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**THE EFFECT OF A DIET ENRICHED WITH CHITOSAN AND WHEY ON
TOTAL PROTEIN AND GLOBULIN FRACTIONS IN THE BLOOD PLASMA OF
BROILERS: MECHANISMS OF IMMUNOMODULATION**

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Abstract: This study investigated the effect of dietary supplementation with 2% chitosan and 5% whey on protein metabolism indicators in the blood plasma of broiler chickens, namely the levels of total protein and globulin fractions (alpha, beta, and gamma). The study was conducted on 60 one-day-old Cobb-500 hybrid broiler chickens over a period of 42 days. The chickens were divided into three groups: Group 1 (control) received a standard basal diet, Group 2's diet was supplemented with 2% chitosan, and Group 3's diet was supplemented with 5% whey. Biochemical indicators in the blood plasma were determined on day 42 of the experiment using the ELISA method. The results showed that in both experimental groups, the levels of total protein and all globulin fractions were significantly higher ($p < 0.05$) compared to the control group. The group supplemented with whey showed the highest values for these indicators. In conclusion, natural nutritional supplements such as chitosan and whey effectively strengthen the protein synthesis function of the liver in broilers and activate humoral immunity, thereby proving to be effective means for enhancing their immunological resistance.

Key words: broiler chickens, chitosan, whey, total protein, globulins, alpha-globulins, beta-globulins, gamma-globulins, immunomodulation, blood plasma, diet.

Introduction

Modern poultry production is based on intensive methods for meat production, where the issues of maintaining high productivity and preserving livestock are of paramount importance [1]. The nutritional factor, including the quality and balance of the diet, is a fundamental component that directly affects the growth, development, and immune status of poultry [2, 3]. In recent years, the imposition of restrictions on the use of antibiotic growth promoters (AGPs) has intensified the search for safe and effective immunomodulators of natural origin in poultry farming [4, 5].

One such promising supplement of plant and animal origin is chitosan and its derivatives. Chitosan, a deacetylated product of chitin, is known for its biocompatibility, antimicrobial, antiviral, and immunostimulatory properties, which have been confirmed in numerous studies [6, 7]. It enhances the overall resistance of the organism by normalizing the gastrointestinal microbiota, strengthening the protective function of the intestinal wall, and increasing the activity of immune cells such as macrophages and lymphocytes [8, 9].

Another promising supplement is whey (milk serum). This is a valuable by-product of cheese production, rich in high biological value proteins (lactalbumin, beta-lactoglobulin), immunoglobulins, lactoferrin, lysozyme, and other bioactive peptides [10, 11]. The components in whey contribute to improving protein metabolism in animals, stimulating protein synthesis in



the liver, and activating humoral immunity, particularly antibody production [12, 13, 25, 26, 27, 28, 29].

For these reasons, we aimed to study how the individual supplementation of broiler diets with chitosan (2%) and whey (5%) affects the levels of total protein and globulin fractions, key indicators of immune status, in their blood plasma. This will help strengthen the scientific basis for using natural immunostimulants in poultry farming.

Objective of the study: To investigate the effect of a diet enriched with chitosan and whey on the dynamics of total protein and globulin fractions in the blood plasma of broiler chickens.

Research tasks:

- To develop a scheme for supplementing broiler diets with chitosan (2%) and whey (5%) under experimental conditions.
- To determine the levels of total protein, alpha-, beta-, and gamma-globulins in the blood plasma of the experimental groups of chickens.
- To compare the obtained results and analyze the mechanisms of action of the studied supplements on the immune system of broilers.

Materials and Methods

The study was conducted in 2023 at the experimental animal base of Zarmed University. One-day-old Cobb-500 hybrid broiler chickens were used as the research object. All animal handling procedures were carried out in accordance with the ethical guidelines of the International Association for the Study of Pain (IASP).

For the experiment, a total of 60 chickens were divided into three groups of 20 each and were reared for 42 days. The chickens were kept in cage conditions in rooms with standard microclimate parameters (temperature, humidity, ventilation). All groups had ad libitum access to feed and water. The experiment was conducted according to the following scheme:

1. Group 1 (Control): Standard basal diet adapted for the feeding phase (starter, grower, finisher).
2. Group 2 (Experimental): Basal diet + 2% chitosan.
3. Group 3 (Experimental): Basal diet + 5% dried whey.

The basal diets were formulated according to the nutritional requirements for broiler feeding phases based on international standards (NRC, 1994). Chitosan and whey were calculated based on the total mass of the diet and thoroughly mixed into the main feed mixture.

Blood samples were taken at the end of the experiment (day 42) before the morning feeding, with a volume of 5 ml from the wing vein (v. metacarpalis) into heparinized tubes. The blood samples were immediately centrifuged at 3000 rpm for 15 minutes to separate the plasma. The obtained plasma was stored at -20°C before biochemical analysis.

The levels of total protein and its fractions (alpha-, beta-, gamma-globulins) in the blood plasma were determined using special ELISA (Enzyme-Linked Immunosorbent Assay) test kits designed for poultry. The analyses were performed at the “ELISOTEST” laboratory in strict accordance with the manufacturer's instructions.

Statistical processing of the obtained data was performed using the “Statistica 10.0” software package. To assess the significance of differences between the groups, a one-way ANOVA was used, and Duncan's multiple range test was applied to determine the significance of the mean values. Differences were considered statistically significant at $p < 0.05$.

Results



The results of the study showed that the supplementation of broiler diets with natural bioactive substances, chitosan and whey, had a positive effect on the indicators of protein metabolism in the blood plasma of broiler chickens (Table 1).

