RESEARCH OF OPPORTUNITIES FOR USING IRON NANOPARTICLES AND AMINO ACIDS IN POULTRY NUTRITION

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ABSTRACT: The goal of the study is the opportunity for the combined use of iron nanoparticles with arginine and other amino acids in poultry nutrition. The iron nanoparticles (nanoFe) were obtained through high-temperature condensation and had a size of 80 ± 5 nm. The duration of the experiment on broiler chickens is 28 days. During the study of the amino acid composition of poultry liver, there was an increase by 3.1-4.2% in the content of arginine when feeding nanoFe in the period of 7-28 days of the experiment; and by 3.5-3.7% of amino acids with nanoFe on day 7 of the experiment. Adding nanoFe in poultry’s diet is accompanied by an increased content of NO-metabolites in the liver by 3.1-3.5%. The enrichment of the food with nanoFe helped to increase the iron concentration in the body of poultry by 5.3% during the experiment; when combined with arginine – by 4.5%. The total pool of iron in the body of poultry has increased by 13.0%; when combined with arginine – 14.6%, amino acid complex – 17.3%. Using nanoFe in compound feed resulted in an increase of erythrocytes in blood, and with the additional feeding of amino acids the increase was more significant. The most body weight of poultry at the end of the experiment is noted when combined feeding nanoFe and the complex of amino acids. The use of amino acids in feeding without the inclusion of nanoFe is accompanied by an increase in the body weight by 6.1-9.4%.

Keywords: Iron nanoparticles, Broiler chickens, Arginine, Lysine, Methionine, NO-Metabolites, Growth

1. INTRODUCTION

The unique properties of nanoparticles of drugs determine the broad practice of their application in medicine and biology. The latest years have seen an increase in the research projects pointing at the need for using iron-containing nanomaterials in treating cancer [1-2], as a contrast medium for high-resolution magnetic resonance imaging of tumors, blood clots, etc. [3-4], with positron emission tomography [5], as microbicides [6-7], in the production of medical dressings [8], and others. Iron nanoparticles are viewed as a good alternative to the currently available preparations based on the microelements [9].

Meanwhile, along with the advantages of nanoparticles, the latter have a number of disadvantages including the ability to produce reactive oxygen intermediate [10] and to stimulate apoptosis [11]. The use of nanoparticles is associated with kidney damage [12-13] which is caused by the potential toxicity of nanoparticles for the renal tubules and glomeruli [14]. In this regard, the use of nanoparticles in practice must be combined with a complex of measures to mitigate negative effects associated with the use of ultratine substances. One of the solutions could be the use of arginine [15].

Arginine is a nonessential amino acid that plays an important role in cell division, healing of wounds and immune function [16-17]. Arginine is considered to be a conditionally essential in inflammatory and oxidative stress [18-19]. In the same time, arginine is one of the factors that regulate the growth of animals [20]. The exchange of arginine is associated with the arrival of the metal nanoparticles from outside. Earlier, the fact of arginine level increase in the liver when feeding with iron nanoparticles was shown at the poultry’s model [21].

The study is a continuation of the research works concerning the development of new drugs with micronutrients for animals based on metal nanoparticles. The goal of the study is the opportunity for the combined use of iron nanoparticles with arginine and other amino acids in poultry nutrition.

2. METHODS AND MATERIALS

2.1 Ethics statement

The experimental research on animals was conducted according to instructions, recommended by the Russian Regulations, 1987 (Order No. 755 of
August 12, 1977 of the USSR Ministry of Health) and “The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996).”

The animals were placed in the attestation vivarium at the Institute of Bioelementology of the Orenburg State University. The vivarium was duly equipped and was run by competent personnel. The veterinary requirements were met.

2.2 Characteristics of iron nanoparticles (nanoFe)

NanoFe were spherical in shape, sized 80 ± 5 nm, Z-potential – 15 ± 0.2 mV. Nanoparticles were obtained through high-temperature condensation on a Migen machine (Gen, & Miller, 1981) and provided by Prof. Glushchenko (the Institute of Problems of Chemical Physics of Russian Academy of Sciences (IPCP RAS), Moscow). The composition of the nanoFe is determined through plasma-chemical method: 99.8 wt% of metallic iron, Fe3O4, α – Fe2O3. The material notification of the preparations included scanning and transmission electron microscopy using the machines like JSM 7401F, JEM-2000FX (JEOL, Japan); X-ray phase analysis on the diffractometer DRON-7 (NPP “Burevestnik”, Russia). The AFM investigation was done on the microscope SMM-2000 (JSC PROTON-MIET, Russia).

