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**EFFECT OF A CHITOSAN AND WHEY POWDER COMPLEX ON THE PHYSIOLOGICAL STATUS OF BROILER CHICKENS: A COMPREHENSIVE SCIENTIFIC AND EXPERIMENTAL ANALYSIS****Farxod Kholbayevich Rakhmonov**

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**Abstract:** This scientific study investigates the physiological, biochemical, and morphofunctional effects of a chitosan and whey powder complex used as a natural alternative to feed antibiotics in industrial poultry farming. The experiment was conducted on Ross-308 broiler chickens over a 42-day rearing period. Hematological and biochemical blood parameters, intestinal morphometry, antioxidant system status, immunological indices, and productive performance were comprehensively evaluated. The results demonstrated that supplementation of broiler diets with chitosan and whey powder significantly optimized protein metabolism, improved intestinal epithelial structure, enhanced antioxidant defense mechanisms, and increased feed conversion efficiency. Experimental groups exhibited elevated levels of total protein, albumin, hemoglobin, and immunoglobulin fractions. The height of intestinal villi increased, while the number of pathogenic microorganisms decreased. Activation of antioxidant enzymes was accompanied by a reduction in lipid peroxidation products. The application of the complex contributed to increased live body weight, improved growth performance, and higher economic profitability. The obtained data confirm the high biological efficiency of natural biopolymer feed additives in antibiotic-free poultry production systems.

**Keywords:** broiler chickens, chitosan, whey powder, physiological status, intestinal morphometry, blood biochemistry, antioxidant defense, feed conversion ratio, prebiotic effect, Ross-308, immunomodulation, hepatoprotection.

**Introduction**

Currently, one of the priority directions in global poultry production is the development of environmentally safe, antibiotic-free, and biologically valuable poultry products [1]. Modern broiler crosses, particularly Ross-308, are characterized by intensive growth and rapid muscle deposition; however, accelerated metabolism is frequently associated with oxidative stress, immune suppression, intestinal dysbiosis, and metabolic disturbances [2; 3]. As a consequence, feed conversion efficiency deteriorates, productivity decreases, and mortality rates increase [4].

In recent years, restrictions on the use of antibiotic growth promoters have significantly increased interest in natural biologically active feed additives [5]. Among them, chitosan and whey powder possess particularly valuable biological properties [6]. Chitosan is a natural polysaccharide derived from chitin-containing marine organisms and exhibits antimicrobial, immunomodulatory, antioxidant, and sorption activities [7; 8]. Positively charged amino groups of chitosan interact with negatively charged bacterial cell membranes, suppressing microbial proliferation and pathogenic activity [9].

Whey powder is a valuable source of biologically active proteins, albumins, globulins, lactose, vitamins, and mineral compounds [10]. Lactose and oligosaccharides contained in whey stimulate the development of beneficial intestinal microflora and exhibit pronounced prebiotic effects [11]. Several studies demonstrated that whey components improve intestinal trophic processes and nutrient utilization efficiency [12].

Although numerous studies have evaluated the independent effects of chitosan and whey products, the synergistic influence of their combined application remains insufficiently investigated [13; 14; 15; 16]. Research conducted by Rakhmonov and co-authors demonstrated positive effects of biopolymer feed additives on metabolic and immunological indices in broiler chickens [2; 5; 21; 25].

Therefore, the aim of the present study was to evaluate the physiological, biochemical, and morphofunctional efficiency of dietary supplementation with a chitosan and whey powder complex in broiler chickens.

### Materials and Methods

The experimental studies were carried out during 2023–2026 under poultry laboratory and farm conditions. A total of 400 one-day-old Ross-308 broiler chickens were used in the experiment. Birds were divided into four analogous groups, each containing 100 chickens.

The control group received a standard basal diet. Experimental groups received additional supplementation with different doses of chitosan and whey powder:

- Control group — basal diet;
- Experimental group I — 30 mg chitosan + 70 mg whey powder/bird;
- Experimental group II — 60 mg chitosan + 140 mg whey powder/bird;
- Experimental group III — 100 mg chitosan + 300 mg whey powder/bird.

Broilers were maintained under standard microclimatic conditions. Ambient temperature gradually decreased from 32°C to 24°C during the growing period. Relative humidity was maintained within 60–70%. The experiment lasted 42 days [17].

Hematological analyses included determination of hemoglobin concentration using the Sahli method, while erythrocyte and leukocyte counts were measured using a Goryaev chamber [18].

Biochemical analyses involved determination of total protein by the biuret method and albumin using bromocresol green reagent. ALT and AST activities were measured by the kinetic method [19].

For histological evaluation, intestinal samples collected on day 35 were stained with hematoxylin-eosin. Villus height and crypt depth were measured using morphometric microscopy techniques [20].

The antioxidant status of liver tissues was assessed by spectrophotometric determination of malondialdehyde (MDA), superoxide dismutase (SOD), catalase, and glutathione levels [21].

Statistical processing was performed using Microsoft Excel and Statistica 12.0 software. Results were expressed as  $M \pm m$ . Differences between groups were evaluated using Student's t-test and considered statistically significant at  $p < 0.05$  [22].

### Results

#### Intestinal Morphometry and Digestive System Status

The functional condition of intestinal epithelium plays a key role in nutrient absorption efficiency. The obtained results demonstrated that supplementation with the chitosan-whey complex significantly improved intestinal trophic morphology.

