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NANOPARTICLES IN COMBINATION WITH AMINO ACIDS CHANGE PRODUCTIVE AND IMMUNOLOGICAL INDICATORS OF BROILER CHICKEN

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Abstract

The prospects of using metal nanoparticles to stimulate productivity of farm animals are widely discussed. However, nano-sized materials exhibit various negative properties, such as prooxidant effects, and can provoke apoptosis and kidney damage. A possible approach is the use of ultrafine materials in combination with agents leveling adverse effects of nanoparticles. For the first time we studied the prospects of joint use of iron and arginine nanoparticles, the mechanism of their interaction and influence on the productivity of poultry and demonstrated that their simultaneous application promote live weight gain. We formed 6 groups (n = 30) of 11-day old broilers of the cross Smena 8. The poultry was injected twice (after 2 week intervals) with iron nanoparticles and fed either with dietary arginine (the amino acid which is known to influence metabolism and immune response and considered as conditionally essential for inflammatory and oxidative stress), or the mixture of arginine, lysine and methionine. Our experiments showed that the joint use of iron nanoparticles and arginine increased the weight gain up to 9.2 % as compared to the control, and moreover, the iron nanoparticles together with a mixture of amino acids provided an increase up to 20 %. Withal, the nanoparticles and amino acids when applied separately resulted in lower weight gain, and at the end of the experiment the body weight of broilers fed with dietary arginine (group II) and those injected with iron nanoparticles (group III) increased by 6.1 and 5.9 % ($P \leq 0.05$), respectively. Intramuscular administration of iron nanoparticles (the poultry groups III, IV and VI) promoted the immune response that was manifested in enhanced level of leukocytes — by 8.12; 10.50 and 3.88 % ($P \leq 0.05$), respectively, on the day 1, and by 7.3; 8.19 and 4.00 % ($P \leq 0.05$), respectively, in a week. The study of NO-metabolites showed an increased level in blood and liver (by 3-4 %) only in groups III, IV and VI. Singly injected iron nanoparticles (group III) changed metabolism of arginine and increased its level by 3.83 % ($P \leq 0.05$). Thus the joint use of iron nanoparticles and the complex of arginine with other amino acids is most likely to be helpful in the poultry meat production.

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

The search for substances with growth-stimulating action is one of the current trends in agricultural biology. A number of studies indicate the prospects of using metal nanoparticles as preparations to increase productivity of farm animals [1, 2]. Essential metal-based nanoparticles have been reported to significantly exceed the analogues in the form of mineral salts in their bioavailability [3], are characterized by less pronounced toxic effect (4), with which the promise of their use as sources of microelements is associated [5].

However, nanoparticles have a number of disadvantages. They stimulate

the production of active forms of oxygen [6, 7], apoptosis [8], and structural and functional reorganization of tissues [9], cause kidney damage [10], affect the elemental status of organs and tissues [11]. Nevertheless, a set of measures for leveling the negative effects of micronutrient nanoparticles can make the practical application of such ultradisperse substances possible.

Previously, we have demonstrated that the intake of iron nanoparticles by animals is associated with an increase in the arginine level in the liver [12]. The mechanism of this is unclear yet. Arginine is known to exhibit various metabolic and immunological effects and is considered conditionally essential for inflammatory and oxidative stress [13, 14]. At the same time, arginine is one of the factors involved in the regulation of animal growth [15]. Mechanisms for triggering arginine synthesis are closely related to the proliferation of white blood cells and NO synthase activation. It is noteworthy that iron homeostasis is closely related to the homeostasis of NO metabolites [16]. The use of arginine to reduce the negative effects of zinc nanoparticles intake has been reported by L.M. Faddah et al. [17].

In this paper, we are for the first time demonstrating that inclusion of arginine in the diet in combination with intramuscular injections of iron nanoparticles increases productivity of agricultural poultry more efficiently than each of these techniques used individually.

Our purpose was to study the prospects for the joint use of the preparations of iron nanoparticles with arginine and other amino acids, the mechanism of their interaction and the impact on productivity in poultry.