2.3 Animals and dosage

The experiment was made on “Smena-7” broiler chickens. At Orenburg poultry plant there was a selection of 240 one-day-old chickens. Selection conditions: well-developed chicks and the difference in live weight is not more than 5%. All selected poultry was tagged (foot plastic tags), weighed and placed in the same conditions. On the basis of the data of individual daily weighing of chicks and cost accounting for food, four groups were formed using the method of analogous pairs (n = 30): control (I) and six experimental (II, III, IV, V, VI, VII). Chickens of I (control) group were fed with basic diet during the experiment. Chickens of II, III, V groups in the period from 14 to 42 days of life in addition to the basic diet received nanoFe at a dosage of 4 mg/kg of feed. The diet of III and IV groups included an additional dose of arginine of 7 g/kg. The complex of amino acids such as arginine, lysine, and methionine added to the diet of the chickens of groups V and VI: arginine – 7 g/kg, lysine – 6 g/kg, methionine – 2 g/kg. The diet of the chickens of VII group included lysine and arginine: arginine – 7 g/kg, lysine – 6 g/kg. In experimental work was presented amino acid special for poultry.

During the preparation, the drag with nanoFe was mixed with water and then dispersed for 30 minutes with ultrasound (f – 35 kHz, N – 300 W, A – 10 µA). After processing, the lysols were mixed with feed.

The basic diet in the period from 8 to 28 hours included 404 g of wheat/kg, 173 g of corn, 100 g of sunflower meal, 200 g of soybean meal, 40 g of corn gluten, 50 g of sunflower oil, 10 g of vitamin and mineral premix, 2.6 g of salt, 18 g of monocalcium phosphate, 10 g of limestone powder/kg. The content of arginine in the basic diet was 1%, lysine – 0.9%, methionine – 0.38%.

The basic diet in the period from 29 to 42 hours included 368.4 g of wheat/kg, 220 g of corn, 100 g of sunflower meal, 200 g of soybean meal, 40 g of corn gluten, 50 g of sunflower oil, 10 g of vitamin and mineral premix, 2.5 g of salt, 18 g of monocalcium phosphate, 10 g of limestone powder/kg. The content of arginine in the basic diet was 1%, lysine – 0.9%, methionine – 0.32%.

The experimental research involved keeping animals in equal conditions in accordance with the existing density, temperature, and humidity standards. The cages are equipped with 2 automatic nipple drinkers with a feeder. The poultry was fed with complete feed taking into account the recommendations of the All-Russian Research and Technological Poultry Institute [22].

The broiler chickens were observed daily for the entire period of the experiment for clinical signs (the dynamics of body weight, general appearance and behavior). On the appointed day of termination (1, 7, 14 and 28 days of the experiment) all the planned chickens were euthanized by Nembutal anesthesia. Then mortem examination was carried out.

Hematologic blood analysis of the broilers was performed on URIT 2900 VETPlus blood hematology analyzer (URIT, China) and CS-T240 biochemical analyzer (DURIU, China). Biochemical analysis was conducted using DiaVetTest Randox veterinary kits (Randox, UK).

The amino acid composition of poultry’ tissue and feed was determined through the method of capillary electrophoresis using “Kapel” system by measuring the proportion of amino acids (Lumex, Russia).

Determining the level of NO-metabolites in the blood’s plasma and tissues was conducted spectrophotometrically with Griess reagent on Infinite PRO F200 (TECAN, Austria) microplate reader at 540 nm. The determination of the amount of NO-metabolites in the liver was carried out within a few hours after the selection of a pre-frozen biological material in plasma immediately after collection.

2.4 Data analysis
All the data obtained during the study were subjected to statistical processing. Statistical analysis was performed by comparing the experimental groups with the control group using the SPSS 19.0 Software (IBM Corporation) and Statistica 10. The value with P ≤ 0.05 was considered to be statistically significant. Such indicators as the weight of the mother's body were subjected to the analysis of variance (ANOVA), and Scheffe's multiple comparison tests.

