Educator Insights: A Journal of Teaching Theory and Practice
Volume 2, Issue 1, January 2026
brightmindpublishing.com
ISSN (E): 3061-6964


# EFFECT OF A CHITOSAN (BOMBYX MORI)–BASED HYDROXYAPATITE COMPOSITE ON THE NUMBER OF LACTO- AND BIFIDOBACTERIA IN THE INTESTINAL MICROFLORA OF ROSS-308 BROILER CHICKS

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**Abstract**
This study evaluated the effect of a hydroxyapatite composite synthesized on the basis of chitosan obtained from Bombyx mori silkworms on the intestinal microbiocenosis of Ross-308 broiler chicks. Under poultry farm conditions, the dynamics of beneficial intestinal microflora indicators—lactobacilli and bifidobacteria (total lacto-bifido group)—were investigated when the additive was included in the diet at doses of 0.4, 0.7, and 1.0 g/kg. The results demonstrated that the chitosan–hydroxyapatite composite biologically supports intestinal microecology, stabilizes populations of beneficial microorganisms, and shows promise as an alternative feed additive to antibiotics. The highest microbiocenotic efficiency was observed at a dose of 0.7 g/kg. The findings are consistent with contemporary concepts regarding the ability of chitosan and its composites to modulate digestive functions and immune responses in animals.

**Keywords**: Chitosan, Bombyx mori, hydroxyapatite, broiler chickens, intestinal microbiocenosis, lactobacilli, bifidobacteria, prebiotic effect, immunomodulation, antibiotic alternative.

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## Introduction

The modernization of poultry production in Uzbekistan, the implementation of resource-efficient feeding technologies, and the increase in safe and competitive product output are among the priority directions of national agrarian policy. Within this framework, maintaining intestinal health in broiler chickens while preserving high growth rates, stabilizing gut microbiocenosis, and rationally reducing the use of antibiotics are issues of significant scientific and practical relevance [1,3].

The intestinal microbiocenosis plays a crucial role in nutrient digestion and absorption, intestinal barrier function, and both local and systemic immunity. An increased proportion of beneficial microflora is directly associated with enhanced resistance and productivity of poultry [6–8]. Modern studies indicate that probiotic and prebiotic approaches stimulate the growth of beneficial bacteria, particularly *Lactobacillus* and *Bifidobacterium* species, inhibit pathogen colonization, and shift the metabolic environment in a favorable direction [14,16].

In recent years, chitosan and its derivatives have been widely discussed as functional bioadditives in animal husbandry and poultry farming. Numerous studies highlight the sorption properties of chitosan, its effects on the intestinal environment, biological compatibility, and its ability to modulate immune processes [5,9,18]. Chitosan-based composites, including organomineral systems with hydroxyapatite, have been reported to exert positive effects on intestinal microecology when used as biocorrectors [2,4,12].

## MAIN PART

The scientific hypothesis of this study is that the chitosan–hydroxyapatite composite acts as a selective substrate and physiological “matrix” in the intestine, supporting the colonization and metabolic activity of beneficial microorganisms. As a result, populations of lactobacilli and bifidobacteria increase, intestinal balance is restored, and the overall resistance of broiler chickens is enhanced [4,6,8].

Evidence suggests that chitosan and chitosan-based formulations improve gastrointestinal function, facilitate feed digestion, and modulate gut microbiota composition [10,11]. Some studies indicate that chitosan may increase the proportion of commensal bacteria, including bifidobacteria and lactobacilli, in the intestinal tract [14,18].

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## METHODOLOGY (MATERIALS AND METHODS)

The experimental study was conducted under production conditions at the "Afrosiyob Parranda" poultry farm (Samarkand, Uzbekistan). One-day-old Ross-308 broiler chicks were used as experimental animals. At 15 days of age, chicks were weighed and divided into four groups using the analog method; each group consisted of 25 birds with an initial body weight difference within ±15 g.

The first group served as the control and was fed a standard farm diet. Groups 2–4 were experimental groups and received a chitosan hydroxyapatite immunomodulator (70/30 fraction) mixed into the feed during two periods: from 15 to 25 days of age (10 days) with the "Rost (RO-6-5)" diet and from 32 to 42 days of age (10 days) with the "Finish (RO-6)" diet. The additive was administered at doses of 0.4 g/kg (Group 2), 0.7 g/kg (Group 3), and 1.0 g/kg (Group 4).

