The environmental-biology aspects of use of chitosan and ultrafine particles of copper and iron in the nutrition of broiler chickens

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Abstract. The natural biopolymer chitosan is currently of great interest to specialists in the field of medicine and veterinary medicine due to properties such as biocompatibility, good sorption and low allergenicity. The aim of the study is to study the environmental-biology effect of chitosan and UFP on growth, productivity and morpho-biochemical blood parameters of broiler chickens. The study was performed on broiler chickens of «ArborAcres» cross (n = 60) in the conditions of vivarium. Biochemical studies of blood serum were performed using an automated analyzer CS-T240 (DIRUI Industrial Co., Ltd, China). The use of chitosan (group I) and UFP (group II) leads to a positive productive effect, however, the joint feeding with chitosan with UFP (group III) does not give a similar result. Feeding with chitosan-containing diet (group I) reduced the concentration of total cholesterol and triglycerides to a greater extent than the use of UFP (group II). Against the background of the introduction of chitosan into food (group I), the activity of catalase increased compared with that of the control. The level of malondialdehyde (MDA), as an indicator of lipid peroxidation, decreased with all variants of feed additives. The use of chitosan and UFP in the diets of broiler chickens is advisable and helps to normalize the metabolism and productivity of the bird. The results indicate a promising approach, and requires further research as a component that can be used in the industrial production of animal feed.

1. Introduction
Chitosan is one of the promising feed additives; it is a natural biopolymer having good biocompatibility, biodegradability, and low allergenicity [1]. It is widespread in nature, as a component of the exoskeleton of shrimp, crabs and insects [2, 3]. Chitosan administered with food is capable of enzymatic cleavage and assimilation in the form of low-molecular-weight compounds. Chitosan oligomers influence nonspecific resistance factors, stimulating the immune system. It is actively used in pharmaceutical research and industry as a carrier for drug delivery and as a biomedical material and sorbent [4]. In addition, the problem of environmental pollution by toxic substances, including heavy metals and the receipt and production of livestock, is still relevant. A lot of attention is paid to the search for integrated solutions aimed at simultaneous sorption and safety, as well as the ability of targeted delivery of a substance. Chitosan was chosen due to its high mucoadhesiveness and ability to enhance the penetration of large molecules through the surface of the mucous membrane [5].

The complex of UFP and chitosan is able to reduce the rate of release of active substance and thereby prolong the effect. Thus, it is possible to modulate the relative parameters to meet the needs
for various nutrients [6]. The aim of the study was to assess the effect of chitosan and UFP on the growth, productivity and morpho-biochemical blood parameters of broiler chickens.

2. Materials and methods
The studies were conducted on broilers of ArborAcres cross (n = 60) in vivarium conditions in accordance with the requirements of the instructions and recommendations of the Russian Regulation, 1987 (Order of the Ministry of Health and Social Development No. 755 of 08/12/1977) and «The Guide for Care and Use of Laboratory Animals (National academy Press, Washington, DC, 1996)». All of the experimental methods and techniques were approved by the Committee on Ethics of the Federal Research Centre of Biological Systems and Agrotechnologies.

In order to study the effect of chitosan growth, productivity and morpho-biochemical blood parameters, four groups were formed (n = 15) (one control and three experimental groups). Throughout the growing period (42 days) starting from the age of seven days, UFP and chitosan were introduced into the diet of the experimental groups. Chickens in the control group received a premix according to the recommendation of VNITIP (2009) with iron sulfate (FeSO$_4$ × 7H$_2$O) and copper (CuSO$_4$ × 5H$_2$O) used as a source of iron and copper. Chickens from group I were fed with chitosan added in the amount of 1 mg/kg feed (Evalar, Russia). In group II – FeSO$_4$ × 7H$_2$O was replaced with Fe UFP at a dose of 17.5 mg/kg feed (Advanced Powder Technologies, Tomsk), and CuSO$_4$ × 5H$_2$O with Cu at a dose of 1.7 mg/kg feed (LLC «Platinum», Moscow). In group III FeSO$_4$ × 7H$_2$O was replaced by FeUFP and CuSO$_4$ × 5H$_2$O with Cu UFP and chitosan.

Morphological parameters were determined using an automatic hematology analyzer model URIT-2900 VetPlus ("URIT Medial Electronic Co., Ltd", China). Biochemical studies of blood serum were performed using an automated analyzer CS-T240 (DIRUI Industrial Co., Ltd, China) with the use of commercial kits for veterinary studies DiaVetTest (Russia) and Randox Laboratories Limited (United Kingdom).

