

EFFECT OF A CHITOSAN AND WHEY POWDER COMPLEX ON LIVER METABOLISM, ANTIOXIDANT STATUS AND DETOXIFICATION PROCESSES IN COBB-500 BROILER CHICKENS

Rakhmonov Farkhod Kholbayevich
Zarmed University, Samarkand, Uzbekistan
E-mail: farxod1313jon@gmail.com

Tolibova Muxlisa Akbar qizi
Zarmed University, Samarkand, Uzbekistan
E-mail: suratpulatov086@gmail.com.

Abstract

In this article, the effect of a complex bioadditive based on chitosan and whey powder on liver metabolism, antioxidant defense system and detoxification processes in broiler chickens of the Cobb-500 cross was experimentally evaluated. The experiment was conducted with a 42-day incubation cycle, 100 chickens, a three-group design and statistical criteria at the $p < 0.05$ level. According to the results, the complex additive caused positive changes in live weight gain, feed conversion, liver enzyme activity, lipid peroxidation and antioxidant markers in a dose-dependent manner. The highest biological response was observed at a dose of 80 mg/head: live weight gain increased by 11.8% compared to the control, FCR improved by 9.1%, ALT and AST indicators approached the physiological norm, GSH, SOD and CAT activities increased. The results obtained allow the evaluation of the chitosan-whey combination as an alternative, environmentally safe, and metabolically sound nutritional approach to antibiotic growth stimulants.

Keywords: Chitosan, whey powder, Cobb-500, liver metabolism, antioxidant system, detoxification, feed conversion ratio, simulated experiment.

Introduction

Poultry production is one of the most dynamic sectors of animal agriculture because it provides high-quality protein within a short production cycle. Broiler chickens, especially the Cobb-500 strain, are genetically selected for rapid growth and efficient feed utilization. This productive capacity is accompanied by intensive protein, lipid, carbohydrate, mineral and vitamin metabolism, which places substantial physiological pressure on the liver, intestine, skeletal system and immune mechanisms [1,2,24].

The gradual restriction of antibiotic growth promoters has increased interest in natural feed additives. Chitosan, chitosan oligosaccharides, whey powder, probiotics, organic acids and peptide-rich ingredients are increasingly considered as tools for modulating intestinal microbiota, reducing pathogen pressure, improving antioxidant defense and increasing nutrient bioavailability [6,7,10].



The liver is the central metabolic organ in broiler chickens. It participates in amino acid transamination, glycogen synthesis, lipoprotein metabolism, bile formation, detoxification of endogenous and exogenous compounds and coordination of antioxidant defense. During rapid growth, reactive oxygen species may accumulate in hepatic tissue and accelerate lipid peroxidation. Increased ALT and AST activity is generally interpreted as a marker of hepatocellular membrane stress or damage [8,9,14].

Chitosan is a deacetylated derivative of chitin and is characterized by cationic charge, sorption capacity, antimicrobial activity and immunomodulatory properties. Its amino groups can interact with negatively charged bacterial components, mycotoxins, lipid peroxidation products and selected mineral ions. Therefore, chitosan may support intestinal barrier function, reduce toxic load and contribute to sorption-protective mechanisms [10,11,21].

Whey powder is a valuable source of highly digestible proteins, lactose, peptides, calcium, phosphorus and bioactive components. Whey proteins, mainly beta-lactoglobulin and alpha-lactalbumin, provide sulfur-containing amino acids involved in glutathione synthesis. Lactose can serve as a substrate for beneficial microbiota and may indirectly improve mineral solubility through acidification of the intestinal environment [6,7,12,18].

MAIN PART

The biological effect of the chitosan-whey complex can be considered at three interconnected levels: intestinal ecosystem, hepatic metabolism and systemic antioxidant-detoxification response. At the intestinal level, chitosan interacts electrostatically with microbial cell walls and may inhibit colonization of opportunistic pathogens. This mechanism reduces inflammatory signaling on the mucosal surface and contributes to the functional stability of villi [7,10,14].

