The effect of an antioxidant on the hematological profile of birds

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Abstract. The research is devoted to the study of the influence of the modern antioxidant of the flavonoid group ‘Dihydroquercetin’ as part of the feed combination on the hematological profile and biochemical parameters of the blood of broiler chickens of the KOBB-500 cross. The content of hemoglobin and erythrocytes in the blood of broilers receiving dihydroquercetin increased, which indicates an increase in the intensity of redox processes in the body. The number of leukocytes in all groups was normal, but in the experimental groups their decrease was noted, which indicates the therapeutic and immunostimulating effect of the drug. Evaluation of protein metabolism by the content of total protein and the fraction of albumin in the blood serum showed its positive dynamics within the normal physiological values, which characterizes an increase in the intensity of assimilation processes in the bird's body. The metabolism of carbohydrates was assessed by the content of glucose in it, and a decrease in its level indicates its increased consumption as an energy component for metabolic processes associated with intensive growth of chickens. An increase in the activity of alkaline phosphatase by 1.3-1.8 times is associated with the active growth of chickens.

1. Introduction

One of the main conditions for the successful cultivation of young poultry in industrial conditions is to maintain its high resistance to negative environmental factors [1]. The productivity of farm animals and birds largely depends on the diet of feeding and the ratio of the main groups of feed in it. With intensive metabolism genetically embedded in a particular bird cross, the enzymatic system cannot fully meet the body's need for a high degree of nutrient digestion of the diet. Therefore, feed additives need to be included in the full-diet feed. At the same time, it is necessary to systematically monitor the state of metabolism in the body, which allows predicting the productivity of poultry at all stages of its cultivation. The creation of only an optimal microclimate is insufficient to neutralize the negative effects of anthropogenic factors. It is therefore necessary to use environmentally friendly adaptogens to stimulate the immune system of chickens when growing [2-4].

The quality of poultry meat and its ecological cleanliness have recently become of particular importance. Food products can be the source and carrier of a large number of potentially hazardous toxic substances of chemical and biological nature [5]. In this regard, an urgent problem of the consumer market of our country is the development of ways to obtain environmentally friendly and safe poultry products [6-8]. To this end, modern biotechnology centers make it possible to obtain
various biologically active preparations available for use and the use of substances of natural origin is of greatest interest [9]. One of them is a natural antioxidant of plant origin – dihydroquercetin [10].

Dihydroquercetin (DHQ), also known as 3,5,7,3,4-pentahydroxy flavanone or commonly as taxifolin, is a kind of bioactive flavonoid. Because the molecular structure of DHQ contains five phenolic hydroxyl groups, it is considered to be one of the best and rarest natural powerful antioxidants in the world [11]. The safety of DHQ also has been investigated since it is a key component of dietary supplements [12]. Additionally, DHQ has a wide range of pharmacological activities, including anti-inflammatory [13], anti-microbial [14] anti-cancer [15], anti-Alzheimer [16], anti-toxoplasmosis effects [17], health-promoting effects on hepatoprotective and cardiovascular systems [18,19]. Due to its excellent pharmacological activity, DHQ is routinely used in pharmaceuticals, health products, foods and agriculture.

Dihydroquercetin (DHQ), an extremely low content compound (less than 3%) in plants, is an important component of dietary supplements and used as functional food for its antioxidant activity [20].

The purpose of the studies is to compare the metabolism and degree of nutrient absorption of the diet in broiler chickens when an antioxidant additive is included in the full-diet feed. The objectives of the study were to study the influence of the above drug on the morphological and biochemical blood indices of experimental birds.

2. Materials and methods

The research was carried out on broiler chickens of the KOBB-500 cross in the production conditions of Limited liability company ‘Zverohochnost Kiznerskoye’ (Russia, Republic of Udmurtia, Kizner village). For the production experiment, 40 heads of day-old broiler chickens of the KOBB-500 cross with an average live weight of 39.80±0.13 g were selected and four groups of 10 heads each were formed from them according to the principle of analogues. All chickens, depending on the periods of the experiment, received a basic ration, consisting, respectively, of starting, growth and final feeds used in the poultry farm.

