Research Article

Impact of Nano-Biological Synbiotic on Performance, Physiological Traits, and Gut Microbiota of Broiler Chickens

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Abstract | This study aimed to evaluate the effects of dietary nano-biological synbiotic supplementation on growth performance, economic efficiency, selected blood biochemical parameters, intestinal microbial balance, and ileal histomorphology of broiler chickens (female Ross 308) during a 35 day rearing period. A total of 225 one-day-old chicks were randomly assigned to five treatments with three replicates per treatment, including a non-supplemented control group (T1) and four experimental groups (T2, T3, T4, and T5) supplemented with nano-synbiotic at levels of 1, 2, 3, and 4 g/kg of feed, respectively. The results showed significant improvements ($P \leq 0.05$) in final body weight, body weight gain, and feed conversion ratio in all supplemented treatments compared with the control group, while feed intake was not significantly affected. This improvement was reflected in enhanced economic efficiency, with the 3 g/kg supplementation level demonstrating the most superior performance among the tested levels. Supplemented birds exhibited significant reductions ($P \leq 0.05$) in serum glucose, cholesterol, AST, and ALT levels, accompanied by significant increases ($P \leq 0.05$) in total protein, albumin, and globulin concentrations relative to the control. Additionally, lactic acid bacteria counts were significantly increased ($P \leq 0.05$), whereas Escherichia coli populations were significantly reduced ($P \leq 0.05$) in all supplemented treatments. Villus height and the villus height-to-crypt depth ratio were also significantly improved ($P \leq 0.05$) compared with the control. These findings indicate that supplementation with 3 g/kg nano-synbiotic achieved the most favorable productive, physiological, microbial, and histological responses under the conditions of this study.

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Introduction

Broiler chicken production plays a pivotal role in global food security, serving as a significant source of high-quality animal protein at a relatively low cost compared to other types of meat. With a growing global population and rising demand for animal-derived protein, sustainable broiler production requires continual improvement in both productivity and health under modern rearing systems (Salah *et al*., 2018). Central to achieving this efficiency are gut health and the intestinal microbial balance, which influence nutrient absorption, immune competence, metabolic processes, and overall growth performance in broiler chickens (Lee, *et al*., 2023).

In pursuit of alternatives to antibiotic growth promoters, synbiotic supplementation combining probiotics and prebiotics has emerged as a potential strategy to enhance gut health and production performance. Synbiotics are designed to support the proliferation of beneficial gut microorganisms, thereby improving digestive efficiency, nutrient utilization, and competitive exclusion of pathogenic bacteria. Several studies have demonstrated that supplementation with commercial synbiotics can lead to significant improvements in body weight, feed conversion efficiency, and selected blood biochemical markers in broiler chickens (Abdel-Wareth *et al*., 2018). However, the effectiveness of synbiotic supplementation has been shown to vary according to the specific strains of probiotics, types of prebiotics, and environmental and management conditions under which broilers are raised.

Physiological and biochemical indicators such as blood glucose, cholesterol, total protein, albumin, and globulin are widely recognized as key parameters for evaluating the health and metabolic responses of broiler chickens to dietary interventions (Abd El Latif, and Omar, 2023). Research indicates that synbiotic supplementation can modulate these parameters in ways that reflect improved metabolic function and reduced oxidative stress (Naeem, and Bourassa, 2025). Furthermore, the composition and balance of the intestinal microbiota particularly the populations of lactic acid bacteria serve as critical indicators of gut health. Recent studies have shown that lactic acid bacteria isolated from broiler chickens can enhance growth performance, improve nutrient absorption, and promote a balanced gut microbial community, highlighting their potential as host-adapted synbiotic candidates (Wang *et al*., 2023).

Despite the positive outcomes reported with commercial synbiotic products, information remains limited regarding synbiotics developed from host-derived microbial communities. Host-adapted synbiotics are hypothesized to exhibit greater compatibility with the native gut microbiota of broiler chickens, potentially resulting in superior functional outcomes in terms of growth performance, physiological regulation, and microbial balance. This represents a significant gap in the current literature, as few studies have directly compared host-derived synbiotic formulations with commercially available products under practical production conditions.

