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Growth Enhancement by Intramuscular Injection of Elemental Iron Nano- and Microparticles

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Abstract

We studied the influence of highly dispersed elemental iron preparations on the productivity and metabolism of broiler chicks. Iron nanoparticles were synthesized using high-temperature condensation. No toxicity of the nanoparticles and their agglomerates was observed in an experiment utilizing a genetically engineered luminescent strain of *Escherichia coli* (K12 TG1). The efficiency of single intramuscular injections in 120 broiler chicks (Smena 7) of preparations containing iron particles with sizes of 80 ± 5 nm (nanoparticles), 923.7 ± 29.6 nm (nanoparticle agglomerates) or 9.8 ± 0.4 nm (microparticles) was compared. The use of these preparations increased the live weight of the chickens. The maximum difference in live weight was observed 4 days after the injection of nanoparticles (9.8%; $P \leq 0.01$), 17 days after the injection of agglomerates (4.97%; $P \leq 0.01$), and 17 days after the injection of microparticles (8.5%; $P \leq 0.01$). Injections with iron preparations were accompanied by increased protein deposition, causing daily weight gain in chickens. This gain was higher by 1.3 - 4.3 g when nanoparticles were used, by 0.6 - 1.4 g for agglomerates, and by 0.1 - 1.4 g for microparticles. The iron preparations promoted an increase in the arginine content of the chicken liver compared with that of control animals. After the use of nanoparticles, the arginine content increase was 2.25% ($P \leq 0.05$) within 1 day and 3.78% ($P \leq 0.05$) 7 days after injection. The arginine content increased by 2.08% ($P \leq 0.05$) 7 days after the use of agglomerates and by 3.86% ($P \leq 0.05$) 21 days after the use of microparticles. Thus, similar biological effects were shown for different sizes of elemental iron particles. These effects were enhanced as the particle size decreased, although further investigation is required before the joint use of metal nanoparticles and arginine.

Keywords: iron nanoparticles, poultry chickens, agglomerates, iron microparticles, intramuscular injection

1. Introduction

Nanodisperse forms of essential metals and their compounds are widely used in animal husbandry as microelement preparations. Compared with traditional preparations, these preparations are notable for the lower toxicity of nanoparticles (Zhang et al., 2005; Hao et al., 2009; Wang et al., 2014) and the greater bioavailability of microelements (Rohner et al., 2007).

Work aimed at creating new iron preparations is of great interest. Iron deficiency, or anemia, is thought to affect the health of more than 1 billion people worldwide (World Health Organization, 2008). Agricultural animals also suffer from anemia.

Modern treatment of anemia includes the oral supplementation of iron compounds, including single Fe^{+2} salts or soluble Fe^{+2} or Fe^{+3} chelates. These preparations have several negative side effects, including gastrointestinal distress (Peña-Rosas et al., 2012; Cancelo-Hidalgo et al., 2013) and changes in the composition of colonic microflora (Zimmermann et al., 2010; Dostal et al., 2012). These methods also result in defects in body structural elements caused by the formation of reactive oxygen species (Kehrer, 2000).

Iron nanoparticles and compounds may be considered a good alternative to existing treatments. Previous work has demonstrated the growth enhancement and wound healing effects of iron nanoparticles (Gluschenko et al., 2002; Sizova et al., 2013), and nano-Fe^{+3} has been used as a food additive (Mohamad et al., 2014).

One way to improve nanopreparations is to control the size of the nanoparticle material. Differences in the biological properties of nano-Fe for particles of different sizes have been demonstrated previously (Yang et al., 2014). Decreasing Fe particle size has been reported to increase the absorption of this element (Rohner et al., 2007), and size-dependent toxicity has been demonstrated (Cho et al., 2009; Prietl et al., 2014).

The objective of this research was to study the action and influence of iron preparations on the metabolism of broiler chicks for a range of particle sizes.

