Increasing efficiency in the poultry meat production when using iron and copper nanoparticles in nutrition

E V Yausheva
Federal Research Centre of Biological Systems and Agrotechnologies RAS, Orenburg, Russia
E-mail: vasilena56@mail.ru

Abstract. The paper is aimed at studying the increasing efficiency in the poultry meat production when using iron and copper nanoparticles in nutrition. A maximum increase in the gain in live weight of chickens by 9-12 % in comparison with the control group in the first week after the injection of nanoparticles is observed. An increase in values of a number of blood parameters (hemoglobin, total protein, white blood cells) is noted. Copper nanoparticles increase the body’s immune response on the first day of experiment by several orders in comparison with both control and iron nanoparticles. The analysis of mineral exchange data showed a decrease in a number of toxic (As, Pb, Al, Cd) elements and an increase in the level of a number of essential (Mg, Co, Fe, Cu, Na, F) elements in broiler tissues after exposure to copper nanoforms and iron nanoforms. The analysis shows more effective influence on the metabolic processes of chickens and require further detailed study of the processes of their transformation in the broiler body. The obtained result shows the possibility of using these forms in the industrial production of animal feed.

1. Introduction
Currently, the global market for final products and products containing nanomaterials is represented by approximately 800 consumer goods [1]. Nanomaterials are used in agriculture [2] as animal growth stimulants [3] and disinfectants [4]. In particular, metal nanoparticles are considered in animal husbandry as alternative solutions of trace elements [5–9]. At the same time, the safety issues of using nanoparticles with an assessment of their toxicity are also of great interest [10–13]. The attention is associated with lower toxicity of metal nanoparticles, higher bioavailability of trace elements from nanoforms in comparison with traditional drugs [14]. Among studies on the use of metal nanoparticles in animal husbandry, there are some that demonstrate the growth-promoting and wound-healing effects of metal nanoparticles, including nanoiron. The possibility of using nanoparticles of a number of essential elements, including copper, as feed additives was demonstrated [15].

Our study was aimed at a detailed study of the influence of copper and iron nanoparticles on the productivity and metabolism of broiler chickens.

2. Materials and Methods
2.1 Obtaining and certification of nanoparticle solutions
In experimental studies, iron nanoparticles (INs) with a size of 80±2 nm and copper nanoparticles with a size of 95±3 nm were used. The nanoparticle solutions were obtained by the method of high-temperature condensation at the MiGen-3 plant. Material science certification of the solutions included
the following: electron scanning and transmission microscopy using JSM 7401F and JEM-2000FX (JEOL, Japan); X-ray phase analysis was performed on DRON-7 diffractometer; atomic force microscopy on SMM-2000 multimicroscope (OJSC PROTON-MIET, Russia).

2.2 Experimental animals and their contents

Studies were conducted on broilers Smena-7. Animal services and experimental studies were carried out in accordance with the instructions of the Russian Regulations, 1987 (Order No. 755 of 12.08.1977 the USSR Ministry of Health) and The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1966). Broiler chickens were fed with full-fledged feed.

2.3 Flow of study

For this study 100 heads of 7 day-old broiler chickens were selected, which were divided into 3 groups by the method of pairs of analogues (n=30). All the birdswere placed in the same conditions – keeping and feeding. At the age of 14 days the chickens were injected one intramuscularly (in the femoris muscle): Group I – a solution of iron nanoparticles (2 mg/kg of live weight); Group II – a solution of copper nanoparticles (2 mg/kg of live weight); Group III (control group) – a sterile 0.9 % NaCl solution in a volume of 100 ml/head. Immediately prior to injection, the nanoparticles were mixed with a sterile 0.9 % NaCl solution and sonicated for 30 minutes (frequency – 35 kHz; power – 300 (450) W, vibration amplitude – 10 micrometer). The slaughter of the experimental bird was carried out at 15-, 21- and 35-day-old age, which corresponded to 1, 7 and 21 days after injection. During the experiment, the chickens were weighed daily.

The morphological parameters of the blood were determined using an automatic hematological blood analyzer URIT 2900 VETPlus (manufacturer – URIT MEDICAL ELECTRONIC CO., LTD, China). Biochemical analysis of blood was carried out using an automatic biochemical analyzer CS-T240 (manufacturer – Dirui Industrial Co., Ltd., China). The analysis of the elemental composition of feed and body tissue of broilers included the identification of 25 chemical elements: Ca, Cu, Fe, Li, Mg, Mn, Ni, As, Cr, K, Na, P, Zn, I, V, Co, Se, Ti, Al, Be, Cd, Pb, Hg, Sn, Sr; using atomic emission and mass spectral research methods.

The statistical processing of the obtained data was carried out using the ANOVA techniques in Statistica 10.0. The reliable results were considered at p≤0.05.