3. RESULTS

3.1 Iron metabolism indicators

Using nanoFe (II group) was accompanied by an increase in the iron content in the blood serum by 4.1% and 6.1% (P ≤ 0.05) on days 14 and 28 of the experiment. Complex amino acids and nanoFe (V group) identified an increase in the iron concentration in the serum on day 7 by 5.7% (P ≤ 0.05), day 14 by 7.3% (P ≤ 0.05), day 28 by 7.7% (P ≤ 0.01). The dynamics of ferritin concentration was varied in a similar way (Table 1).

Table 1 Content of iron and ferritin in the serum of chickens

<table>
<thead>
<tr>
<th>Group</th>
<th>Fe, umol/l</th>
<th>Ferritin, μg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days of the experiment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>1</td>
<td>26.2±0.38</td>
<td>24.7±0.17</td>
</tr>
<tr>
<td>7</td>
<td>27.1±0.2</td>
<td>21.6±0.8</td>
</tr>
<tr>
<td>14</td>
<td>24.5±0.29</td>
<td>21.6±0.6</td>
</tr>
<tr>
<td>28</td>
<td>26.0±0.4</td>
<td>23.4±0.1</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>5*</td>
</tr>
<tr>
<td>28</td>
<td>4</td>
<td>2*</td>
</tr>
<tr>
<td>Note: * – the results are statistically significant (p≤0.05); ** – the results are statistically significant (p≤0.01).</td>
<td></td>
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</table>

Receiving nanoFe with food helped to increase the concentration of iron in the body of poultry by 5.3% (P ≤ 0.05) in group II and by 4.5% (P ≤ 0.05) in group III.

The size of iron pool in the body of poultry increased by 13.0% (P ≤ 0.05) in group II, by 14.6% (P ≤ 0.001) in group III, and by 17.3% (P ≤ 0.001) in group V.

Table 2. Characteristics of the iron pool in the body of poultry (28 days of experiment)

<table>
<thead>
<tr>
<th>Group</th>
<th>Indicator</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iron concentration, mmol/kg of weight</td>
<td>1.32±</td>
<td>1.38±</td>
<td>1.39±</td>
<td>1.33±</td>
<td>1.36±</td>
<td>1.33±</td>
<td>1.33±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.001</td>
<td>0.003*</td>
<td>0.004*</td>
<td>0.001</td>
<td>0.008</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Size of iron pool in organism, mmol</td>
<td>2.53±</td>
<td>2.86±</td>
<td>2.90±</td>
<td>2.71±</td>
<td>2.97±</td>
<td>2.79±</td>
<td>2.74±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.07</td>
<td>0.07*</td>
<td>0.05*</td>
<td>0.12</td>
<td>0.05*</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>Note: * – the results are statistically significant (p ≤ 0.05); ** – the results are statistically significant (p ≤ 0.01).</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

3.2 The amino acid composition

Adding nanoFe to food without amino acids is accompanied by an increase of arginine content in the poultry’s liver of group II compared to the control group by 3.3% (P ≤ 0.05) on day 7, by 4.2% (P ≤ 0.05) on day 14, and by 3.05% (P ≤ 0.05) on day 28 of the experiment (Figure 1).
analysis of the amino acid composition of poultry’s liver after the 7th and 14th days of the experiment showed similar results.

Fig. 1. The amino acid composition of poultry’s liver of groups II, III and V compared to the control one (Day 28 of the experiment).

The exception is the increase in arginine concentration in poultry’s liver of groups III and V on the 7th day of the experiment by 3.5% ($P \leq 0.05$) and by 3.7% ($P \leq 0.05$) compared to the control group. The content of arginine in group II of the poultry’s liver remained at high level during the experiment.

The analysis of the amino acid composition of poultry’s liver in the groups that did not receive nanoFe showed no significant changes in the analyzed indicators (Figure 2).

Fig. 2. The amino acid composition in liver of poultry of groups IV, VI and VII compared to the control values.

3.3 Content of NO-metabolites

Adding nanoFe to the diet of poultry in group II is accompanied by the increased content of NO-metabolites in the liver compared to the control group from 7 to 28 days of the experiment by 3.1-3.5% ($P \leq 0.05$) (Figure 3).

The combination of nanoFe with arginine and the amino acid mixture contributed to the growth of NO-metabolites indicators in the liver by 2.8 and 3.6% ($P \leq 0.05$) only on day 7 of the experiment.