2. Method

2.1 Acquisition and Certification of Iron Preparations

Nanoparticles were synthesized by high-temperature condensation using Migen-3 at the Institute of Chemical Physics (Russian Academy of Sciences, Moscow, Russia). The synthesis strategy used has been described previously (Zhigach et al., 2000). Metallic Fe (puriss p.a. 99.99%) was used for synthesis. Microparticle preparations were purchased from Alfa Aesar GmbH & Co., RG (Lancashire, UK). These materials were assessed by electron scanning and transmission microscopy using the following equipment: a JSM-7401F and a JEM-2000FX, respectively («JEOL», Tokyo, Japan). X-ray phase analysis was performed with a DRON-7 diffractometer (RPE Burevestnik, St. Petersburg, Russia). These assessments showed that the iron nanoparticles were 80.0 ± 5 nm in size. The particle cores were composed of approximately 96.0 ± 4.5% ($P \leq 0.05$) crystalline metal and 4.0 ± 0.4% ($P \leq 0.05$) metal oxide; the thickness of the oxide film on the surface of the nanoparticles was 6 nm. The iron microparticles were 9.8 ± 0.4 nm in size (puriss p.a. 99.5%, and the oxide film was 7 nm thick.

2.2 Preliminary Study of Iron Preparations

Iron nanoparticle preparations were tested for dispersion and biological toxicity. Nanoparticle suspensions were exposed to ultrasound for 0.33 - 30 min [frequency 35 kHz, sound power 300 (450) W, oscillation amplitude 10 mcm].

Morphometric indices of the particle samples were obtained using by atomic force microscopy in contact mode using an SMM-2000 "Factory PROTON-MIAT" microscope (Moscow, Russia). MSCT-AUNM cantilevers (Park Scientific Instruments, Santa Clara, USA) with a spring constant of 0.01 nm and diameter of 15 - 20 nm were used during scanning. Quantitative morphometric analysis of the images was performed using standard microscope software.

The biological activity of the resulting iron nanoparticle powder samples was assayed *in vitro* by measuring the inhibition of bacterial bioluminescence. The genetically engineered luminescent strain *E. coli* K12 TG1 was used; this train was engineered to constitutively express the luxCDABE genes of the natural marine microorganism *Photobacterium leiognathi* 54D10 and was produced by Immunotech (Moscow, Russia).

The test was implemented according to an algorithm proposed by Deryabin and Polyakov (2004). Fe preparations, in 150 mcl volumes at the concentration used in intramuscular injections (0.08 M), were poured into the wells of 96-well plates made of opaque plastic. Next, a 150-mcl suspension of luminescent bacteria was added to each well. The luminescence intensity of these mixtures was measured using a luminometer LM-01T («Immunotech», Prague, Czech Republic) at 3 min intervals for 180 min. The influence of Fe powder on bacterial luminescence intensity (I) was assessed with the following equation:

$$I = \frac{Ik_{0\min} vol o_{nmin}}{Ik_{nmin} vol o_{0\min}} \quad (1)$$

where *Ik* and *Io* indicate the luminescence intensity of control and experimental samples at 0 and n min, respectively, of measurement.

2.3 Study Design

Studies were performed *in vivo* on «Smena-7» broiler chicks in the experimental biological clinic of the Orenburg State University. Experimental research with animals was performed according to Russian Regulations (1987) and “The Guide for the Care and Use of Laboratory Animals” (National Academy Press Washington, D.C., 1996).

Poultry chickens were fed and housed according to recommendations (Fisinin et al., 2000). Chicks were fed with complete feed throughout the study, including the periods from 14 to 21 days and from 21 to 35 days (Table 1). Chickens were provided with *ad libitum* access to water in nipple drinkers.