Broiler chickens were fed with complete feed, in accordance with the recommendations of the manufacturer of this cross [21]. In terms of energy and nutrient content, they were similar and differed between groups in the amount of supplement administered. The chickens of the control group received only the combined (main diet), the I experimental group received an additional antioxidant supplement ‘Dihydroquercetin’ (LLC‘Kakhor’, Zima, Irkutsk region) in the amount of 0.50 g per 100 g of feed, the II group – 0.75 g per 100 g of feed and III – 1.00 g per 100 g of feed (table 1).

Dihydroquercetin – an antioxidant, bioflavonoid of natural origin, obtained from Siberian and Daurian larch, has a decongestant, capillary protective, antioxidant property.

Small crystal or amorphous powder from light yellow to yellow, odorless, slightly bitter taste.

Dihydroquercetin regulates metabolic processes, has a positive effect on the functional state of the internal organs of the body, creates mechanisms for protecting healthy body cells from pathologies caused by chemical poisoning, exposure to electromagnetic radiation and radiation, by neutralizing radical activity, viral and bacterial processes. It is not toxic, harmless, has high activity at low concentrations, is resistant to thermal and mechanical effects.

Dihydroquercetin is necessary for birds in their breeding, production of poultry production in areas technogenic for heavy metals and radionuclides, as well as industrial enterprises susceptible to pollution of chemical, metallurgical, petrochemical and other types of industry.

Due to capillaroprotective and antioxidant properties of dihydroquercetin, metabolism at the cell and capillary interface and correction of the body's antioxidant status are significantly improved. The antioxidant effects of dihydroquercetin, like other flavonoids, are one of the non-specific mechanisms for realizing many of its other biological properties.

Increases the average daily increase in live mass, while young birds more intensively increase productivity with age, reduces the cost of feed for the increase in live mass, improves the quality of production, increases egg production and the average weight of the egg.
Table 1. Production experience diagram.

| Groups         | Number of goals | Experience diagram                              |
|----------------|-----------------|------------------------------------------------|
| Control        | 10              | Full-Sized Feed (FSF)                           |
| I experimental | 10              | (FSF) + 0.50 g DGC per 100 g feed stock         |
| II experimental| 10              | (FSF) + 0.75 g DGC per 100 g feed stock         |
| III experimental| 10              | (FSF) + 1.00 g DGC per 100 g feed stock         |

The duration of the experiment was 40 days, and to find out the effectiveness of increasing the duration of the poultry feeding period, it was increased to 60 days. Throughout the period of cultivation, poultry created the same zoohygienic conditions of maintenance and care.

Chickens under the age of 7 days: air temperature – 32-34 °C, humidity – 40-60%, illumination – 15-20 lux. Chickens under the age of 14 days: air temperature – 27 °C, humidity – 60-70%, illumination – 15-20 lux. Chickens under the age of 21 days: air temperature – 24 °C, humidity 65-70%, illumination – 15-20 lux. Chickens under the age of 28 days: air temperature – 21 °C, humidity – 65-70%, illumination – 10-15 lux. Chickens under the age of 35 days: air temperature 19 °C, humidity – 65-70%, illumination – 7-10 lux.

Before taking blood, the bird is fixed with two hands by the wings and limbs. Blood is taken from a vein on the inside of the wing above the elbow joint by puncture. It is clearly visible, as it is the largest vessel in this area. The place of blood collection, closer to the elbow joint, is cleaned of small feathers and down. Before taking blood, the vein is wiped with a cotton swab soaked in ethyl alcohol. Due to the high blood clotting, the puncture site is wiped with an anticoagulant liquid (heparin 2.0-2.5 U/ml, trilon B) and the required amount of blood is collected in a test tube, into which any of the above anticoagulants are previously introduced. After taking blood, the vein is clamped with a cotton swab for 1-3 minutes.