Technique. Iron nanoparticles (NP) of d = 80±5 nm were used (the particles are a core of crystalline iron with an oxide shell of Fe_3O_4 on the surface). Preparations of nanoparticles were obtained by high-temperature condensation at the MiGen assembly (Institute for Energy Problems of Chemical Physics, RAS, Moscow). Material certification of preparations included electronic scanning and translucent microscopy using a JSM 7401F and JEM-2000FX (JEOL, Japan), X-ray diffraction analysis using a diffractometer DRON-7 (NPP Burevestnik, Russia), atomic force microscopy using a multi-microscope SMM-2000 (OJSC PROTON-MIET, Russia). For injection, iron nanoparticles were prepared by mixing with saline solution, after which they were subjected to UV sterilization and processed for 30 minutes using ultrasonic disperser UZDN-2T (NPP Akadempribor, Russia) (35 kHz, 300-450 W, amplitude of oscillation of 10 μm).

Dietary methionine (JSC Volzhsky Orgsintez, Russia); lysine monochlorohydrate (Ha-ngzhou Greensky Biological Tech Co., Ltd., China), and arginine hydrochloride (Tianjin Tiyanyan Pharmaceutical Co., Ltd., China) were used as amino acid preparations.

Experiments were performed in the vivarium (Orenburg State University) with broiler chicken cross Smena 8. The keeping conditions and experimental procedures met the instructions and recommendations provided for by the Russian regulations (Order of the USSR Ministry of Health No. 755 of 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. The 11-day old chickens (n = 168) were selected for the experiment and divided into six groups by analogous pair method (n = 28). At the age of 15 and 29 days, birds from groups III, IV, and VI were injected with Fe nanoparticle (NP) lysozoles (2 mg/kg live weight) intramuscularly [18]. Starting from the age of 15 days, daily arginine at a dose of 7 g/kg of feed [19] was added to the basic diet (BD) in groups II and IV. Arginine (7 g/kg), lysine (6 g/kg), and methionine (2 g/kg) were administered in group V and VI. Control poultry (group I) received BD. Diets were

formed taking into account feeding recommendations [20].

During the experiment, all poultry were kept in the same conditions, the microclimate in the room conformed VNITIP recommendations and requirements [21]. Poultry were fed twice daily, feed consumption was controlled daily. Water was provided ad libitum. The growth and development of chickens (examination and individual weighing) were evaluated. The birds were decapitated under nembutal ether at days 1, 7, and 14. Blood samples for hematological studies were collected into vacuum tubes with anticoagulant (EDTA-K3), for biochemical studies into vacuum tubes with a coagulation activator (thrombin).

Hematological parameters (number and type of leukocytes) were estimated using an automatic hematological analyzer URIT-2900 Vet Plus (URIT Medical Electronic Group Co., Ltd, China).

Concentration of plasma NO metabolites (the total amount of nitrate and nitrite ions) was determined spectrophotometrically with the Griss reagent using the microplate reader Infinite PROF200 (Tecan Austria GmbH, Austria) at $\lambda = 540$ nm. Blood was pre-deproteinized by adding a 2-fold excess of 96 % ethyl alcohol to 1 ml of blood. Since the method allows only nitrite ions to be determined, vanadium chloride (III) was added to the plasma to reduce nitrates, after which the tubes were placed in a water bath (37 °C, 30 min). The resulting optical density values of the samples were compared with the calibration curve. To construct it, a series of $NaNO_2$ solution delutions (5 to 100 μM) was prepared, and the samples were processed similarly to the experimental samples [21]. Similarly to test samples NO metabolites were also measured in tissue samples. In this case, sample preparation included processing biomaterial in phosphate buffer solution (pH 7.45) using a homogenizer TissueLyser LT (Qiagen N.V., Germany).

Weight proportions of arginine, lysine, methionine, tyrosine, phenylalanine, histidine, leucine-isoleucine, valine, proline, threonine, serine, alanine and glycine were estimated in the study of amino acid composition of poultry tissues and feeds. In the preparation of liver tissue and feed samples, the material was homogenized (TissueLyser LT, Qiagen N.V., Germany), dried at 60-70 °C and milled. Test samples were subjected to acid or alkaline (for tryptophan determination only) hydrolysis at a temperature of 110 °C for 14-16 hours. After acid hydrolysis, the sample was filtered (decalcified slowly filtering blue ribbon filters, Melior XXI LLC, Russia), after alkaline hydrolysis, filtration was not performed. Hydrolyzates were mixed with reagents (sodium carbonate, JSC Bashkir soda company, Russia; phenyl isothiocyanate, Shandong Hailan Chemical Industry Co., Ltd, China) and evaporated in a warm air stream. The dry residue was diluted in distilled water (0.5 ml) and centrifuged (5 min, 5000 rpm). The resulting supernatant was examined by capillary electrophoresis using the Kapel system (Lumex-Marketing Ltd, Russia; GOST 55569-2013).