Therefore, the present study aims to evaluate the effects of host-derived synbiotic supplementation compared to commercial synbiotic formulations on productive performance, selected physiological and biochemical traits, and intestinal microbial balance in broiler chickens. By addressing this gap, the study seeks to provide scientifically grounded recommendations for practical application in commercial broiler production, ultimately contributing to improved bird health, welfare, and production efficiency.

Materials and Methods

Broiler rearing and experimental design

The experiment was conducted in a research poultry facility in Thi Qar Governorate, southern Iraq, during November 2025 for a period of 35 days. The study included 225 one-day-old female Ross 308 broiler chicks, with an average initial body weight of 43 g per chick, ensuring uniformity at the start of the experiment and a balanced growth initiation. Chicks were housed in a three-tier battery system, with each tier containing cages measuring 1.5× 1 m^2, and environmental conditions including temperature, lighting, and ventilation were managed according to Aviagen (2025) management handbook standard growth requirements.

The chicks were randomly allocated to five experimental treatments, each with three replicates of 15 chicks per replicate, as follows:

- T1 (Control): no supplementation.
- T2: supplementation with synbiotic at 1 g/kg of feed.
- T3: supplementation with synbiotic at 2 g/kg of feed.
- T4: supplementation with synbiotic at 3 g/kg of feed.
- T5: supplementation with synbiotic at 4 g/kg of feed.
- Fresh water and feed were provided *ad libitum*

throughout the experimental period. Feeding was conducted in two phases:

Starter phase (1–21 days): feed contained 23.02% crude protein and 2984 kcal/kg of metabolizable energy (ME) to meet the high nutrient requirements for early growth. Grower phase (22–35 days): feed contained 20.03% crude protein and 3105 kcal/kg ME to support continued growth and efficient feed conversion. The diets were formulated according to the nutritional requirements of Ross 308 broilers with minor modifications to suit local experimental conditions. The detailed composition of both diets is presented in Table 1.

Table 1: *Formulated starter and grower diets for broiler chicks and their calculated chemical composition*

Feed Ingredient (%)	Starter (1–21 d)	Grower (22–35 d)
Yellow corn	41	49
Wheat	17	16
Soybean meal (48%)	33	25
Protein concentrate (40%)	5	5
Premix (6%)	1	1
Vegetable oil (sunflower)	1.2	2.5
Limestone	1.5	1.2
Salt	0.3	0.3
Total	100	100
Calculated Chemical Composition		
Crude protein (%)	23.02	20.03
Metabolizable Energy (kcal/kg)	2984	3105
Lysine (%)	1.32	1.15
Methionine + Cystine (%)	0.95	0.82
Calcium (%)	1.02	0.9
Available phosphorus (%)	0.45	0.4

A commercial vitamin–mineral premix (Laymix®, Erbil, Iraq) was included at 1% of the diet according to the manufacturer's specifications (6% CP, 4331.57 kcal/kg ME, 1.50% lysine, 5.90% methionine, 5.00% methionine + cystine, 24.05% Ca, and 10.20% available phosphorus), and its contribution was incorporated into the final nutrient calculations of the experimental diets.

Diets were formulated according to NRC (1994) recommendations.

Experimental nano-synbiotic material

The nano-synbiotic material used in the present study consisted of nano-sized *Taraxacum officinale* root powder loaded with lactic acid bacteria. The formulation was obtained from a reliable scientific source in its ready-to-use form and had undergone prior processing, sterilization, and physicochemical characterization to ensure suitability for dietary application.

The material had been previously evaluated for stability, bacterial viability, and functional efficiency as reported by Abaas et al. (2026), and it was incorporated into the experimental diets without further modification. Figure 1 shows the final composition of this symbiotic nanomaterial.

Figure 1: *Nano-synbiotic formulation used in this study*

Performance and economic efficiency

Weekly measurements of body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and economic efficiency (EE) in Iraqi dinars were recorded at 35 days of age, as indicated by Ogbuokiri (2018a) and Dev *et al.* (2020) according to the following equations.

BWG (g) = Final Body Weight (g) - Initial Body Weight (g)

$$(FCR) = \frac{Feed\ Intake\ (g)}{Body\ Weight\ Gain\ (g)}$$

$$EE\ (IQD/kg\ BWG) = \frac{Cost\ of\ Feed\ Intake\ (kg) \times Feed\ Price\ (IQD/kg)}{Body\ Weight\ Gain\ (kg)}$$

Note: Feed cost was calculated based on a market price of 1000 IQD per kg of feed during the experimental period in Iraq.

