in Group I (control). The conversion index for protein and energy value of the feed reveals a result similar to the indices in the growth dynamics of the studied carp population. The data on the bioavailability of the elements show that introduction of the nanoparticles into feed adds to the iron and cobalt bioavailability compared with Group II by 16.4% and 3.1%, respectively.

## Discussion

Hypothesis about higher bioavailability of iron and cobalt from alloy nanoparticles in comparison with mineral salts has been confirmed. It does not contradict previous studies on the use of iron nanoparticles (Ho *et al* 2011), and naturally connected with a separate mechanism of cellular uptake of nano-metals compared to soluble forms (Aslam *et al* 2014). Lower ability of erythrocytes to keep metal after nanoparticle introduction plays a certain role as compared with ions (Pereira *et al* 2013). The possible influence of alloy nanoparticles on metabolism of other elements is determined by their chemical instability and degradation in the body (Ho *et al* 2011). Accordingly, elements in the flow body leads to a change in the metabolism of other elements. The increase in the relative amounts of the toxic

elements could be accounted for by a reduction in the essential elements (Kudrin *et al* 2000).

There is no reason to assume that the mechanism of regulation of iron metabolism obtained from alloy Fe + Co will be different from that of iron obtained from the content of FeSO<sub>4</sub>. Accordingly, the increase in the common pool of iron in body when using nanoparticle preparation should be considered as a consequence of the relatively slow release of iron from nanoFe<sup>3+</sup>. It may be an advantage in terms of preventing the generation of nontransferrin bound Fe that can be observed following absorption of therapeutic doses of soluble iron (Dresow *et al* 2008; Hurrell, 2011; Lomer *et al* 2012; Schumann *et al* 2012; Aslam *et al* 2014). Lower accumulation of cobalt in organism when: CoCl<sub>2</sub>; CoSO<sub>4</sub> is used despite nanoparticles per os can be explained by the antagonism of Fe and Co during their absorption from the digestive tract (Smith *et al* 1987). Decrease in cobalt pool after intramuscular introduction of Fe and Co alloy confirms this fact.

It has been established by our research that alloy nanoparticles of Fe and Co and salts will influence on the size of pools of some elements in body of animal in different ways. For example, it affected on the pools of cadmium, lead and aluminum in body of chicken. The use of nanoparticles promoted their increase. An overwhelming number of essential and conditionally essential elements decrease at intramuscular injection of nanoparticles. It was accompanied by a significant reduction in the growth rate of chicken at the age of 24-28 days (10-14 days after injection). The similar results were obtained for iron nanoparticles earlier (Sizova *et al* 2015).

For various reasons, including the potential impact of the salts on the micro-ecological status of the chickens (Yudina *et al* 2003) and competition among various ions at the stage of absorption (Smith *et al* 1987), among others, the total pool of the majority of the essential microelements in the chickens (they were fed with iron and cobalt in a form of salt) was observed to decrease. It can be connected with the fact that there is no antagonism of Fe and these elements during their absorption. A negative correlation between Fe intake from food and content of cadmium and lead in blood is known (Wang *et al* 2012). It occurs because iron ions inhibit absorption of other elements in intestine (Fung *et al* 1997; Watts, 1998; Choi *et al* 2005). Competition for the common transporters of iron and other divalent metals in intestine are one of reasons (Barany *et al* 2005; Ranganathan *et al* 2011). Due to high penetration power of nanoparticles, metals in their composition can penetrate in intestinal cells omitting traditional ways of fixation and transfer by proteins. It is confirmed (Pereira *et al* 2013; Latunde-Dada *et al* 2014).

The importance of intestinal absorption during the assessment of inter-element relationships was confirmed by the fact of increasing pools of cadmium, lead and aluminum in body of chickens after intramuscular injections in comparison with feeding with nanoparticles (Table 1). It is logical these metals increase in body only through the feed because they lack in the intramuscular medication.

At the same time, transfer of iron, derived from nano Fe (III), is ferroportin-mediated (Aslam *et al* 2014). Thus, changing pools of chemical elements could result in Fe and Co homeostasis. It is known that Fe concentration is strictly controlled in biological fluids. Too much iron may generate active oxygen

(Braun *et al* 1999). Earlier it was demonstrated that ferroportin is synthesized together with an additional entry of iron in blood (Lieu *et al* 2001; Delaby *et al* 2005). Probably, the ability of ferroportin to transfer other metals including cadmium (Chung *et al* 2004; Troadec *et al* 2010) can lead to the change of their common pool in organism.

At the same time, changes in pools of chemical elements after the use of nanoparticles are determined by the response of body to the entry of cobalt. Antagonistic interrelations of Co and B and synergism of Al, Fe, Si are known from the studies (Zaksas *et al* 2013).

## Conclusion

It was established that *in vivo* bioavailability of Fe and Co nanoparticles from their alloy would be higher in comparison with mineral salts. The greatest difference was registered in chicken. The experiments suggest evidence of a potential promising future for substances containing an antagonist metal alloy of iron and cobalt that could be used in animal feed. Facts obtained by biological assessment of nanoparticle medication containing iron and cobalt alloy can be useful in biology and medicine.

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