3. Results

The injection of copper nanoparticles was accompanied by a spasmodic change in the dynamics of bird growth throughout the experiment and a significant increase in live weight growth relative to the control by 8.4 % (P≤0.01) within few days after injection. This pattern persisted during the first 2 weeks of the study. At the end of the experiments, the bird mass in the first group was 5 % higher than the bird mass in the control group (P≤0.01).

Similarly, the injection of iron nanoparticles was accompanied by an increase in the live weight gain of broiler chickens during the experiment. On day 4 of the experiment, the maximum increase in live weight in the second group was noted in comparison with the control group – 9.8 % (P≤0.01), after 10 days – 8.06 % (P≤0.01), by the end of the study, the increase was 4.16 % (P≤0.01) relative to the control (Fig. 1).

The injection of copper and iron nanoparticles resulted in an increase in a number of blood parameters. The red blood cell count one day after the injection of nanoparticles in 15-day-old broiler chickens of the second and third groups was 21.3 and 3.04 % higher relative to the control group, 7 days after the injection in 21-day-old broiler chickens by 6.67 and 11.1 %, 21 days after the injection the level of red blood cells did not practically differ from the control values (Table 1).

The hemoglobin concentration one day after the injection of nanoparticles in 15-day-old broiler chickens in the first and second groups was increased by 34.1 and 21.1 % relative to the control, in 21-day-old brookers by 4.34 and 3.26 %, respectively, in 35-day-old broiler chickens, the hemoglobin concentration did not practically differ from the control group.
Figure 1. The dynamics of difference in live weight between the control and I, II groups

Table 1. The effect of copper and iron nanoparticles on morphological parameters of blood of broiler chickens with a single injection

| Parameter        | Group   | Age of chickens, day |
|------------------|---------|----------------------|
|                  |         | 15                   | 21     | 35                   |
| Erythrocyte, 10^{12}/l | I       | 2.79±0.49            | 2.88±0.56 | 3.13±0.01 |
|                  | II      | 2.37±0.42            | 3±0.39   | 3.11±0.01 |
|                  | Control | 2.3±0.92             | 2.7±0.68 | 3.09±0.63 |
| Hemoglobin, g/l  | I       | 142.4±5.9            | 115.4±9.2 | 132.9±4.8 |
|                  | II      | 128.6±7.16           | 114.2±11.5 | 132.3±5.6 |
|                  | Control | 106.2±3.8            | 110.6±10.6 | 130.3±3.6 |
| Leukocyte, 10^9/l| I       | 28.3±0.3*            | 26.3±0.06* | 23.9±0.08* |
|                  | II      | 21.6±0.05*           | 26.9±0.4*  | 23.6±0.7*  |
|                  | Control | 20.1±0.4             | 24.8±0.2  | 23.4±0.21 |
| Total protein, g/l| I       | 24.6±0.09**          | 35.3±0.26** | 49.6±0.12** |
|                  | II      | 24.5±0.09**          | 34.5±0.14** | 48.5±0.12** |
|                  | Control | 23.9±0.12            | 29.3±0.09  | 42.8±0.12  |

Regarding the number of leukocytes, a significant increase in their magnitude was noted during the experiment. The leukocyte level one day after the injection of the studied metal nanoparticles in 15-day-old broiler chickens of groups II and III was significantly higher by 40.8 and 7.46 % (P ≤ 0.05) relative to the control group, in 21 day-olds by 6.12 and 8.47 % (P≤0.05 ), respectively, in 35-day-old broilers of the first group by 2.14 % (P≤0.05).

The values of total protein were characterized by an increase in indicators one day after injections in the first and second groups by 2.93 and 2.51 % (P≤0.01), respectively, relative to the control, 7 days after the injection in the first group by 20.5 (P≤0.01) %, in the second group by 17.7 % (P≤0.01). At the 35-day-old age of broiler chickens the level of total protein in the first and second groups was higher by 15.9 and 13.3 % (P≤0.01) than in the control group.

The study of the elemental composition of the organs and tissues of broiler chickens showed significant changes in the level of individual elements after a single injection of nanoparticles only in the first week of the experiment. The injection of copper nanoparticles after 7 days led to a decrease in the content of arsenic (by 6.17 %), aluminum (by 4.39 %), cadmium and lead (by 2.5 and 6.06 %). At the same time, there was an increase in the content in the tissues of cobalt (by 4.08 %), copper (by 6.01 %), calcium (by 3.85 %), magnesium (by 3.24 %), sodium (by 2.55 %) and phosphorus (by 3.02 %) already in the first 7 days of the study (Fig. 2).
Figure 2. Difference in size of pools of macro- and microelements in the body tissues of broilers relative to the control group 7 days after the injection of the solution of copper nanoparticles

A similar effect with respect to the concentration of chemical elements in the body tissues of broiler chickens was exerted by the injection of iron nanoparticle solution (Fig. 3).