Biochemical indices of blood

At 35 days of age, three birds per replicate were randomly selected for blood sampling via the wing vein. Blood was collected into heparinized tubes and centrifuged at 3000 rpm for 15 minutes to obtain plasma. Biochemical analyses, including Total Protein, Albumin, Globulin, Glucose, and Cholesterol, were performed using commercial kits from BIOLABO and measured with a spectrophotometer. Liver enzyme activities (ALT, AST) were also evaluated to monitor metabolic responses to dietary treatments (Al-Salhi, 2025).

Intestinal microbial counts

Microbial analyses and colony counts of cecal contents were performed in the ceca according to Al-Salhi *et al.* (2022). To comply with halal standards, birds were euthanized at 35 days of age according to Islamic procedures. Serial dilutions were prepared, and populations of lactic acid bacteria (LAB) and coliforms were enumerated, with results expressed as $\log_{10}$ CFU/g, serving as an indicator of intestinal microbial balance and the effect of dietary supplementation on promoting beneficial bacteria.

Histological analysis of the ileum

After slaughtering the Broilers according to Islamic procedures at 35 days of age, samples were collected from the ileum of the same birds used for microbial analyses. The samples were fixed in 10% formalin and processed following standard histological procedures, then stained with hematoxylin and eosin. Villus height and crypt depth were measured as indicators of nutrient absorption capacity and digestive efficiency in the small intestine, following standard histological methods (Uni *et al*., 1998).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) in IBM SPSS Statistics, Version 25 (IBM Corp., Armonk, NY, USA, 2018). The experiment consisted of five treatments, each with three replicates. Results are presented as mean ± standard error (SE), and differences among means were considered significant at P ≤ 0.05.

Results and Discussion

Effect of nano-synbiotic supplementation on growth performance

Table 2 indicates significant differences (P≤0.05) among treatments in final body weight, total body weight gain, and feed conversion ratio, whereas no significant differences were observed in feed intake.

Final body weight was significantly influenced by dietary supplementation (P≤0.05), with T4 recording the highest mean value, while the control group (T1) showed the lowest performance. The remaining supplemented treatments exhibited intermediate values, demonstrating a progressive response to increasing inclusion levels. Notably, all experimental treatments outperformed the control group in final body weight.

A similar pattern was observed for total body weight gain, where T4 achieved superior growth compared with the other treatments (P≤0.05), and the control group remained the lowest. All supplemented groups showed improved weight gain relative to the control.

Feed intake was not significantly affected by the treatments, despite slight numerical variations among means. This finding suggests that the improvement in growth performance was not driven by increased feed consumption. Feed conversion ratio was significantly improved by nano-synbiotic supplementation (P≤0.05). T4 demonstrated the most efficient feed utilization, whereas the control group exhibited poorer conversion efficiency. All supplemented treatments showed better feed efficiency compared with the control. In general, nano-synbiotic supplementation enhanced productive performance, and all experimental treatments surpassed the control group in growth-related traits, with T4 showing the most pronounced improvement.

Table 2: *Effect of nano-synbiotic supplementation on productive traits (Mean ± SE)*

Treatment	Body weight (g)	Weight gain (g)	Feed intake (g)	FCR (g feed / g gain)
T1	1775.29± 85.58 e	1732.29± 78.22 e	3329.67± 64.96	1.92±0.04 a
T2	1820.11 ±61.25 d	1777.11± 96.34 d	3285.61± 51.88	1.85±0.06 b
T3	2026.78 ±78.75 c	1983.78± 68.65 c	3271.32± 38.37	1.65±0.07 c
T4	2455.15± 57.75 a	2412.15± 55.95 a	3348.11± 48.61	1.38±0.03 e
T5	2228.74± 42.44 b	2185.74± 55.58 b	3305.79± 55.77	1.51±0.05 d
Sig.	*	*	N.S	*

**Different letters within the same column indicate significant differences (P ≤ 0.05).*

N.S.: Indicates no significant differences between the Means.

Effect of nano-synbiotic supplementation on economic efficiency

As illustrated in Figure 2, nano-synbiotic supplementation significantly influenced economic efficiency (P≤0.05). The economic return showed a clear improvement in the supplemented groups compared with the control treatment.