Erythrocytes were counted in the Goryaev counting chamber (LLP ‘Minimed’, Russia), the total number of leukocytes – in the Goryaev counting chamber by the test tube method, the hemoglobin concentration was determined using the Sahli hemometer (Health Equipment Plant, Moscow, Russia). A leukogram was derived by counting different types of white blood cells stained according to Romanovsky-Giemsa, with the calculation of the percentage content of individual types of white blood cells.

The levels of total protein and its fraction, glucose, cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase and total bilirubin were measured using an automated biochemical and enzyme immunoassay analyzer Chem Well Combi (Awareness Technology, USA).

Processing of the digital data on the research results was carried out by the method of variation statistics as for the reliability of differences between the compared indicators (P<0.05-0.001) using Microsoft Office Excel application software.

3. Results and discussion

The results of the study of the hematological profile of blood were included in the diagnostic algorithm of a number of pathological processes. According to the results of our studies, it was revealed that the morphological parameters of the blood of chickens of the experimental groups were at the level of physiological norms [21] for a number of parameters (table 2).

The morphological composition of the blood reflects the processes occurring in the body, and by the intensity of redox processes in the body, one can judge the content of erythrocytes, leukocytes and hemoglobin. Hematological indicators of blood reflect the processes that take place in the body, and by the intensity of redox processes in it, it is possible to conclude that there are red blood cells, white blood cells and hemoglobin [22].

The amount of hemoglobin and red blood cells in the blood of broilers who received dihydroquercetin with feed corresponded to the physiological norm, but had a steady upward trend -
an adequate level of the drug. It was found that at the end of the scientific and economic experiment in the I, II and III experimental groups, an increase in the concentration of hemoglobin in the blood was observed by 37.77 (P<0.05); 53.46 (P<0.01) and 78.75% (P<0.001). By the end of the feeding period, the level of red blood cell concentration in the blood of chickens of the experimental groups tended to increase in comparison with the control – in groups I and II by 6.45%, and in group III – by 16.13%. This indicates the activation of metabolic processes in the tissues and, accordingly, an increase in their supply of oxygen and the excretion of metabolic products. The permissible level of hemoglobin in chickens from the control group indicates a possible lack of oxygen in the tissues.

| Table 2. Blood Morphological Composition of broiler chickens. |
|---------------------------------------------------------------|
| **Rates** | **Groups experimental** | **Reference values** |
| --- | --- | --- |
| **Control** | **I** | **II** | **III** |
| Hemoglobin, **g/l** | 59.30±2.16 | 81.70±4.71 | 91.00±0.71** | 106.00±7.48** | 80-120 |
| Erythrocytes, **×10^{12}/l** | 3.10±0.22 | 3.30±0.29 | 3.30±0.18 | 3.60±0.18 | 3-4 |
| Leukocytes, **×10^{9}/l** | 40.00±1.19 | 39.60±0.79 | 28.00±1.59** | 24.10±2.12** | 20-40 |
| Lymphocytes, **unit** | 50.70±1.08 | 52.00±1.41 | 55.70±2.04 | 54.30±1.78 | 52-60 |
| Platelets, **×10^{9}/l** | 33.00±3.24 | 66.00±7.87 | 122.70±6.34* | 140.70±4.55** | 32-150 |
| Hereinafter: *P<0.05, **P<0.01, ***P<0.001 |

An increase in the concentration of red blood cells and hemoglobin in the blood occurs due to the activation of erythropoiesis and an increase in hematopoiesis in bone marrow tissue. Activation of hematopoiesis, apparently, is a consequence of the use of an antioxidant in poultry feeding, which to a certain extent stimulates an increase in the number of red blood cells and a natural proportional increase in the hemoglobin content in the blood.

The purpose of white blood cells is to prevent the harmful effects of the environment on the bird's body, forming cellular and humoral non-specific and specific factors. White blood cells have phagocytic activity and form the humoral immunity of the body. An increase in the level of leukocyte concentration indicates the presence of pathological processes in the body.

It was found that the number of white blood cells in all groups of broiler chickens is within the physiological norms. At the end of rearing chickens in the experimental groups, there was a decrease in the concentration of leukocytes in the blood serum compared to the control by 1.00; 30.00 (P<.01) and 39.75% (P<.01), respectively. This indicates a therapeutic and immunostimulating effect.