Microbiological evaluation focused on beneficial intestinal microflora, with the total number of lactobacilli and bifidobacteria determined per 1 g of sample and expressed as colony-forming units (CFU). Results were presented as mean values ± standard deviation (M ± SD).

**Statistical analysis.** Statistical significance was assessed using the Welch Student's *t*-test. The sample size was $n = 25$ per group. Differences were considered statistically significant at $p < 0.05$. It should be noted that the presented *p*-values represent approximate estimates of statistical significance relative to the control group.

## RESULTS AND DISCUSSION

Administration of the chitosan (*Bombyx mori*) hydroxyapatite composite resulted in a pronounced increase in beneficial intestinal microflora. In the control group, the number of lacto- and bifidobacteria increased up to 20 days of age, declined slightly by day 30, and increased again by day 40. This pattern reflects normal microbiocenosis development and feed phase transitions in broilers [7,8].

In contrast, experimental groups receiving the additive exhibited more stable and significantly higher counts of beneficial bacteria during supplementation periods (15–25 and 32–42 days). The most pronounced increase was observed in Group 3 receiving 0.7 g/kg of the composite.

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**Table 1. Effect of the chitosan (*Bombyx mori*) hydroxyapatite composite on the number of lacto- and bifidobacteria in the intestinal microflora of broiler chicks (CFU/g; M ± SD)**

| Age | Control group | Group 2 (0.4 g/kg) | *p* | Group 3 (0.7 g/kg) | *p* | Group 4 (1.0 g/kg) | *p* |
|---|---|---|---|---|---|---|---|
| 1 day | $2.16\times10^{7} \pm 0.4\times10^{7}$ | $2.16\times10^{7} \pm 0.4\times10^{7}$ | >0.05 | $2.16\times10^{7} \pm 0.4\times10^{7}$ | >0.05 | $2.16\times10^{7} \pm 0.4\times10^{7}$ | >0.05 |
| 20 days | $1.34\times10^{8} \pm 0.7\times10^{8}$ | $4.36\times10^{7} \pm 0.4\times10^{7}$ | <0.001 | $2.45\times10^{8} \pm 0.1\times10^{8}$ | <0.001 | $2.11\times10^{8} \pm 0.2\times10^{8}$ | <0.01 |
| 30 days | $2.29\times10^{7} \pm 1.2\times10^{7}$ | $2.40\times10^{8} \pm 1.3\times10^{8}$ | <0.001 | $3.21\times10^{7} \pm 1.7\times10^{7}$ | <0.05 | $1.80\times10^{8} \pm 0.4\times10^{8}$ | <0.001 |
| 40 days | $1.37\times10^{9} \pm 0.3\times10^{9}$ | $4.15\times10^{10} \pm 0.5\times10^{10}$ | <0.001 | $4.88\times10^{10} \pm 1.1\times10^{10}$ | <0.001 | $3.98\times10^{10} \pm 0.6\times10^{10}$ | <0.001 |

**Note:** *p* — statistical significance compared with the control group (Welch *t*-test).

## DISCUSSION

The data demonstrate that the chitosan-based hydroxyapatite composite exerts a positive, dose-dependent effect on beneficial intestinal microflora. The most stable and pronounced microbiocenotic response was observed at the 0.7 g/kg dose, indicating an optimal biological level of supplementation. Lower doses showed weaker effects, while higher doses did not further enhance microbial growth, suggesting a typical dose–response relationship.

This phenomenon can be explained by fermentation kinetics of prebiotic substrates, intestinal transit time, mucosal interactions, and competitive microbial dynamics [6,8]. Similar patterns have been reported in studies examining functional feed additives and gut microbiota development in broilers [7,14].

## RESULTS

Under poultry farm conditions, the chitosan (*Bombyx mori*) hydroxyapatite composite effectively stimulated beneficial components of intestinal microbiocenosis in broiler chicks. The highest increase in lacto- and bifidobacteria counts was recorded in the group receiving 0.7 g/kg of the additive, with statistically significant differences compared to the control ($p < 0.05$).

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## CONCLUSION

The chitosan (*Bombyx mori*) hydroxyapatite composite exerts a beneficial effect on the intestinal microbiocenosis of Ross-308 broiler chicks by increasing populations of lactobacilli and bifidobacteria, stabilizing intestinal physiological balance, and enhancing overall resistance of the organism. These findings support the potential use of this bioactive additive as a prophylactic and functional alternative to antibiotics in poultry farming. Future studies should include comprehensive microbiota profiling (16S rRNA metagenomic analysis), intestinal morphology, immune markers, and production parameters such as feed conversion ratio and survival rate.

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