In the control group, the total protein level in the blood plasma averaged 50 ± 2.5 g/L. In Group 2, supplemented with 2% chitosan, this indicator increased to 60 ± 2.0 g/L, which is a 20% increase compared to the control group. In Group 3, supplemented with 5% whey, this increase was more significant, with the total protein level reaching 65 ± 1.8 g/L, which is 30% higher than the control.

The dynamics of the globulin fractions showed a similar trend. Gamma-globulins, the fraction representing immunoglobulins and the primary indicator of humoral immunity, were 15 ± 1.0 g/L in the control group, but increased to 19 ± 1.5 g/L in Group 2 and 20 ± 1.0 g/L in Group 3. The levels of alpha- and beta-globulins were also significantly higher in the experimental groups, indicating the activation of acute-phase proteins and components of the complement system.

Table 1. The effect of chitosan and whey on protein fractions in the blood plasma of broilers ($M \pm m$, g/L)

Indicators	Group 1 (Control)	Group 2 (2% Chitosan)	Group 3 (5% Whey)
Total protein	50 ± 2.5	$60 \pm 2.0^*$	$65 \pm 1.8^*$
Alpha-globulins	15 ± 1.0	$18 \pm 1.2^*$	$20 \pm 1.5^*$
Beta-globulins	20 ± 1.5	$23 \pm 1.8^*$	$25 \pm 1.0^*$
Gamma-globulins	15 ± 1.0	$19 \pm 1.5^*$	$20 \pm 1.0^*$

Note: The asterisk (*) denotes a significant difference ($p < 0.05$) compared to the indicators in the control group.*

Discussion

The obtained results confirm the high efficacy of chitosan and whey in stimulating protein synthesis and enhancing immune functions in the body of broilers.

The increase in total protein levels primarily indicates an improvement in the liver's function of protein synthesis. Whey is a "complete" protein source containing all essential amino acids necessary for tissues [10, 14, 16]. This fully provides the building blocks for the synthesis of albumin and globulins in the liver. The effect of chitosan has a different mechanism. It strengthens the gut barrier, suppresses pathogenic flora, and thus improves the digestion and absorption of nutrients [6, 18, 20, 23].

As a result, the body utilizes protein more efficiently, leading to an increase in the total protein concentration in the blood plasma. Our results are consistent with the research of CIS scientists on the effectiveness of whey and chitosan in improving feed quality [22, 23, 24, 25, 28, 29, 30].

The increase in alpha- and beta-globulin levels indicates the activation of the innate (non-specific) branch of the immune system. Alpha-globulins contain various acute-phase proteins, such as transferrin and seromuroid. An increase in their levels may indicate subclinical mild inflammatory processes or the preparedness of the immune system for stress. It is known that chitosan activates macrophages, stimulating the production of cytokines (interleukin-1, interleukin-6), which in turn accelerates the synthesis of these proteins [9, 13].



Beta-globulins include components of the complement system and some transport proteins. They are involved in the processes of opsonization and destruction of bacteria. Therefore, an increase in this fraction indicates the strengthening of the body's incoming defense mechanisms against infections. Lactoferrin, a component of whey, also contributes to this process by binding iron and inhibiting bacterial growth, thereby affecting the activity of the complement system [11, 17].

The most significant indicator, gamma-globulins (mainly immunoglobulins IgY, IgM, IgA), showed a marked increase, confirming the unique immunomodulatory properties of the studied supplements. The presence of immunoglobulins in whey and its role in forming local immunity in the gastrointestinal tract are well-studied [12, 15]. Furthermore, its bioactive peptides can directly influence the differentiation of B-lymphocytes and stimulate antibody production [17]. Chitosan is known as an immunoadjuvant that enhances the immune response to antigens, thereby increasing the effectiveness of vaccines [5, 21, 26, 27].

It is absorbed by lymphoid tissues and increases the activity of B-lymphocytes, consequently raising the concentration of immunoglobulins in the blood serum. Our observations fully align with previous studies on chitosan's ability to enhance antibody production [7, 19].

Comparing the experimental groups, all indicators, especially total protein and gamma-globulins, were slightly higher in the whey-supplemented group (Group 3) than in the chitosan group (Group 2). This suggests that the direct supply of high-quality proteins and bioactive peptides from whey may be more effective in stimulating immunoglobulin synthesis than the immunoregulatory effect of chitosan. However, the role of chitosan in normalizing gut microbiota and its long-term benefits for immune strengthening should not be underestimated.

Conclusion

Based on the conducted experimental study, the following conclusions can be drawn:

Supplementation of broiler chicken diets with 2% chitosan and 5% whey increases their blood plasma total protein levels by 20% and 30%, respectively, indicating an improvement in the liver's protein synthesis function.

The studied supplements significantly increase ($p < 0.05$) the levels of alpha-, beta-, and gamma-globulins, thereby activating both branches of the chicken's immunity – innate and acquired (humoral).

The significant increase in gamma-globulins (immunoglobulins) confirms that chitosan and whey are effective immunomodulators for enhancing the immunological resistance of broilers.

The slightly higher effect of whey on indicators such as total protein and gamma-globulins can be explained by its content of high-quality proteins and directly acting bioactive peptides.

The presented results provide a scientific and practical basis for the widespread use of chitosan and whey as natural immunostimulants in poultry farming as an alternative to antibiotics. This, in turn, contributes to the production of environmentally safe and high-quality poultry products.

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