Treatment T4 achieved the highest economic efficiency (P≤0.05), reflecting its superior growth performance and improved feed conversion ratio. In contrast, the control group recorded the lowest economic return. The remaining supplemented treatments showed intermediate economic values, *yet al*l of them exceeded the control treatment.The improvement in economic efficiency corresponds directly with the enhancement observed in productive traits, particularly body weight gain and feed conversion ratio, without a significant increase in feed intake .This indicates that the economic benefit resulted primarily from better feed utilization rather than increased feeding cost.

Overall, nano-synbiotic supplementation enhanced profitability, with T4 representing the most economically efficient treatment under the conditions of this study.

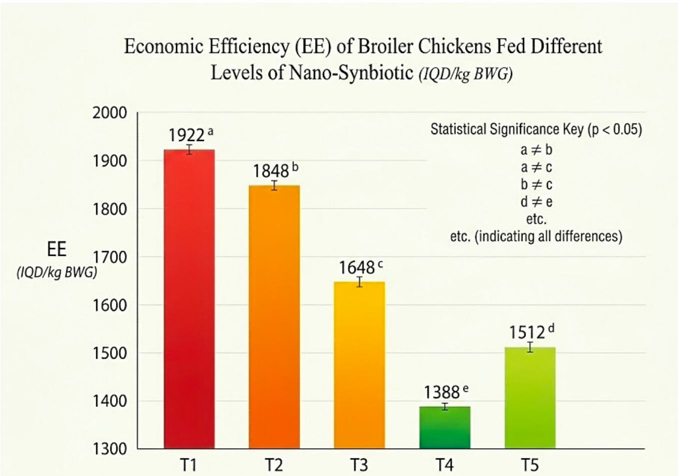

Figure 2: *Effect of nano-synbiotic supplementation on economic efficiency*

Discussion of growth performance and its economic implications

The significant improvement observed in growth performance parameters, particularly in the fourth treatment (3 g/kg), can be attributed to this inclusion level representing the optimal biological dose of the nano-synbiotic supplement. At this concentration, a functional synergism between the probiotic and prebiotic components was likely achieved, resulting in maximal enhancement of the intestinal environment. This synergistic interaction may have promoted the stabilization of beneficial microbial populations, improved intestinal epithelial integrity, and enhanced digestive and absorptive efficiency. Consequently, nutrients were utilized more effectively for muscle deposition, which was reflected in superior body weight gain and improved feed conversion ratio. The absence of significant differences in feed intake among treatments further supports that the observed improvements were driven by enhanced nutrient utilization efficiency rather than increased feed consumption. Although the fifth treatment (4 g/kg) maintained performance superiority over the control group, its response was comparatively lower than that of the fourth treatment. This pattern suggests the possible attainment of a biological saturation threshold at the higher inclusion level. Beyond the optimal dose, excessive supplementation may lead to microbial competition, altered metabolic equilibrium, or physiological regulatory adjustments that limit further enhancement in digestive efficiency. Such a non-linear response pattern reinforces the concept of an optimal inclusion level beyond which proportional improvements in performance are not achieved (Abaas *et al*., 2026).

These findings are consistent with previous research. Dev *et al*. (2020) reported that synbiotic supplementation improves growth performance by enhancing gut microbial balance and nutrient utilization efficiency. Similarly, Abdel Hafeez *et al*. (2017) indicated that increasing synbiotic levels beyond the optimal range does not necessarily produce proportional improvements in productive traits. Khosravi *et al*. (2025) also demonstrated that moderate synbiotic inclusion levels yielded superior feed conversion efficiency compared with higher levels, confirming the existence of a biologically optimal dosage.

The productive superiority observed in the fourth treatment was directly reflected in economic efficiency. The treatment that achieved the best feed conversion ratio and highest market body weight, without a concomitant increase in feed intake, generated the greatest economic return. This relationship is logically consistent with commercial poultry production systems, where feed cost constitutes the largest proportion of total production expenses. Improvements in feed conversion efficiency therefore translate directly into reduced cost per unit of weight gain and enhanced profitability. Ogbuokiri (2018b) similarly emphasized that even modest improvements in feed conversion ratio can significantly increase net economic return under practical production conditions. In summary, the 3 g/kg inclusion level of the nano-synbiotic supplement represented the optimal biological and economic dosage under the conditions of the present study. Increasing the level to 4 g/kg did not yield proportional additional benefits, highlighting the importance of precise dosage optimization in functional feed additive applications to achieve maximal productive and economic efficiency.