Table 1. Ingredients and chemical composition of diet for growing **chickens (as-fed basis) (P. 5)**

Feedstuff	As fed % CP	As fed % CF
Wheat	14,58	3,50
Corn	8,17	3,70
Wheat brad	15,00	9,00
Soybean meal	44,62	18,00
Sunflower meal	38,80	12,10
Corn gluten	62,00	5,00
Sunflower-seed oil	0,00	0,00
Solt	0,00	0,00
Monocalcium phosphate	0,00	0,00
Lime	0,00	0,00
Vitamin and mineral premix[1]	0,00	0,00

Supplied per kilogram of diet: 1,000 IU vitamin A, 0,2 IU vitamin D3; 2500 g/ton vitamin E, 200 g/ton IU vitamin K; 200 g/ton vitamin B1; 400 g/ton vitamin B2; 1200 g/ton vitamin B3; 3000 g/ton vitamin B5; 200 g/ton vitamin B6; 3 g/ton vitamin B12; 70 g/ton vitamin Bc; 5000 g/ton vitamin C; 5000 g/ton Zn; 4.8 g/ton Cu; 100 g/ton Co; 100 g/ton.

A total of 150 one-day-old hens from the cross flock “Smena-7” were purchased from the hatchery of the poultry production farm “Orenburgskaya”. Four groups of 30 broiler chicks were formed based on the results of 10-day growth and development monitoring. All chicks were housed and fed in the same conditions. At the age of 14 days, the chicks received 1 intramuscular injection the thigh of iron nanoparticles (80 ± 5 nm; Group I), iron nanoparticle agglomerates (923.7 ± 29.6 nm; Group II), iron microparticles (9.8 ± 0.4 nm; Group III), or sterile saline (Group IV) per head. Iron solutions for the injections were prepared by mixing nanoparticles (or microparticles) with physiological saline to a volume of 200 μl. The resulting preparation was sterilized with ultraviolet light and then treated with ultrasound [frequency 35 kHz, sound power – 300 (450) W, oscillation amplitude – 10 mic]. The ultrasound treatment was 30 min for groups I and III and 20 s for group II. The iron dosage was 2 mg*kg^{-1}*d of live weight. These parameters are supported by earlier research (Sipaylova et al., 2011).

Chicks were weighed daily for the duration of the experiment.

The birds were slaughtered at the ages of 15, 21 and 35 days (n = 5 for each timepoint). These timepoints corresponded to 1, 7 and 21 d, respectively, after injection with Fe. The feathers, skin, flesh, viscera, the gastrointestinal tract, visceral fat, and blood of each chick were weighed. The protein content and crude fat in each tissue were determined using the Kjeldahl method and the Soxhlet method, respectively. Body protein and fat accretion were estimated based on the data obtained.

To test the hypothesis presented by Faddah et al. (2012) regarding the effect of metal nanoparticles on arginine (Arg) metabolism, the amino acid composition of the liver was estimated. The mass content of amino acids in the liver was determined by capillary electrophoresis using the Kapel system (Lumex, St. Petersburg, Russia). Samples were prepared as follows: liver tissues were homogenized, dried at 60-70 °C and ground. Then, acid and alkaline (only for tryptophan) hydrolysis of the obtained liver samples was performed. Hydrolysis occurred at 110 °C within 14-16 h. The samples were filtered after the completion of acid hydrolysis (no filtration was performed after alkaline hydrolysis). Hydrolysates were mixed with reagents and evaporated under a warm air stream, and the dry residue was dissolved in distilled water and centrifuged. The obtained supernatant was studied by capillary electrophoresis.

To determine the morphological indices, blood (1 mL) was collected in vacuum tubes with EDTA (EDTA content 1.2-2 mg/ml). For biochemical indices, vacuum tubes with a clot activator (SiO_2) were used. The samples were centrifuged at 1.6×g for 10 min at 20 °C. The concentrations of erythrocytes, platelets, and monocytes were determined using a Sysmex XT2000 (Sysmex Corporation, Tokyo, Japan). The concentrations of hemoglobin, corpuscular hemoglobin, hematocrit, iron, and total protein were determined using a Cobas-8000 system (Roche, Bazel, Switzerland).