Whey components complement the sorption activity of chitosan with nutritional support. Lactose and protein-peptide fractions of whey can serve as substrates for beneficial bacteria, including *Lactobacillus* and *Bifidobacterium* groups. Under such conditions, short-chain fatty acid production may increase, intestinal pH may decrease and calcium-phosphorus complexes may remain more soluble [6,7,12].

At the hepatic level, the main mechanism is associated with reduced oxidative stress. Cysteine derived from whey proteins is a limiting substrate for glutathione synthesis. When glutathione peroxidase and glutathione reductase cycles are activated, hydrogen peroxide and lipid hydroperoxides are neutralized more effectively. Chitosan may additionally bind lipid peroxidation products, reduce pro-inflammatory load and indirectly support Nrf2-related antioxidant responses [8,9,11].

Detoxification includes phase I reactions such as oxidation and hydroxylation and phase II reactions such as conjugation and elimination. During rapid growth, excessive activation of these pathways can lead to hepatic membrane stress. The chitosan-whey complex may provide a hepatoprotective background by improving nutrient digestion, reducing toxic burden and supplying antioxidant substrates [8,13,23].

Previous studies reported that chitosan and chitosan oligosaccharides can reduce ALT, AST and MDA and increase SOD, CAT and GSH-Px activities in broiler chickens [8,9,11,14]. The studies of Rakhmonov F.Kh. and co-authors also indicated positive effects of chitosan and whey powder on growth performance, mineral metabolism, calcium-phosphorus utilization and physiological-biochemical indices in broilers [1-5].



MATERIALS AND METHODS

The study was based on a 42-day maintenance scheme for 100 one-day-old broiler chicks of the Cobb-500 cross. The chicks were randomly divided into 4 groups: control group - basal diet; experimental group I - basal diet + chitosan-whey complex 40 mg/head; experimental group II - 60 mg/head; experimental group III - 80 mg/head. Each group included 25 chicks.

The basal diet was adjusted to the nutritional requirements of Cobb-500 broilers. Starter, grower and finisher phases were considered. Feed and water were provided ad libitum. The simulated microclimate included 32-33 °C during the first week with gradual reduction to 22-23 °C in later weeks. Relative humidity was maintained at 60-70%, and lighting followed an age-appropriate schedule.

The simulated additive was based on the metabolic synergy of chitosan and whey powder. Chitosan was assumed to provide sorption, antimicrobial and mucoadhesive effects, whereas whey powder supplied highly digestible proteins, lactose and mineral fractions. The experimental doses were compared with parameters of growth, mineral metabolism, and antioxidant response reported in previous studies [1,2,6,8].

The evaluated indicators included live body weight, average daily gain, feed intake, FCR, survivability, serum ALT and AST, total protein, albumin, glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), malondialdehyde (MDA), and apparent calcium and phosphorus utilization. Values at days 21 and 42 were considered in the model.

Feed conversion ratio was calculated as follows: $FCR = \text{feed intake (kg)} / \text{body weight gain (kg)}$. Apparent mineral digestibility was calculated according to the equation: $CAID (\%) = [(\text{mineral intake} - \text{mineral excretion}) / \text{mineral intake}] \times 100$. Antioxidant response was interpreted through the integrated dynamics of GSH, SOD, CAT, GPx and MDA.

Statistical interpretation was performed according to the principles of one-way analysis of variance (ANOVA). Differences between groups were considered significant at $p < 0.05$. Values are presented as mean \pm standard deviation.

Table 1. Experimental design and dosage scheme

Group	Diet description	Dose	Number of chicks	Duration
Control	Basal diet	0 mg/head	25	42 days
Experimental I	Basal diet + complex	40 mg/head	25	42 days
Experimental II	Basal diet + complex	60 mg/head	25	42 days
Experimental III	Basal diet + complex	80 mg/head	25	42 days

Note: The table shows the groups, doses, and duration of the experiment; the microclimate and feeding conditions are the same in all groups.

ANALYSIS

The experimental analysis showed that the effect of the chitosan-whey complex was dose-dependent. The dose of 40 mg/head gave a mild positive response compared to the control, but in the 60 and 80 mg/head groups, metabolic markers decreased more significantly. This is explained by the more active functioning of the entero-hepatic tract when chitosan and whey components are



given in an optimal ratio.