The data are presented as the arithmetic mean (M) with the standard error of the mean (m). Statistical analysis was performed using ANOVA (Statistica 10.0 software package, StatSoft Inc., USA). Differences were considered statistically significant at $P \leq 0.05$.

Results. Increased arginine content in group II diet resulted in an increase in the live weight of the birds versus control by 4.0 % in 1 day, by 5-6 % in 2 weeks, and by 6.1 % ($P \leq 0.05$) at the end of the study (Table 1). Intramuscular Fe NP injection in group III chickens resulted in an increase in live weight by 6.2 % ($P \leq 0.05$) versus control at day 1 and by 9.4 % ($P \leq 0.05$) by day 4. At week 2, a decrease in growth down to 3.5 % versus control was observed. A repeated injection of nanoparticles after 2 weeks, like the first one, increased the living weight in group III by 5.9 % ($P \leq 0.05$) versus control in 1 day. This difference was practically maintained for 2 weeks, and by the end of the experi-

ment, the corresponding parameter in group III exceeded the control one by 7.1 % ($P \leq 0.05$). In group IV, a combination of Fe NP injections with additional arginine in the diet promoted similar changes in the live weight in the first week of studies, as in group III. Thus, in group IV, these parameters exceeded the control ones by 7.8 % in 1 day ($P \leq 0.05$), and by 7.5 % ($P \leq 0.05$) after 1 week. During week 2, group IV chickens exceeded control ones in the weight gain (increase by 6.0 %, $P \leq 0.05$). Repeated administration of Fe NP increased the live weight gain, and by the end of the study the difference between group IV and control broilers in the weight reached 9.2 % ($P \leq 0.05$).

1. Changes in the live weight (g) **in cross Smena 8 broilers with intramuscular Fe nanoparticle** (NP) **injections against the background of amino acid feed additives** ($M \pm m$, $n = 7$, vivarium)

Groups by experimental embodiment	Week 1	Week 2	Week 3
I (control, BD)	886.6±14.9	1268.0±17.4	1608.1±23.6
II (BD + arginine)	835.1±9.21	1347.9±19.5	1663.6±17.9*
III (BD, Fe NP injections)	868.8±8.69*	1312.8±12.7*	1672.7±20.3
IV (BD + arginine, Fe NP injections)	866,2±6,32*	1344.1±11.5*	1693.3±15.4*
V (BD + arginine + lysine + methionine)	905.9±8.39*	1392.2±10.8*	1775.0±16.3*
VI (BD + arginine + lysine + methionine, Fe NP injections)	924.0±10.3*	1431.6±14.7*	1937.2±13.8*

N o t e. BD — basic diet.
* Differences versus control are significant at $P \leq 0.05$.

2. Changes in WBC numbers ($\times 10^9$/l) **in cross Smena 8 broilers with intramuscular Fe nanoparticle** (NP) **injections against the background of amino acid feed additives** ($M \pm m$, $n = 7$, vivarium)

Groups	Leucocytes	Lymphocytes	Monocytes	Granulocytes
		Day 1		
I (control)	22.2±0.58	12.1±0.02	1.37±0.040	8.69±0.700
II	21.7±1.07	12.7±0.32	1.34±0.030	7.63±1.360
III	23.9±0.08*	12.6±0.02*	1.58±0.010*	9.83±0.030*
IV	24.5±0.12*	12.8±0.01*	1.61±0.050*	10.05±0.110*
V	22.6±0.31	12.3±0.23	1.35±0.020	8.98±0.430
VI	23.0±0.03*	12.4±0.03*	1.50±0.020*	9.15±0.050*
		Day 7		
I (control)	25.2±0.62	13.2±0.29	1.62±0.070	10.50±0.440
II	25.8±0.63	13.2±0.22	1.77±0.020	10.80±0.410
III	27.1±0.06*	14.6±0.10*	1.69±0.020*	10.80±0.130*
IV	27.3±0.08*	14.6±0.09*	1.66±0.010*	11.00±0.050*
V	25.9±0.63	13.4±0.40	1.76±0.050	10.80±0.180
VI	26.2±0.04*	13.8±0.10*	1.66±0.020*	10.70±0.060*
		Day 14		
I (control)	25,5±0,29	12.9±0.26	2.01±0.020	10.50±0.100
II	25,0±0,37	13.2±0.23	1.89±0.010	9.94±0.260
III	26.1±0.28	13.8±0.27	2.01±0.030	10.30±0.100
IV	26.2±0.29	13.7±0.39	2.04±0.020	10.50±0.090
V	26.0±0.12	13.5±0.14	2.00±0.030	10.50±0.150
VI	25.2±0.35	13.3±0.26	2.03±0.030	9.83±0.130

N o t e. Group I control (basic diet, BD), group II — BD + arginine, group III — BD, Fe NP injections, group IV — BD + arginine, Fe NP injections, group V — BD + arginine + lysine + methionine, group VI — BD + arginine + lysine + methionine, Fe NP injections (see details in section *Technique*).
* Differences versus control are significant at $P \leq 0.05$.