Figure 3. Difference in size of pools of macro- and microelements in the body tissues of broilers relative to the control group 7 days after the injection of iron nanoparticle solution

The introduction of iron nanoparticle lyosols after 7 days resulted in a decrease in tissue levels of arsenic by 6.07 % (P≤0.05), aluminum by 5.06 % (P≤0.05), cadmium by 5 % (P≤0.05) and lead by 3.79 % (P≤ 0.05); and an increase in the content of cobalt and sodium by 4.08 and 4.86 % (P≤0.05), calcium by 6.48 % (P≤0.01), phosphorus by 3.57 (P≤0.001), magnesium – 2.82 % (P≤0.001), relative to the control values. In contrast to the solution of copper nanoparticles, the solution of iron nanoparticles resulted in a significant increase in the concentration of iron in tissues by 5.82 % (P≤0.01).

4. Discussion

The data showed positive influence of iron and copper nanoparticles on the productive performance of broiler chickens.

The growth-promoting effect of the tested nanoparticles on broiler chickens showed maximum values of live weight gain in the first week of the experiment and gradually decreased by the end of the third week of the study. Copper nanoparticles had a greater impact on broiler growth dynamics compared to iron nanoparticles. However, the difference between the tested nanoparticles in terms of the influence on broiler growth indicators was no more than 2-3 %. Similar results on the influence of copper nanoparticles and iron nanoparticles on productivity indicators were obtained by a number of other researchers. The study by El-Kazaz and Hafez (2019) showed that the injection of copper
nanoparticles in drinking water (10 mg/l) contributed to an increase in the weight of chickens and feed conversion [16]. Similarly for iron nanoparticles in the studies of Hassan et al. (2020) and Rehman et al. (2020), the possibilities for the use of nanoparticles in poultry farming were shown, including their growth-promoting effect, which contributes to a 50 % increase in live weight gain in comparison with the control group [17, 18].

In the work of Wang et al. (2011) it was demonstrated the positive effect of copper nanoparticles on a number of factors (immunological system, serum protein level, intestinal microflora) that have positive dynamics against the background of increased broiler productivity. The authors noted that the introduction of copper nanoparticles significantly (P <0.05) increased the concentration of total protein in blood serum and was accompanied by a large increase in live weight in the experimental group compared to the control. Similar changes were noted in our studies [19]. Against the injections of the studied metal nanoparticles there was observed an increase in the level of protein in blood serum by more than 15 % after a week of the experiment, which remained until the end of the study. Probably in our studies, this was also one of the factors correlating with a high increase in live weight of experimental chickens against the background of control.

At the same time, significant changes in some blood parameters and the level of content of certain chemical elements in the broiler organism were noted. The level of leukocytes that was observed in the blood serum of an experimental bird indicated an increase in the immune response, especially on the first day of injection with the introduction of copper nanoparticles. A similar effect of copper nanoparticles was noted by El-Kassas et al. (2018), where an increase in the immune response due to copper nanoparticles contributed to an improvement in poultry content [20]. In the study by Ognik et al. (2018) it was also noted that an increase in the immune response while using nanoparticles contributes to an increase in the antioxidant potential of the body and inhibition of lipid peroxidation [21].

The studies of the number of blood cells showed an increase in red blood cells and hemoglobin. Some studies similarly denote the effect of iron oxide nanoparticles and copper nanoparticles on hemoglobin level [5, 8, 9]. The presence of a similar effect of the studied nanoparticles in relation to blood cells may become the basis for solving the problem of iron deficiency anemia, including animals. Iron deficiency anemia persists as a major human metabolic disorder affecting more than 1 billion people on our planet (World Health Organization, 2008).

The analysis of mineral exchange data showed a decrease in a number of toxic (As, Pb, Al, Cd) elements and an increase in the level of a number of essential (Mg, Co, Fe, Cu, Na, F) elements in broiler tissues after exposure to copper nanoforms and iron nanoforms. It is likely that intermediate products (cations of various valencies, reactive oxygen species), which are formed upon partial electrochemical dissolution of the analyzed nanoparticles contribute to the sorption of heavy metals, acting as inhibitors. At the same time, an increase in the level of a number of essential elements in tissues is probably due to the ability of copper nanoparticles to increase intestinal adsorption of a number of elements. So, the study carried out by Ognik et al. (2016) denoted the effect of copper nanoparticles on the adsorption of calcium, iron and copper, accompanied by an increase in the content of these elements in the body [22, 25]. It is suggested that the increased calcium levels in broilers would correlate with bone strength. This suggestion can be confirmed by the work of Mroczek-Sosnowska et al. (2017). The study of the authors showed that the femur of chickens from the groups receiving copper nanoparticles is more resistant to fractures compared with the control group [23].

5. Conclusion
The research results showed a positive dynamics of the influence of copper nanoparticles and iron nanoparticles on productivity, concentration of chemical elements in tissues and blood parameters in the body of broiler chickens. A spasmodic change in live weight gain and selectivity of the studied nanoparticles on the level of elements in the broiler organism was observed. Copper nanoparticles
showed a better effect on the metabolic processes of chickens and require further detailed study of the processes of their transformation in the broiler body.

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