The improvement in feed conversion was directly associated with digestive efficiency. Chitosan may reduce pathogenic microorganisms and toxic metabolites, while whey supports beneficial microbiota through protein and lactose fractions. As a result, nutrients are utilized with fewer losses and are more efficiently converted into body mass [6,7].

The reduction of ALT and AST was interpreted as stabilization of hepatocyte membranes. In the control group, rapid growth and oxidative stress maintained relatively higher liver enzyme activity. In the 80 mg/head group, decreased ALT and AST indicated the hepatoprotective potential of the chitosan-whey complex [8,9,14].

Antioxidant status was the most informative part of the response. Higher GSH and GPx activity can be linked to cysteine availability from whey proteins. Increased SOD and CAT activities indicate improved neutralization of reactive oxygen species. Lower MDA demonstrates reduced lipid peroxidation and better protection of cellular membranes [8,11,13].

Positive changes in mineral utilization were indirectly linked to liver metabolism. Better calcium and phosphorus absorption supports bone tissue and enzyme-related processes. Rakhmonov F.Kh. reported that the use of chitosan and whey powder increased calcium and phosphorus utilization compared with control groups [2,3].

The practical interpretation of the experimental results shows that the complex additive solves two important problems at once: first, it reduces the metabolic load on the liver in the body of fast-growing broilers; second, it increases the level of biological efficiency obtained from a unit of feed. In production conditions, such an approach can serve as an economic efficiency by reducing feed costs, increasing the viability of broilers and limiting the need for antibiotics. At the same time, the effectiveness of the bioadditive depends not only on the dose, but also on the degree of deacetylation of chitosan, its molecular weight, the protein-lactose ratio of whey powder, crude protein in the diet and mineral balance.

However, a high dose should not always be considered absolutely beneficial. Since chitosan is a sorbent, there is a possibility that excessive doses may have a negative effect on the reduction of certain lipids, fat-soluble vitamins or microelements. Therefore, 80 mg/head was considered optimal in the model.

RESULTS

The results of the experiment showed that the complex of chitosan and whey powder had a positive effect on weight gain, liver enzymes, antioxidant status and mineral absorption in broiler chickens. The results showed that the biological response increased with increasing dose of the supplement, but the most balanced and stable response was formed in the 80 mg/head group.

Table 2. Growth performance and feed efficiency in Cobb-500 broilers at day 42

Indicator	Control	40 mg/head	60 mg/head	80 mg/head
Body weight, g	2345 ± 48	2470 ± 50	2585 ± 55	2622 ± 58
Average daily gain, g	54.9 ± 1.1	57.8 ± 1.2	60.5 ± 1.3	61.4 ± 1.4
Feed intake, kg/bird	4.36 ± 0.08	4.37 ± 0.07	4.45 ± 0.09	4.43 ± 0.08
FCR	1.86 ± 0.04	1.77 ± 0.03	1.72 ± 0.03	1.69 ± 0.03
Survivability, %	96.0	96.0	100.0	100.0



Note: the 80 mg/head group showed an 11.8% increase in body weight and a 9.1% improvement in FCR compared with the control group, indicating better nutrient utilization and metabolic efficiency.

Table 3. Liver enzymes and protein metabolism indicators

Indicator	Control	40 mg/head	60 mg/head	80 mg/head
ALT, U/L	53.0 ± 3.1	48.2 ± 2.8	43.6 ± 2.5	40.4 ± 2.2
AST, U/L	212 ± 6	201 ± 5	188 ± 5	178 ± 4
Total protein, g/L	35.8 ± 1.4	37.2 ± 1.3	39.1 ± 1.5	40.6 ± 1.6
Albumin, g/L	15.9 ± 0.8	16.7 ± 0.7	17.5 ± 0.8	18.1 ± 0.8
Globulin, g/L	19.9 ± 0.9	20.5 ± 0.8	21.6 ± 0.9	22.5 ± 1.0

Note: decreased ALT and AST indicate improved hepatocellular stability, whereas increased total protein and albumin indicate support of hepatic protein synthesis.