2.4 Statistical Analysis

Statistical processing of the data was performed using the software package Statistica 6.0. The calculated values included the arithmetic mean value (M) and the standard error of the mean (m). Results with $P \leq 0.05$ were

considered significant.

3. Results and Discussion

Ultrasonic treatment of iron suspensions destroyed agglomerations of iron nanoparticles. Complexes with sizes of 923.7 ± 29.6, 383.7 ± 83.4, 257.5 ± 49.2, 211.9 ± 31.1, 175 ± 34.5, 151.2 ± 43.2, 142.2 ± 38.5 and 119.8 ± 23.8 nm were obtained after dispersal for 20 s, 2, 3, 4, 5, 6, 7 and 8 min, respectively.

Samples containing iron nanoparticles of the original size (80.5 ± 5.5 nm) were obtained following dispersion for more than 15 min. The percentage of nanoparticle agglomerates in the samples ranged from 3 to 5%.

No toxicity of the iron preparations used was revealed by our study. Biological testing of these preparations was performed according to the study published by Keenan et al. (2009) regarding the toxicity of iron nanoparticles with zero valence. Increasing the surface area of preparations while decreasing the particle size enhances the production of active oxygen forms (Li et al., 2003; Moller et al., 2010). In our study, the samples used for intramuscular injection were prepared at a concentration of 0.09 M. The exposure of *E.coli* K12 TG1 to 923 nm iron agglomerates or 80.5 nm nanoparticles did not cause a loss in bacterial luminescence (Figure 1).

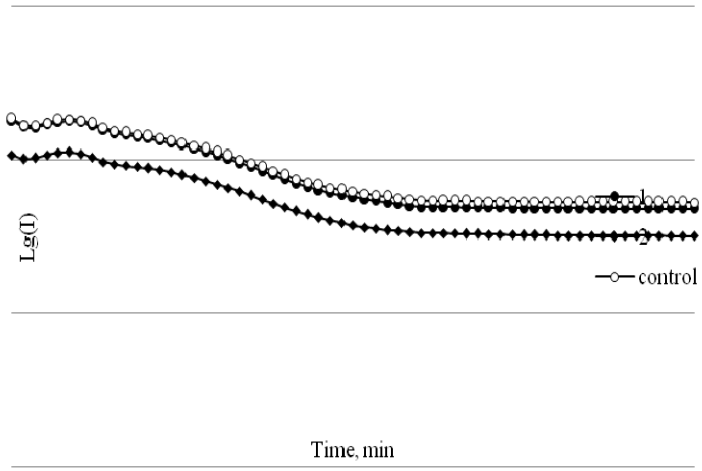

Figure 1. Change of luminescence of *E.coli* with cloned *luxCDABE*-genes *P. leiognathi* after contact with iron nanoparticles d = 80 ± 5 nm (1) and agglomerates of iron nanoparticles d = 923.7 ± 29.6 nm (2). Along the x-axis – contact time, min; along the y-axis – registered values of luminescence intensity. (P.7)

Earlier data confirm that these iron preparations are not toxic (Deryabin et al., 2011).

A growth-enhancing effect of iron preparations was demonstrated in our study with chicks (Figure 2).