Statistical processing. Data are expressed as mean values ± standard error of the mean (M ± m). Statistical analysis was performed using Statistica 10.0 (StatSoft Inc., USA) and Microsoft Excel (Microsoft, USA). Significance of the group differences was estimated using Student’s t-test with p≤0.05 considered as significant.

3. Results
3.1. Research results
Assessing feed intake, it was found that the maximum eatability was noted in group I (Table 1). Low feed eatability was characteristic of broilers of group III.

Analysis of the dynamics of live weight per experiment showed differences in growth rate of broiler chickens. Thus, chickens of group I exceeded their peers from the control group by 4.4% (Figure 1). In turn, the difference with the control was 2.6% in group II.

Live weight of chickens was minimal in group III among all groups with a difference of 3.7% from the control (p≤0.05) by the end of the experiment.

For the period of the experiment, 1.48 kg/head (p≤0.05) was obtained in group I, an increase at a feed cost of 2.36 kg, it was 5.8% higher than the same value in the control group.

Thus, the chickens of group I were distinguished by the maximum feed intake and feed costs per 1 kg of live weight gain, which ensured maximum indicators of live weight.
The difference in live weight between the experimental and control groups, %

| Group            | Live weight gain per an experiment, kg | % of control | Feed costs per 1 kg of live weight gain, kg | % of control | Feed intake, g |
|------------------|---------------------------------------|--------------|---------------------------------------------|--------------|----------------|
| Control          | 1.4±0.04                              | 100.0        | 2.23                                        | 100.0        | 2238           |
| I experimental   | 1.48±0.06*                            | 105.7        | 2.36                                        | 105.8        | 2363           |
| II experimental  | 1.44±0.1*                             | 102.8        | 2.34                                        | 104.9        | 2344           |
| III experimental | 1.35±0.1                              | 96.4         | 2.14                                        | 95.9         | 2141           |

Note: * – at p≤0.05 comparing experimental samples with control

The combined feeding of chitosan and UFP (group II) ensured an increase in live weight gain up to 1.44 kg, it was higher by 2.8% (p≤0.05), respectively, exceeding the same indicator in the control. At the same time, in the III experimental group, we noted a decrease in feed consumption and production costs of 1 kg of live weight gain by 4.1% compared with the control, which led to low productivity indicators. Thus, the inclusion of chitosan in feed is accompanied by a positive effect on the growth of broiler chickens.

The use of the studied additives was reflected in the dynamics of hematological parameters. So, the number of red blood cells in blood of broiler chickens increases by 4.5% (p≤0.05) after feeding with UFP (group II) compared with the control. In the remaining groups, the number of red blood cells is close to the control values (Table 2).

The hematocrit was reduced in groups II and II by 4.4 and 7.8% (p≤0.05), respectively, relative to the control. In group III, vice versa, hematocrit increased by 2% (p≤0.05).

Table 2. Morphological parameters of blood of broilers «ArborAykres» at the age of 42 days (experiment in vivarium conditions, M ± m, n = 15)

| Indicator          | Group I            | Group II            | Group III           | Control           |
|--------------------|--------------------|---------------------|---------------------|-------------------|
| Erythrocytes, 10¹²/l | 3.47±0.03          | 3.75±0.09*          | 3.57±0.02           | 3.54±0.01         |
| Hemoglobin, g/l    | 108.3±1.5*         | 105±1.3             | 114±2.9             | 111.8±1.5         |
| Hematocrit, %      | 19.4±0.3           | 18.7±0.1*           | 20.7±0.8            | 20.3±0.8          |
| Leukocytes, 10⁹/l  | 37.2±1.9           | 33.9±2.5*           | 43.08±3.2*          | 39.3±2.5          |

Note: * – at p≤0.05 comparing experimental samples with control
Analyzing the results, a decrease in the level of leukocytes in blood of broiler chickens was noted by 5.3% in group I and by 13.7% (p ≤ 0.05) – in group II compared with the control. An increase in the number of leukocytes was characteristic of group III by 9.6% (p ≤ 0.05).

The use of different variants of the combination of the studied additives led to a change in biochemical parameters of blood serum of broiler chickens (Table 3).

The amount of total protein, urea cholesterol and triglycerides in blood serum tended to decrease in all groups relative to the control. So, low cholesterol and triglycerides were observed in group III with a difference from the control of 20 and 33% (p ≤ 0.05), respectively.

The absence of oxidative stress was indicated by the dynamics of the activity of catalase (CT) and total superoxide dismutase (T-SOD) (Table 4).