A lymphocyte is a type of white blood cell present in normal blood. The lymphocyte is engaged in the fight against viruses and some bacteria and belongs to the link of humoral immunity. A significant increase in its concentration level is characteristic of viral infections and blood diseases. It decreases with various immunodeficiency disorders characteristic of kidney failure, with prolonged fasting or overwork [23]. The study of hematological parameters of the blood of broiler chickens indicates that the content of lymphocytes increases slightly, within normal limits, as a result of the use of an antioxidant when feeding broilers by 2.56% in group I, by 9.86% in group II and by 7.10% in group III. At the same time, it was found that the bird of the control group had a lower indicator.

A decrease in the number of platelets may indicate cirrhosis of the liver or anemia. The results of the studies showed that the use of the test drug provided an increase in this indicator in the I-III experimental groups, compared with the control group, by 2.0; 3.7 and 4.3 times (P<0.05-P<0.001), respectively, were also at the level of the physiological norm.

As a result of biochemical blood tests, it was found that the use of the drug contributes to an increase in the intensity of redox processes, as a result of the activation of metabolism and energy.
The effect of the studied additive is aimed at normalizing the metabolism in the body of broilers in order to increase the conversion of feed into products and more fully realize the potential of productivity. The most significant indicators of protein metabolism that have clinical significance are the content of total plasma protein and its distribution by individual protein fractions (table 3).

The assessment of protein metabolism by the concentration of total protein and the albumin fraction of poultry blood serum indicates a positive trend. In the chickens of the experimental groups, the protein and albumin synthetic liver functions were more intense, which is obviously associated with a better assimilation of nitrogenous substances of the food consumed, and, consequently, a greater intake of protein products into the blood. Accordingly, the total protein concentration in the blood serum of birds of the experimental groups by the end of the rearing period was, on average, 21.21% higher (P<0.05) than in the control.

However, it is impossible to objectively assess the level of nutrition by the level of total protein, since this indicator can change under the influence of many factors that are not directly related to nutrition, but are characteristic of some metabolic disorders and liver function. In this regard, the concentration of serum albumin is monitored.

The main functions of albumins are to maintain oncotic pressure, transport free fatty acids, bilirubin, steroid hormones, magnesium, calcium, and so on.

It is noted that a violation of the concentration of albumin protein, during the period of the experiment, is better mobilized for the synthesis of tissues of a growing organism. The level of albumin concentration in the blood serum of birds of the experimental groups was higher, on average, by 21.10% (P<0.05) than in the control, but it was at the normal level, which characterizes an increase in the intensity of assimilation processes in the body.

**Table 3. Biochemical blood indices of broiler chickens.**

| Rates                  | Control     | I            | II           | III          | Reference values |
|------------------------|-------------|--------------|--------------|--------------|------------------|
| Total protein, g/l     | 29.70±1.78  | 31.70±2.48   | 36.00±0.71*  | 40.30±2.94   | 32-47            |
| Albumin, g/l           | 26.70±1.08  | 30.00±0.71   | 32.30±1.08*  | 34.70±2.27*  | 31.4-36.1        |
| Glucose, mmol/l        | 15.30±0.33  | 13.20±0.28*  | 10.70±0.08***| 10.10±0.25** | 9.3-16.5         |
| Cholesterol, mmol/l    | 4.50±0.07   | 4.00±0.11*   | 3.40±0.11**  | 3.30±0.11**  | 3.4-4.6          |
| AST, units/l           | 321.50±6.27 | 311.70±3.32  | 310.30±1.51  | 310.20±5.22  | 107-481 (~330)  |
| ALT, units/l           | 5.90±0.29   | 4.40±0.63    | 2.00±0.07*** | 1.40±0.18*** | 1.2-6.8          |
| Alkaline phosphatase,  | 530.70±9.23 | 693.70±65.67 | 862.00±44.97*| 976.30±25.31***| 770-1100        |
| units/l                |             |              |              |              |                  |
| Bilirubin total, mg%   | 0.163±0.063 | 0.140±0.074  | 0.127±0.015  | 0.120±0.044  | 0.01-0.5         |