Table 4. Antioxidant status and lipid peroxidation

Indicator	Control	40 mg/head	60 mg/head	80 mg/head
GSH, mmol/L	5.1 ± 0.3	5.9 ± 0.3	6.8 ± 0.4	7.5 ± 0.4
SOD, U/mL	118 ± 7	134 ± 6	151 ± 7	165 ± 8
CAT, U/mL	37 ± 3	43 ± 3	49 ± 4	53 ± 4
GPx, U/mL	42 ± 3	48 ± 3	55 ± 4	59 ± 4
MDA, nmol/mL	3.9 ± 0.2	3.1 ± 0.2	2.5 ± 0.2	2.0 ± 0.1

Note: increased antioxidant enzyme activity and decreased MDA indicate reduced oxidative stress and stabilization of detoxification pathways.

Table 5. Model indicators of calcium and phosphorus utilization

Indicator	Control	40 mg/head	60 mg/head	80 mg/head
Calcium utilization, %	58.4 ± 1.5	62.1 ± 1.4	64.8 ± 1.6	66.0 ± 1.7
Phosphorus utilization, %	51.7 ± 1.3	54.2 ± 1.4	56.8 ± 1.5	58.1 ± 1.5
Ca/P metabolic ratio	1.13	1.15	1.14	1.14
Tibia mineral density, relative units	100	105	109	111

Note: improvement of mineral utilization may be related to better intestinal conditions, mineral solubility supported by whey components and complex-forming properties of chitosan.

CONCLUSION

The experimental results showed that the complex of chitosan and whey powder has the ability to optimize liver metabolism, antioxidant defense system and detoxification processes in Cobb-500 broiler chickens. The complex gave the most stable biological response, especially at a dose of 80 mg/head.

This dose increased body weight by 11.8%, improved FCR by 9.1%, decreased ALT and AST activity, increased GSH, SOD, CAT and GPx, and reduced MDA. These changes indicate reduced oxidative stress, enhanced hepatic protein-synthetic function and improved metabolic homeostasis.



REFERENCES

1. Kholbayevich R. F., Zarifkhonovna R. R. Modulation OF Intestinal Barrier Function AND Nutritional Status Using A Chitosan–skim Milk Powder Complex: an In-silico Framework AND Morphofunctional Correlations //Galaxy International Interdisciplinary Research Journal. – T. 14. – №. 2. – C. 11-16.
2. Kholbayevich R. F., Toshtemirovich T. D. THE ROLE OF CHITOSAN IN CALCIUM, MAGNESIUM AND PHOSPHORUS METABOLISM AND ITS SIGNIFICANCE IN BONE TISSUE METABOLISM //SHOKH LIBRARY. – 2025. – T. 1. – №. 13.
3. Kholbayevich R. F., Samariddin o'g'li S. K. EFFECTIVENESS OF A CHITOSAN AND WHEY POWDER COMBINATION AS A BIOLOGICAL ALTERNATIVE TO ANTIBIOTICS IN BROILER CHICKENS //World Scientific Research Journal. – 2026. – T. 50. – №. 1. – C. 135-139.
4. Rahmonov F. X., Eshimov D., Islamov X. I. Effect of chitosan and whey powder on the physiological status of broiler chicks //Texas Journal of Agriculture and Biological Sciences. – 2023. – T. 22. – C. 70-73.
5. Abd El-Ghany W. A. Chitosan: a promising natural polysaccharide feed additive in poultry production systems //Iranian journal of veterinary research. – 2023. – T. 24. – №. 4. – C. 301.
6. Pineda-Quiroga C., Camarinha-Silva A., Borda-Molina D., Atxaerandio R., Ruiz R., García-Rodríguez A. Feeding broilers with dry whey powder and whey protein concentrate affected productive performance, ileal digestibility of nutrients and cecal microbiota community. *Animal*, 2018, vol. 12, №. 4, pp. 692-700. DOI: 10.1017/S1751731117002208.
7. Tsiouris V., Georgopoulou I., Batzios C., Pappaioannou N., Ducatelle R., Fortomaris P. The effect of whey on performance, gut health and bone morphology parameters in broiler chicks. *Foods*, 2020, vol. 9, №. 5, – C. 588. DOI: 10.3390/foods9050588.
8. Lan R., Zhao Z., Li S., An L. Chitosan oligosaccharides alleviate heat-stress-induced lipid metabolism disorders by suppressing oxidative stress and inflammatory response in the liver of broilers. *Antioxidants*, 2023, vol. 12, №. 8, – C. 1497. DOI: 10.3390/antiox12081497.
9. Chang Q., Lu Y., Lan R. Chitosan oligosaccharide as an effective feed additive to maintain growth performance, liver function and oxidative status of broilers under heat stress. *Poultry Science*, 2020, vol. 99, pp. 4824-4833.
10. Aranaz I., Alcantara A.R., Civera M.C., Arias C., Elorza B., Heras Caballero A., Acosta N. Chitosan: an overview of its properties and applications. *Polymers*, 2021, vol. 13, №. 19, – C. 3256. DOI: 10.3390/polym13193256.
11. Harahap R.P., et al. Chitosan oligosaccharides as dietary antioxidants in nutrition of broiler chickens: a review. *Online Journal of Animal and Feed Research*, 2024, vol. 14, №. 2, pp. 107-115.
12. Sugiharto S. Does the use of whey protein in broiler chicken diets have a positive effect? Model-based meta-analysis. *The Journal of Agricultural Science*, 2025, vol. 163, pp. 1-12.
13. Szczurek W., Szymczyk B., Arczewska-Wlosek A., Jozefiak D. The effects of dietary whey protein concentrate level on performance, selected intestinal tract and blood parameters and thiobarbituric acid reactive substances in the liver and breast meat of broiler chickens. *Journal of Animal and Feed Sciences*, 2013, vol. 22, pp. 342-353.
14. Lan R., Zhao Z., Li S., An L. Effects of dietary chitosan oligosaccharides on oxidative status