Combined use of iron nanoparticles and a mixture of amino acids proved to be optimal: at the end of the experiment, the live weight of group VI chickens exceeded this parameter in the control group by 20.5 % ($P \leq 0.001$) and was by 9.1 % ($P \leq 0.01$) greater compared to group V, which demonstrates a pronounced synergy in the effects of these substances on poultry growth. Methionine is known to be actively involved in metabolic processes, in particular, it increases iron absorption in the gastrointestinal tract. A combination of methionine and arginine is effective for productivity growth [22]. Lysine and arginine are antagonists, but when combined, they stimulate production of growth hormone. Part of the energy required for protein synthesis is formed due to lysine

oxidation [23]. This explains the pronounced effects in the combined use of Fe NP with a complex of arginine, lysine, and methionine amino acids.

Analysis of morphological and biochemical parameters of the blood revealed changes only in the groups that received preparations of iron nanoparticles (Table 2). Thus, in groups III, IV, and VI, the number of leukocytes increased, versus control, respectively, by 8.12, 10,5, and 3.88 % ($P \leq 0,05$) at day 1, by 7.30, 8.19, and 4.00 % ($P \leq 0.05$) after 1 week, and no significant differences versus control were observed after 2 weeks. Similar changes in the parameters were observed for certain types of leukocytes.

In groups III, IV, and VI, the number of lymphocytes increased versus control, respectively, by 3.63. 5.91, and 2.20 % ($P \leq 0.05$) after 1 day, the number of monocytes increased by 15.40, 17.90, and 10.20 % ($P \leq 0.05$), the number of granulocytes — by 13.10, 15.60, and 5.24 % ($P \leq 0.05$). After 1 week, in these groups, the number of lymphocytes significantly increased by 10.90. 10.90, and 5.40 % ($P \leq 0.05$), the number of monocytes increased only by 4.30, 2.47, and 2.47 % ($P \leq 0.05$), the number of granulocytes — by 3.61, 5.25, and 2.55 % ($P \leq 0.05$).

These data are consistent with the results of our earlier assessment of the effects of iron nanoparticles on the productivity and physiological status of broiler chickens [12]. A similar effect of iron and amino acid nanoparticles on productivity has been described by other authors [24, 25]. The data on the morphological composition of the blood presented in this paper are consistent with the conclusions about the ability of metal nanoparticles and their compounds to promote the immune response [26, 27]. Probably, the observed change in the composition of leukocytes upon administration of nanoparticles is a short-term "physiological leukocytosis" which is especially evident against the background of amino acid additives (protein food). Our findings also indicate the close relationship between the gain of leukopoiesis and the growth stimulating effect of the preparations. Arginine metabolism is known to be closely related to the proliferation of monocytes and lymphocytes and the development of oxidative stress through the synthesis of polyamines and protein [28-32].

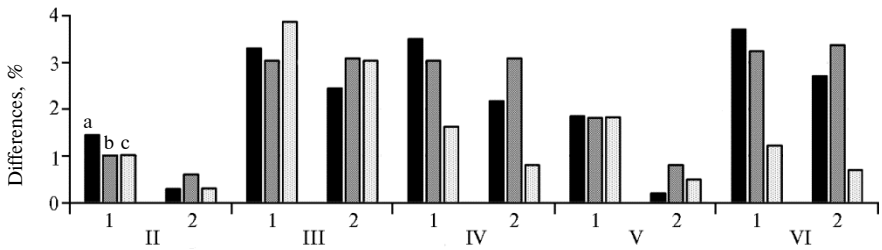

Fig. 1. Differences (%) **versus control** (group I) **in the blood** (1) **and liver** (2) **levels of NO metabolites in cross Smena 8 broilers of various ages with intramuscular Fe nanoparticle injections against the background of amino acid feed additives:** II, III, IV, V, and VI — groups by experiment embodiment (see details in section *Technique*); a, b, c — poultry age of 16, 21, and 35 days (experimental day 1, week 1, and week 2) ($n = 7$, vivarium).