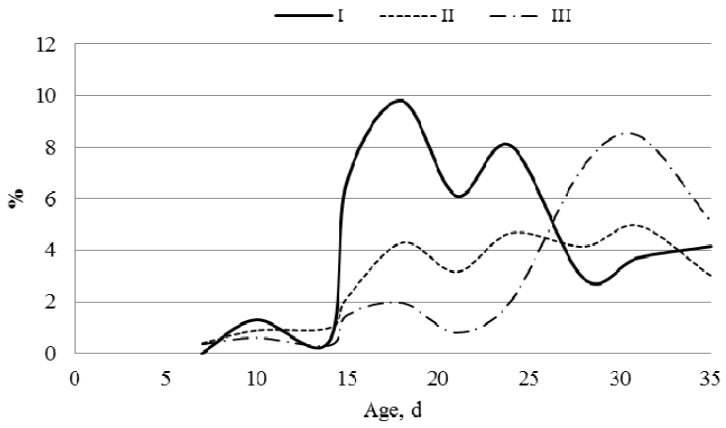

Figure 2. Change in live weight differences (%) between control and experimental groups of broiler chicks (n=30). 14-day old chicks were once intramuscularly injected (thigh) with elemental iron sized: in the I group 80 ± 5 nm (P ≤ 0,5), in the II group 923.7 ± 29.6 nm (P ≤ 0.5), in III group – 9.8±0.4 μm (P ≤ 0.5) (P.8)

After the introduction of iron, the live weight in Group I was significantly greater the control values by 6.7% after 1 day ($P \leq 0.01$), by 8.06% after 10 days ($P \leq 0.01$), and by 4.16% after 21 days ($P \leq 0.01$). The maximum difference in live weight (9.8%; $P \leq 0.01$) was observed on the fourth day following injection. The injection of iron nanoparticle agglomerates was accompanied by a significant increase in live weight 10 and 17 days after injection (+4.69 to 4.97%, $P \leq 0.01$). The preparation containing iron microparticles had the smallest effect on growth. These changes were observed only 3 wk after microparticle injection (+7 to 8%, $P \leq 0.01$).

The growth-enhancing effect of iron nanoparticles on chickens was previously described (Nikonov et al., 2011), and the different growth-enhancing actions of particles of different size was substantiated by the results of additional studies (Yu et al., 2012). The growth-stimulating effect of iron nanoparticles may be explained by metabolic changes in the chick.

The iron concentration in the blood serum increased in Group I by 15.8% within 1 day of injection ($P \leq 0.01$) and by 5.15% after 7 days ($P \geq 0.05$). By day 21, the serum iron content in Group III had only increased by 8.71% (Table 2).

Table 2. Content of iron and total protein in blood serum of broiler chicks (P.8)

Parameter	Group[1]	Age, d					
		15	P-value	21	P-value	35	P-value
Iron, mkmol/l	I	21.2±0.12**	4.8	20.4±0.12**	5.5	19.4±0.06	-
	II	19.1±0.09**	5.1	19.6±0.03	-	19.7±0.09	-
	III	18.6±0.09	-	19.9±0.07*	2.9	21.2±0.03	-
	IV	18.3±0.09	-	19.4±0.06	-	19.5±0.09	-
Total protein, g/l	I	24.5±0.09**	4.4	34.5±0.14**	4.3	48.5±0.12**	5.8
	II	24.3±0.07	-	30.9±0.17*	3.1	43.9±0.15**	5.2
	III	24.2±0.03	-	33.4±0.15*	2.6	46.8±0.15**	4.7
	IV	23.9±0.12	-	29.3±0.09	-	42.8±0.12	-

[1]group I = iron nanoparticles (80 ± 5 nm); group II = iron nanoparticle agglomerates (923.7±29.6 nm); group III = iron microparticles (9.8 ± 0.4 μm); group IV (control) – sterile saline 200 μl

The growth-enhancing effect of the iron preparations was reflected by changes in the concentration of total protein in the blood serum (Table 2). The total protein content in the chick body tissue changed in a similar manner (Table 3).