*P<0.05; **P<0.01; ***P<0.001

Plasma carbohydrates are mainly glucose. As a result, the content of its glucose in the blood of birds of all groups had a carbohydrate metabolism. This indicator depends on the balance of the bird's diet, on the state of the endocrine system, the tension of the nervous system and a number of other factors [24].
If there is a shortage of easily digestible carbohydrates in the feed, there are signs of hypoglycemia, as well as when feeding highly concentrated feeds to poultry. An increase in the concentration of glucose in the blood is noted against the background of manual feeding of poultry with sugar feed.

Our studies have noted that the glucose content is within the physiological norm. By the end of the feeding period, the blood glucose level in the poultry of the experimental groups was lower than in the control group, on average by 25.93% (P<0.01). A decrease in glucose levels indicates an increased consumption of glucose as an energy component of metabolic processes associated with intensive chick growth.

In our studies, it was found that the glucose concentration was in the range of the physiological norm. By the end of the feeding period, the concentration of glucose in the blood of the experimental group was lower than in the control, on average by 25.93% (P<0.01). A decrease in the concentration of glucose indicates an increased consumption of glucose as an energy component of metabolic processes associated with the intensive growth of poultry.

Lipids are heterogeneous substances with different structural and metabolic properties. In addition, they are different in structure and properties. In addition to fatty acids, lipids have cholesterol in their composition. In the blood of birds of the experimental groups, their concentration was reduced by 0.5-1.2 mmol/l (P<0.05 – P<0.01) compared to the control. The revealed reaction of the body to the altered nutritional factor is due to the fact that the tested drug, according to known data [8], contributes to lowering cholesterol in the peripheral blood.

To identify the optimal level of dihydroquercetin administration in the diet of experimental groups of poultry on the overall metabolism in their body, the content of serum enzymes that characterize the functional capabilities of the liver and the nature of metabolic processes was studied. The most frequently studied enzymes are AST and ALT, which play an important role in the exchange of amino acids, and alkaline phosphatase, which cleaves the residues of phosphoric acid from its organic compounds, but is not strictly an organ-specific enzyme [25]. Blood enzymes react quickly to the state of biochemical homeostasis. A non-specific reaction of the body is a change in their activity [26]. The high growth rate of poultry largely determines the direction of metabolism in the body and the activity of ALT and AST transamination enzymes, which play a key role in the synthesis and cleavage of amino acids in the body [27]. A decrease in the level of enzymes and bilirubin in the blood of birds of the experimental groups within the normal range indicates an improvement in the functional state of the liver, which is due to the use of an antioxidant [28].

Alkaline phosphatase takes part in the metabolism of phosphoric acid, promotes the transport of phosphorus in the body and affects bone growth. As a result, an increase in the level of dihydroquercetin in bird feeding causes activation of alkaline phosphatase in the blood of chickens of the experimental groups by 1.3-1.8 times (P<0.01 – P<0.001) against the background of their rapid growth.

4. Conclusion
It is established that the modern antioxidant of the flavonoid group does not have a negative effect on the body of broiler chickens.

The content of red blood cells in the blood of chickens of the experimental groups, at the end of the fattening period, tended to increase, relative to the control in groups I and II by 6.45%, and in group III by 16.13%, which indicates the activation of hematopoiesis and metabolic processes.

In the experimental groups, a decrease in the number of white blood cells in the blood, relative to the control group, was found by 1.00; 30.00 (P<0.01) and 39.75% (P<0.01), respectively, which indicates the immunostimulating effect of the drug.

The total amount of protein in the blood serum of broiler chickens of the experimental groups, by the end of the growing period, was higher, on average by 21.21% (P<0.05), and albumins by 21.10% (P<0.05) than in the control group, which is associated with better assimilation of nitrogenous substances of the feed consumed.
An increase in the activity of alkaline phosphatase by 1.3-1.8 times is associated with the active growth of chickens of the experimental groups.

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