**Table 1. Intestinal morphometric parameters (35 days,  $\mu\text{m}$ ,  $M \pm m$ ,  $n=15$ )**

Parameters	Contro I	Experimenta I II	Experimenta I III	Difference (%)
Duodenal villi height	145 $\pm$ 11	162 $\pm$ 9*	172 $\pm$ 10*	+18.6
Jejunal villi height	128 $\pm$ 10	148 $\pm$ 11*	165 $\pm$ 12*	+28.9
Crypt depth	89 $\pm$ 7	98 $\pm$ 6	112 $\pm$ 8*	+25.8
Mitotic	12.4 $\pm$	15.8 $\pm$ 1.4*	18.2 $\pm$ 1.6*	+46.8

Parameters	Contro I	Experimenta I II	Experimenta I III	Difference (%)
index (%)	1.2			

$p < 0.05$  compared with the control group.

In Experimental group III, villus height in the jejunum increased by 28.9% compared with the control group [23]. Enlargement of the absorptive surface area contributed to improved nutrient assimilation. Lactose and oligosaccharides stimulated the growth of *Lactobacillus* and *Bifidobacterium* species, thereby improving intestinal microbiological balance [24].

Chitosan molecules formed electrostatic interactions with bacterial cell membranes, reducing populations of pathogenic microorganisms such as *Escherichia coli* and *Clostridium perfringens* [25]. As a result, intestinal barrier function and nutrient digestibility significantly improved [26].

#### Blood Biochemistry and Protein Metabolism

Blood biochemical composition reflects the metabolic and physiological condition of poultry organisms. Significant improvements in protein metabolism were observed in the experimental groups.

**Table 2. Blood plasma protein spectrum (35 days, g/L, M $\pm$ m, n=20)**

Protein fractions	Control	Experimental II	Experimental III
Total protein	34.12 $\pm$ 0.9	37.45 $\pm$ 0.6*	38.22 $\pm$ 0.5*
Albumin	12.56 $\pm$ 0.3	14.18 $\pm$ 0.4*	14.65 $\pm$ 0.3*
$\alpha$ - and $\beta$ -globulins	9.84 $\pm$ 0.4	10.23 $\pm$ 0.3	10.41 $\pm$ 0.3
$\gamma$ -globulins	11.72 $\pm$ 0.5	12.94 $\pm$ 0.4*	13.16 $\pm$ 0.4*

$p < 0.05$  compared with the control group.

Total protein concentration increased by 9.7%, indicating activation of protein synthesis processes [27]. Elevated albumin levels reflected improved functional activity of hepatocytes [28]. Increased  $\gamma$ -globulin concentrations indicated stimulation of immunoglobulin synthesis and enhancement of immune responsiveness [29].

Hemoglobin concentration reached  $94.8 \pm 1.2$  g/L, suggesting intensified erythropoiesis. Reduced ALT and AST activities confirmed stabilization of liver functional status and hepatoprotective effects of the feed additive complex [30].

#### Antioxidant System Status

Intensive growth in broiler chickens is frequently accompanied by excessive free radical formation and oxidative stress. Dietary supplementation with the chitosan-whey complex significantly activated antioxidant defense mechanisms.

**Table 3. Liver antioxidant system status (42 days)**

Parameters	Control	Experimental II	Experimental III
MDA (nmol/mg protein)	2.48 $\pm$ 0.19	2.28 $\pm$ 0.16*	2.14 $\pm$ 0.17*
SOD (U/mg protein)	132 $\pm$ 10	144 $\pm$ 11*	156 $\pm$ 12*
Catalase (U/g tissue)	89 $\pm$ 7	96 $\pm$ 6	102 $\pm$ 8*

Parameters	Control	Experimental II	Experimental III
GSH ( $\mu\text{mol/g}$ tissue)	4.12 $\pm$ 0.3	4.56 $\pm$ 0.4*	4.89 $\pm$ 0.3*

$p < 0.05$  compared with the control group.

Reduction in MDA concentration indicated suppression of lipid peroxidation processes. Simultaneously, increased activities of SOD and catalase demonstrated activation of antioxidant defense systems [18; 23].

### Discussion

The obtained results confirm the synergistic biological effects of combined chitosan and whey powder supplementation in broiler chickens. International studies also reported positive effects of chitosan on intestinal morphology and immune function [7; 16]. Wang et al. observed a 22% increase in villus height [27], whereas in the present study the increase reached 28.9%.

Bioactive proteins and lactose contained in whey powder stimulated beneficial intestinal microbiota development and improved intestinal ecological balance [19]. Sorption properties of chitosan promoted detoxification of toxic metabolites and free radicals [20].

Increased relative weights of immune organs confirmed immunoregulatory effects of the feed additive complex [24]. Enhanced antioxidant enzyme activity contributed to stabilization of metabolic processes and reduction of oxidative stress [21].

Growth Performance and Economic Efficiency

**Table 4. Growth performance and feed conversion parameters (42 days)**

Parameters	Control	Experimental II	Experimental III
Final body weight (g)	1936 $\pm$ 45	2102 $\pm$ 38*	2184 $\pm$ 42*
Daily weight gain (g/day)	45.8 $\pm$ 1.2	49.6 $\pm$ 1.1*	51.3 $\pm$ 1.0*
Feed conversion ratio (FCR)	1.58	1.49*	1.46*
Feed consumption (kg/bird)	3.06	2.89	2.84
Mortality (%)	4.2	2.1	1.8

$p < 0.05$ .

Experimental group III demonstrated a 12.8% increase in live body weight. Feed conversion efficiency significantly improved, while mortality rates decreased. Economic calculations indicated increased profitability due to additional meat production and reduced feed costs.

### Conclusion

The obtained results demonstrated that dietary supplementation with a chitosan and whey powder complex possesses high physiological and economic efficiency in broiler chickens. The complex improved protein metabolism, hematological indices, intestinal trophic status, antioxidant protection, and productive performance. Experimental groups exhibited increased live body weight, improved feed conversion efficiency, and reduced mortality rates. The findings confirm the перспективность of chitosan and whey powder as natural biologically active feed additives for antibiotic-free poultry production systems.

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