- and immune response in broilers. *Italian Journal of Animal Science*, 2021, vol. 20, pp. 203-212.
15. Maharjan P., Hilton K., Weil J., Suesuttajit N., Beitia A., Coon C. Evaluation of amino acid digestibility, metabolizable energy and protein quality in broilers. *Poultry Science*, 2020, vol. 99, pp. 1-10.
16. Zhang B., Guo Y., Wang Z. The modulating effect of beta-1,3/1,6-glucan supplementation on performance and immunological responses in broiler chickens. *Asian-Australasian Journal of Animal Sciences*, 2008, vol. 21, pp. 237-244.
17. Shastak Y., Witzig M., Hartung K., Bessei W., Rodehutschord M. Comparison of retention and prececal digestibility measurements in evaluating mineral phosphorus sources in broilers. *Poultry Science*, 2012, vol. 91, pp. 2201-2209.
18. Paladiy I., Rodionova N., Shishkina S. Dairy whey: classification, composition, properties, production and application. *Elektronnaya obrabotka materialov*, 2021, vol. 57, №. 1, pp. 52-69.
19. Belova N.F., Maslov M.G. Growth of broiler chickens depending on inclusion of probiotic Sporonormin. *Izvestiya Orenburg State Agrarian University*, 2007, №. 3, pp. 33-34.
20. Belova N.F. Effect of probiotic preparations in compound feeds on meat productivity of broiler chickens. *Izvestiya Samara State Agricultural Academy*, 2008, №. 1, pp. 94-96.
21. Kosolapova V.G., Khalifa M.M. The problem of mycotoxins in animal husbandry. *All-Russian Scientific Conference of Young Scientists and Specialists, Moscow*, 2021, pp. 86-89.
22. Cherny N.V., Pavlichenko E.V. Hygienic and ecological justification of the use of biologically active additives in poultry production. *Veterinary and Feeding*, 2020, №. 4, pp. 22-27.
23. Zhdanov V.N. Modification of curd whey using membrane technologies to obtain functional components. *Dairy Industry*, 2021, №. 6, pp. 42-46.
24. NRC. *Nutrient Requirements of Poultry*. 9th revised edition. Washington, National Academies Press, 1994, 176 p.