In the groups without nanoFe, no significant changes in NO-metabolite concentrations in poultry’s liver were observed (Figure 4).

Fig. 3. The content of NO-metabolites in the poultry’s liver of groups II, III and V compared to the control values.

Using nanoFe together with amino acids increased the concentration of NO-metabolites in the blood and liver of chickens only on the 7th day of the experiment by 2.0-3.5%; using nanoFe without amino acids boosted the growth of NO-metabolites indicators by 3-4% (Figure 5) during the experiment.

Fig. 5. The content of NO-metabolites in the poultry’s blood in groups II, III and V compared to the control values.
In the groups without nanoFe, no significant changes in the concentration of NO-metabolites in the blood of poultry were observed (Figure 6).

Fig. 6. The content of NO-metabolites in the poultry’s blood in groups IV, VI and VII compared to the control values.

Table 3. The morphological blood parameters of broiler chickens

<table>
<thead>
<tr>
<th>Indicator</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes, 10^12/l</td>
<td>2.7±0.01</td>
<td>2.92±0.02*</td>
<td>2.93±0.02**</td>
<td>2.73±0.26</td>
<td>3.04±0.01**</td>
<td>2.73±0.02</td>
<td>2.71±0.03</td>
</tr>
<tr>
<td>Hemoglobin, GM/DL</td>
<td>146±0.58</td>
<td>152±0.81*</td>
<td>151.7±0.58*</td>
<td>145±2.08</td>
<td>161±0.64**</td>
<td>146.5±0.2</td>
<td>145.2±0.5</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>29.6±0.69</td>
<td>32.6±0.29*</td>
<td>32.8±0.57*</td>
<td>30.9±0.58</td>
<td>33.3±0.75*</td>
<td>31.6±0.83</td>
<td>28.6±0.98</td>
</tr>
<tr>
<td>Mean cell hemoglobin, pg</td>
<td>53.2±0.44</td>
<td>54.7±0.01*</td>
<td>55.3±0.06*</td>
<td>52.5±0.53</td>
<td>55.9±0.06*</td>
<td>56.8±0.39</td>
<td>53.9±0.38</td>
</tr>
</tbody>
</table>

3.4 The morphological composition of the blood of the test chickens

Using nanoFe in food resulted in an increase in the level of erythrocytes of groups V, III and II on day 7 of the studies by 7 (P ≤ 0.01), 5 and 4.7% (P ≤ 0.01). On day 14, the number of erythrocytes in groups V, III and II relatively to the control one was increased by 11.3 (P ≤ 0.05), 6.42 and 5.81% (P ≤ 0.05). The concentration of erythrocytes on day 21 of the experiment in groups V, III and II was increased by 12.6 (P ≤ 0.001), 8.5 and 8.2% (P ≤ 0.01).

3.5 Dynamics of live weight poultry

The addition of amino acids in the diet was accompanied by an increase in broiler chickens body weight in groups IV, VI and VII by 6.1, 9.4 and 7.2% compared to the control values (P ≤ 0.05) (Figure 7).

Using nanoFe in the diet of group II resulted in a significant increase in body weight on day 8 by 8.1% (P ≤ 0.05) in relation to the control one.

Fig. 7. Dynamics of broiler chickens body weight in groups IV, VI and VII compared to the control values.
In the period from the 10th to 25th day of the experiment there was a similar difference between 4 and 7.2% (Figure 8). Feeding chickens of group III with nanoFe along with arginine has determined an increase in body weight by 8.3% (P ≤ 0.05) on day 5.

Adding nanoFe to the diet with “arginine + lysine + methionine” complex was accompanied by an increase in live weight of chickens after 7 days of the experiment by 12% (P ≤ 0.01), day 14 by 14% (P ≤ 0.001), day 21 by 16.2% (P ≤ 0.001), day 28 by 13.9% (P ≤ 0.001) compared to the control values.

**4. DISCUSSION**

Iron nanoparticles and its compounds have good prospects in creating new drugs of microelements [9] in the magnetic resonance imaging [23-24] and as a vehicle for drugs [25], etc. Meanwhile, along with the advantages of the drugs with nanoparticle, there are some disadvantages that affect the central nervous system [26], the excretory system [27], and others. This determines the prospects of research aimed at reducing the adverse effects of nanoparticles [28-29] including the use of drug nanoparticles along with arginine [15].