Table 3. Content of protein in body and daily weight gain of chicks, g/head (P.9)

Group[1]	Body			Daily weight gain		
	age, d			age, d		
	15	21	35	15	15-21	21-35
I	82.9±1.33	157.7±3.51	303.9±6.35	11,77	10.69	10.44
II	80.0±0.57	141.3±1.44	297.7±2.76	8.87	8.75	11.17
III	79.9±0.58	137.3±1.32	315.7±5.11	8.77	8.20	12.74
IV	78.6±0.86	135.7±1.59	294.9±4.87	7.47	8.15	11.37

[1]group I = iron nanoparticles (80 ± 5 nm); group II = iron nanoparticle agglomerates (923.7±29.6 nm); group III = iron microparticles (9.8 ± 0.4 μm); group IV (control) – sterile saline 200 μ

The total protein content of the chicks in Group I exceeded the control level by 5.5% on the 15th day ($P \leq 0.05$), by 16.2% on the 21st day ($P \leq 0.01$), and by 3.1% on the 35th day. These differences were reflected in the higher weight gain of experimental birds. Previously, fixation of 1 gram of protein was shown to lead to a body weight increase of up to 5 grams (Miroshnikov, 2008).

We hypothesized that the influence of iron on protein metabolism could be determined by codetermining the amino acid composition of the liver. Treatment with iron nanoparticles was accompanied by a significant increase in Arg in the liver compared with that in the control group at 1 day (2.25%; $P \leq 0.05$) and 7 days (3.78%; $P \leq 0.05$) following administration (Fig. 3).

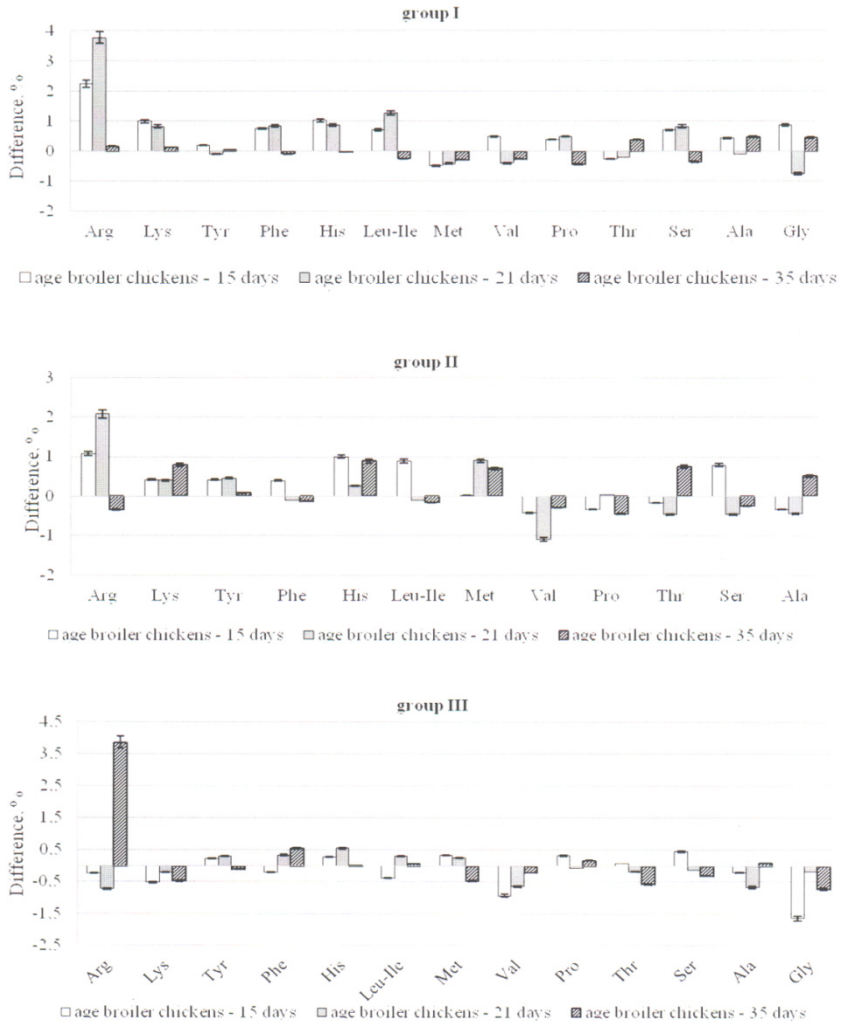

Figure 3. Difference in amino acid content in liver of chicks at the age of 15, 21 and 35 days after a single injection (14-day old) with iron nanoparticles (group I), their agglomerates (group II) and iron microparticles (group III) in comparison with the control group; group I = iron nanoparticles (80 ± 5 nm); group II = iron nanoparticle agglomerates (923.7±29.6 nm); group III = iron microparticles (9.8 ± 0.4 nm); group IV (control) – sterile saline 200 µl; (P. 9)