### Table 3. Biochemical parameters of blood serum of broilers «ArborAcres» at the age of 42 days (experiment in vivarium conditions, M ± m, n = 15)

| Indicator      | Group I     | Group II    | Group III   | Control    |
|----------------|-------------|-------------|-------------|------------|
| Glucose, mmol/l | 14.4±0.3    | 14.6±0.9    | 14.02±0.7*  | 17.1±3.2   |
| Total protein, g/l | 38.4±1.6*   | 38.7±1.7*   | 38.6±1.9*   | 42.8±1     |
| Creatinine, mmol/l | 16.6±0.8    | 18.2±0.9*   | 18.05±0.8   | 17±0.7     |
| Cholesterol, mmol/l | 3.5±0.1*    | 3.1±0.2*    | 3.2±0.1     | 4.06±0.4   |
| Triglycerides, mmol/l | 0.33±0.03   | 0.37±0.07   | 0.25±0.02*  | 0.35±0.05  |
| Urea, mmol/l    | 0.48±0.1    | 0.32±0.1*   | 0.52±0.2*   | 0.63±0.1   |

Note: * – at p≤0.05 comparing experimental samples with intact samples

### Table 4. Indicators of antioxidant system of blood of broilers «ArborAcres» at the age of 42 days (experiment in vivarium conditions, M ± m, n = 15)

| Indicator | Group I | Group II | Group III | Control |
|-----------|---------|----------|-----------|---------|
| SOD,%     | 63.31±2.920 | 494.17±22.25 | 0.03±0.004 |         |
| CAT, μM H2O2/minute | 66.79±2.217 | 364.97±26.03 | 0.03±0.004 |         |
| MDA, μM/L | 60.22±2.632 | 422.24±12.563 | 0.04±0.013 |         |

We did not find peak values of their concentration. Against the background of the introduction of chitosan into food (group I), the activity of catalase on day 42 increases by 17% compared with that of the control.

The level of malondialdehyde (MDA), as an indicator of lipid peroxidation, decreases with the use of all variants of feed additives.

### 4. Discussions

Chitosan and UFP used in the experiment are compatible and biologically active. UFP are widely used in animal husbandry [7] as growth stimulants and sources of trace elements [8]. Depending on physicochemical characteristics, UFP can have an antibacterial effect [9] and induce apoptosis [10], affect the structure of target organs [11].

Chitosan just like UFP can stimulate the activity of certain biochemical processes in metabolic chain [12–14]. The experiment proved that body weight and food intake by chickens that were fed with chitosan-containing diets were different from control animals.

Enrichment of diet with chitosan improved broiler growth rates. The effect was associated with an increase in average daily feed intake and digestibility of nutrients. Similar beneficial effects of chitin-containing additives have been described previously [15].

However, some studies have shown that chitosan can interfere with the digestion and absorption of fat [16]. Such results are due to a decrease in the feeling of fullness in broiler chickens, an increase in feed intake and, as a result, a duodenal sprain [17].
The observed positive effect of chitosan on productivity is probably associated, on the one hand, with an action as a sorbent that promotes the adsorption of toxins and heavy metals in the gastrointestinal tract and enhances their excretion from animals. On the other hand, as an enveloping substance, normalizing the qualitative and quantitative composition of intestinal microflora [18, 22].

Chitosan affects not only the growth rate, but also the biochemical parameters of blood [19]. Thus, some indicators (total protein, triglycerides) had a tendency to a decrease after the use of chitosan. This is probably due to the increased viscosity and mucoadhesiveness of the contents of the gastrointestinal tract, as well as to the ineffective enzymatic assimilation of chitosan [20].

Chitin-containing additives affect cholesterol and triglycerides, causing them to decrease. Due to the presence of adsorbing properties, chitosan can reduce their absorption in the intestine [21]. However, there are opposite results indicating the absence of changes in cholesterol levels. Thus, Nuengjamnong C. [20] argues that chitosan at a low level (1–2 g/kg) cannot affect the concentration of cholesterol and triglycerides in broilers.

Chitosan did not have a significant effect on counts of leukocytes in blood of chickens. However, in animals that consumed chitosan in combination with UFP, the number of leukocytes increased. Meng et al. [17] confirm this by discovering that birds fed with a mixed diet had a higher white blood cell count than other groups aged 42 days.

5. Conclusion
The use of chitosan and UFP in the diets of broiler chickens is advisable and contributes to an increase in body weight, and therefore productivity, normalization of metabolism and poultry. The obtained results indicate the prospects of the approach, and requires further research.

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