The blood levels of NO metabolites increased in 1 day after administration of nanoparticles in groups III, IV, and VI (by 3.3, 3.5, and 3.7 %) (Fig. 1). In 1 week, this parameter was increased in the same groups (by 3.0-3.4 % versus control), in 2 weeks, increased NO metabolites (by 3.85 % versus control) were maintained in group III only. The changes of the level of NO metabolites in the liver were similar: regular changes were observed in groups III, IV, and VI only. Thus, in group III, as a result of nanoparticle administration, this parameter was increased by 2.0-3.0 % in group III versus control, and this difference persisted

throughout the observation period. In group IV, Fe NP injections combined with arginine dietary additives increased the level of liver NO metabolites in the poultry in the first 7 days only (by 2.0-3.4 % versus control). Similar changes were observed in the same time frame in group VI (level increase by 2.7-3.7 % versus control).

Liver amino acid composition was changed in group III only: by the end of the study, arginine levels were increased versus control by 3.83 % ($P \leq 0.05$) (Fig. 2).

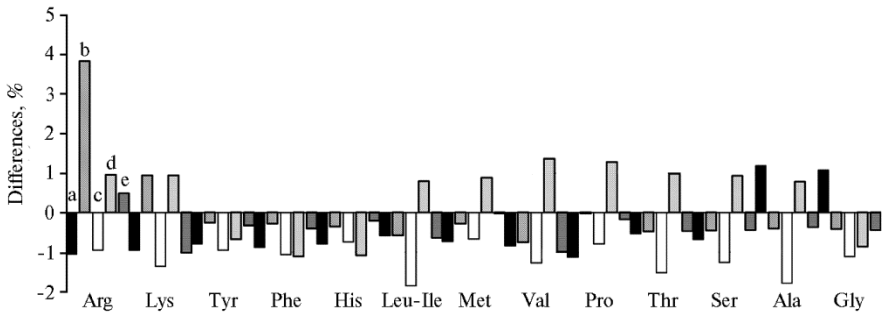

Fig. 2. Differences (%) **versus control** (group I) **in the liver amino acid composition in cross Smena 8 broilers aged 42 days old with intramuscular Fe nanoparticle injections against the background of amino acid feed additives:** a, b, c, d, e — groups II, III, IV, V, VI by experiment embodiment (see details in section *Technique*) (n = 7, vivarium).

Formation of arginine can be initiated by several mechanisms, including activation of the metabolism, as well as through the synthesis of nitric oxide (NO), as shown experimentally [33, 34[. We have found an association between increased formation of nitric oxide and the entry of iron nanoparticles, as well as its dependence on the amount of arginine present in the diet. In particular, with combined use of nanoparticles and amino acids, the blood and liver levels of NO metabolites was increased in chickens in the first week of the experiment only, whereas in the absence of amino acid additives, nanoparticles caused an increase in the amount of NO metabolites throughout the observation period. Additionally, we note that an increase in the amount of arginine in the diet, eliminating the need for its synthesis in the body resulted in a considerable and significant increase in the productivity of the poultry.

Thus, intramuscular injections of iron nanoparticles are followed by a change in the arginine metabolism in the poultry body. Combined use of a preparation of iron nanoparticles and arginine complex with other amino acids results in an increase in the productivity of broiler chickens.

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Events

3D CELL CULTURE
TECHNOLOGICAL INNOVATION AND CLINICAL SUCCESS

(22-23 February 2017, London, United Kingdom)

3D Cell Culture 2017 will address the latest developments of 3D cell culture techniques; the ways in which 3D methods are presently paving the way to future technologies, and the ways in which they are currently revolutionising cancer research, stem cell and regenerative medicine. The 3D cell culture market is predicted to reach $3702.2 million by 2021 with main increase seen in novel technologies and culture methods. This event will highlight emerging technologies, like 3D and 4D bio imaging, and their application to furthering research and medical practice.

In addition, we aim to focus on the involvement of 3D culture methods in drug development and screening, a topic of great current commercial interest to pharmaceutical and research bodies. 3D Cell Culture 2017 will bring together leading professionals and researchers in the industry to discuss the latest developments and future potential of this technique.

We aim to provide a unique and exciting conference on 3D cell culture, covering cutting edge technologies like CRISPR. There will also be emphasis on the success and bright future of 3D methods in cancer research and stem cell research, the potential of organoids, and drug development besides.

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