An increase in Arg content was found in Group II by 7 days after injection (2.08%; $P \leq 0.05$). An increase of 3.86% was observed in Group III at 21 days after injection ($P \leq 0.05$). Arg is the most abundant nitrogen carrier in the body and is the prime factor for maximizing growth potential in young animals (Wu et al., 1999, 2004; Flynn et al., 2002). It can be assumed that the increase in liver Arg content was accompanied by a redistribution of energy storage between the protein and fat in the chick bodies. In a broiler chick model (Cobb 500; Fouad et al., 2013), dietary L-Arg supplementation reduced abdominal fat content by modulating lipid metabolism. In our study, we observed a tendency toward decreasing fat deposition during weight gain in Group I chicks from the 15th to the 21st day (Table 4).

Table 4. Fat content in body and weight gain of chicks, g/head (P.9)

Group[1]	Body			Daily weight gain, g/head		
	age, d			period, d		
	15	21	35	15	15-21	22-35
I	49.1±2.3	79.1±4.07	193.7±6.23	3.61	4.28	8.18
II	51.3±1.34	88.7±5.15	184.7±3.65	5.81	5.35	6.85

III	48.9±1.36	85.7±2.89	195.0±3.26	3.41	5.25	7.81
IV	50.0±2.33	81.3±3.97	167.7±5.67	4.51	4.45	6.17

[1]group I = iron nanoparticles (80 ± 5 nm); group II = iron nanoparticle agglomerates (923.7±29.6 nm); group III = iron microparticles (9.8 ± 0.4 μm); group IV (control) – sterile saline 200 μl

Increasing liver Arg concentrations may result from active macrophage synthesis in response to the introduction of iron particles. The synthesis of polyamines and Arg-containing proteins promotes the proliferation of monocytes and lymphocytes (Suchner et al., 2002). In our studies, the quantity of monocytes increased significantly by 1 day after injection: by 16.3 and 12% ($P \leq 0.01$) in Groups I and III, respectively, and 2.17 times ($P \leq 0.01$) in the Group II compared to the quantity in the control group. The monocyte content 7 days after injection with finely dispersed iron particles was higher by 5.56 and 8.89% in Groups I and III, respectively, and 1.53 times ($P \leq 0.01$) in Group II compared to the content in the control. The monocyte-level in 35-day-old chicks increased only in Group III by 2.19% (Table 5).

Table 5. Blood indices of chicks (P.9)