Possibly, the activation of arginine metabolism in the course of intake of nanomaterials is the norm. We can assume it based on the fact of increasing the content of arginine in the liver of animals when receiving the iron nanoparticles [21]. The mechanism for running an additional arginine synthesis can be initiated in several ways. Arginine is considered to be a conditionally essential at the inflammatory and oxidative stress [18, 30]. The action of arginine is linked with the modulation of inflammatory mediators, release of cytokines, etc. [31].

This study is a continuation of previously performed experiments and it is carried out within the framework of works on the development of new drugs for animals based on metal nanoparticles. As follows from the obtained data, adding *nanoFe* drugs in poultry’s diet was accompanied by a significant increase in arginine concentration in group II poultry’s liver from the 7th to the 28th days of the experiment. It was noted the increase of the level of arginine in poultry’s liver on the 7th day of the experiment when combined *nanoFe* with amino acids.

The activation of arginine synthesis can be initiated by the synthesis of nitric oxide (NO). Iron and NO homeostasis are connected to each other [32]. Iron affects the expression of inducible NO-synthase 2 [33]. Fpn1 NO-induced transcription reduces cellular iron content [34]. Large amounts of NO are released from inducible NO-synthase isoform in response to inflammatory stimuli in various types of cells [35-36]. It happens due to the action of nanoparticles of different metals accompanied by the development of oxidative stress [15]. In its turn, NO is produced by means of oxidation of one of the terminal nitrogen atoms in the guanidine group of L-arginine. This determines the close relationship between the NO production and the exchange of arginine.

This relationship is also described in our studies. Increasing the content of arginine in the liver of animals when receiving *nanoFe* is accompanied by an increase in the concentration of NO-metabolites in the poultry’s liver and blood of group II by 3.0-3.5% (P ≤ 0.05) and 3.5-4.0% (P ≤ 0.05) starting from the first week of the experiment. A significant increase in the content of NO-metabolites in the liver and blood of the poultry in groups III and V fed with *nanoFe* and amino acids was noted only in the first week of the experiment. The content of arginine in the poultry’s liver of these groups did not differ from the control values at the end of the study. This differs in some way from the earlier obtained data when receiving L-arginine orally [37].

At the same time, the formation of physiological levels of nitric oxide from arginine can have an indirect positive effect on the productivity of animals [38]. Our study has confirmed this fact. The use of arginine and other amino acids in the diet without drugs of iron resulted in an increase in the body weight of poultry (Figure 7) and confirmed the link of arginine with growth stimulation processes.

However, using *nanoFe* with arginine and other amino acids was more efficient. The poultry that got a complex of three amino acids and *nanoFe* surpassed its analogues in live weight (Figure 8). Similar results were obtained earlier for zinc nanoparticles when used together with methionine. Adding the nanozink-methionine complex to the diet helped to improve a growth rate and feed conversion of broilers.
The combination of methionine and arginine is effective for increasing productivity. Lysine and arginine are antagonists but together they stimulate a growth hormone. Part of the energy needed for protein synthesis is produced by means of oxidation of lysine. This explains having of florid effects when used nanoFe with "arginine + lysine + methionine" complex (Figure 8).

The combination of growth stimulation action of arginine and nanoFe together with the acceleration of migration of iron by the action of methionine contributed a significant jump in weight gain as compared to the group that did not receive methionine. In the groups fed with nanoFe together and without arginine, the concentration of iron in the body tissues is significantly higher (4.5-5.3%) than in groups where nanoFe was used together with the amino acid complex.

5. CONCLUSION

The most efficient use of iron nanoparticles in the diet of chickens is in combination with arginine, lysine and methionine. This provides efficient use of iron and is accompanied by the increased growth of poultry. During the study of the amino acid composition of poultry liver, there was an increase by 3.1-4.2% in the content of arginine when feeding nanoFe. Also adding nanoFe in poultry’s diet is accompanied by an increased content of NO-metabolites in the liver by 3.1-3.5%. The enrichment of the food with nanoFe helped to increase the iron concentration in the body of poultry by 5.3% during the experiment. Using nanoFe in compound feed resulted in an increase of erythrocytes in blood, and with the additional feeding of amino acids the increase was more significant.

6. ACKNOWLEDGEMENTS

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7. REFERENCES


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