Parameter	Group[1]	Age, d					
		15	P-value	21	P-value	35	P-value
Erythrocytes, 10^{12}/l	I	2.37±0,42	-	3±0.39	-	3.11±0.01	-
	II	1.96±0.56	-	2.27±0.63	-	3.06±0.01	-
	III	2.31±0.7	-	3.08±0.51	-	3.18±0.02	-
	IV	2.3±0.92	-	2.7±0.68	-	3.09±0.63	-
Hemoglobin, g/l	I	128.6±7.16	-	114.2±11.5	-	132.3±5.6	-
	II	87.1±8.69	-	95.1±5.1	-	130.7±7.5	-
	III	110.2±7.2	-	127.6±6.8	-	135.4±6.8	-
	IV	106.2±3.8	-	110.6±10.6	-	130.3±3.6	-
MCH, pg	I	62.1±9.6	-	57.7±6.4	-	53.7±4.7	-
	II	51.2±7.6	-	51.4±8.6	-	52.5±7.5	-
	III	53.9±7.3	-	62.6±4.2	-	54.01±8.2	-
	IV	53.2±9.4	-	56.4±8.9	-	52.9±8.9	-
Hematocrit, %	I	25.8±1.9	-	29±2.4	-	29.2±2.1	-
	II	21.8±2.6	-	22.8±2.4	-	28.7±1.5	-
	III	23.8±3.7	-	27.5±0.87	-	31±2.6	-
	IV	23.8±2.4	-	25.2±0.9	-	28.8±2.8	-
Platelet, 10^{12}/l	I	60±4.2	-	83.9±7.5	-	100.5±6.2	-
	II	61.8±5.2	-	67.7±6.4	-	100.4±6.2	-
	III	65.9±4.8	-	84.5±5.6	-	100.7±8.4	-
	IV	67.4±4.8	-	88.3±6.8	-	100.7±8.7	-
Monocytes, 10^3 mm^3	I	1.5±0.06**	4,7	1.9±0.02	-	1.84±0.07	-
	II	2.8±0.23**	4,1	2.75±0.12**	5,2	1.8±0.06	-
	III	1.68±0.04**	4,6	1.96±0.09	-	1.87±0.07	-
	IV	1.29±0.01	-	1.8±0.04	-	1.83±0.07	-

[1]group I = iron nanoparticles (80 ± 5 nm); group II = iron nanoparticle agglomerates (923.7±29.6 nm); group III = iron microparticles (9.8 ± 0.4 μm); group IV (control) – sterile saline 200 μl

The hemoglobin concentration a day after the introduction of nanoparticles increased by 21.1% compared with that in the control (7 days after the injection, this change was reduced to 3.26%). Similar results for nano-Fe particles were observed in the studies performed by Skorkina et al. (2010).

The injection of iron nanoparticles and agglomerates led to a decrease in platelet content (Table 4). A similar effect was reported following the injection of titanium dioxide nanoparticles (Nemmar et al., 2008; Shumakova et al., 2014).

Our hypothesis regarding the influence of iron particle size on the growth and development of chicks has been confirmed. Growth-enhancing effects of iron preparations were observed in our experiments at different time points after intramuscular injection and were dependent on the particle size. We also observed corresponding

changes in the chicken bodies. Enhanced growth was accompanied by a protein increase and weight gain in chickens, as well as an increase in the Arg concentration in the liver and in the monocyte concentration in the blood.

The mechanism underlying increased Arg synthesis can be triggered in several ways, among which are boosting metabolism and the synthesis of nitric oxide (NO). Iron homeostasis and nitric oxide (NO) production are inversely related (Nairz et al., 2013).

Iron affects the expression of induced NO synthase 2 (Weiss et al., 1994; Dlaska and Weiss, 1999), and NO-induced Fpn1 transcription resulted in iron deprivation. Moreover, iron content is decreased by macrophages (Fritsche et al., 2003). In turn, NO is formed by the oxidation of 1 end atom of nitrogen in guanidine complexes of L-Arg. These processes cause the close connection between NO production and Arg delivery. The generation of physiological levels of nitrogen oxide from Arg may also have an indirect positive influence on animal growth. The anti-inflammatory role of NO in the gastrointestinal tract is well known (Li et al., 2007).

The growth-enhancing effects of L-Arg are associated with a change in the balance of the energy consumed and used for fat burning and a decrease in the production of white fat. L-Arg stimulates mitochondrial biogenesis and the production of brown adipose tissue (McKnight et al., 2010). In the future, Arg and nanoparticle preparations might be frequently used in practice, and previous experiments have shown a decrease in the toxic effects of nano ZnO using Arg (Faddah et al., 2012). In addition, dietary L-Arg has protective effects against oxidative stress and inflammation (Huang et al., 2008; Mostafavi-Pour et al., 2008).

Thus, similar biologic effects have been shown for different sizes of elemental iron particles. These effects increase as the particle size decreases. Further investigation is required before metal nanoparticles and Arg are used together.

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