Table 3: *Effect of nano-synbiotic supplementation on glucose, cholesterol and total protein (Mean ± SE)*

Treat-ments	Glucose (mg/dL)	Cholesterol (mg/dL)	Total Protein (g/dL)
T1	215.4 ± 3.81 a	168.7 ± 4.27 a	3.42 ± 0.07 d
T2	204.1 ± 3.43 b	156.3 ± 3.90 b	3.78 ± 0.06 c
T3	191.6 ± 3.25 c	145.9 ± 3.59 c	4.12 ± 0.05 b
T4	178.9 ± 2.90 d	132.4 ± 3.11 d	4.48 ± 0.06 a
T5	181.7 ± 3.14 d	135.8 ± 3.44 d	4.39 ± 0.07 a
Sig.	*	*	*

**Different letters within the same column indicate significant differences ($P \leq 0.05$).*

Effect of nano-synbiotic supplementation on blood biochemical parameters

Table 3 and Table 4 show that nano-synbiotic supplementation resulted in significant improvements ($P \leq 0.05$) in blood biochemical parameters compared with the control treatment.

Serum glucose and cholesterol levels were significantly reduced ($P \leq 0.05$) in all supplemented treatments relative to the control, with the greatest reduction observed in T4, followed by the other treatments according to supplementation level, indicating a dose-related response.

Total protein concentration was significantly increased ($P \leq 0.05$) in all supplemented groups compared with the control. This increase was accompanied by significant elevations ($P \leq 0.05$) in both albumin and globulin levels, with varying magnitudes among treatments. T4 recorded the highest values, followed by the remaining treatments according to inclusion level. Similarly, AST and ALT activities were significantly decreased ($P \leq 0.05$) in all supplemented treatments compared with the control, with the most pronounced reduction observed in T4 and a graded response among the other treatments. Overall, all supplemented treatments demonstrated significant improvement in these parameters compared with the control, with T4 exhibiting the most marked response.

Table 4: *Effect of nano-synbiotic supplementation on albumin, globulin, AST, ALT (Mean ± SE)*

Treat-ments	Albumin (g/dL)	Globulin (g/dL)	AST (U/L)	ALT (U/L)
T1	1.62 ± 0.04 d	1.80 ± 0.05 d	286.5 ±6.41 a	52.8 ±1.65a
T2	1.78 ± 0.05 c	2.00 ± 0.04 c	262.7 ± 5.87 b	47.6 ± 1.57 b
T3	1.95 ± 0.04 b	2.17 ± 0.02 b	236.8 ± 5.25 c	42.9 ± 1.38 c
T4	2.16 ± 0.05 a	2.32 ± 0.05 a	209.6 ± 4.73 d	37.5 ± 1.26 d
T5	2.12 ± 0.05 a	2.27 ± 0.03 a	214.3 ± 4.90 d	38.9 ± 1.35 d
Sig.	*	*	*	*

**Different letters within the same column indicate significant differences ($P \leq 0.05$).*

Discussion of blood biochemical parameters

The significant modulation of blood biochemical parameters observed in Table 3 and Table 4 reflects a clear metabolic improvement associated with nano-synbiotic supplementation, particularly at the 3 g/kg inclusion level (T4). The reduction in serum glucose and cholesterol concentrations suggests improved metabolic regulation and enhanced nutrient utilization efficiency. Lower circulating glucose levels may indicate improved peripheral uptake and metabolic turnover, while reduced cholesterol concentrations reflect a favorable modulation of lipid metabolism, possibly mediated through microbial deconjugation of bile salts and altered lipid absorption dynamics.

The elevation in total protein, albumin, and globulin levels further supports the hypothesis of improved protein metabolism and enhanced physiological status. Increased albumin concentration is generally associated with better hepatic synthetic activity and improved nutrient assimilation, whereas elevated globulin levels may reflect enhanced immune responsiveness and systemic resilience (Al-Salhi and Al-Shatty, 2023). The superior response observed in T4 suggests that this inclusion level optimized the metabolic–immunological balance without inducing physiological stress.

In contrast, although T5 (4 g/kg) maintained significant improvements compared with the control, its response was not consistently superior to T4, reinforcing the concept of an optimal biological inclusion level. Beyond this threshold, additional supplementation may not proportionally enhance metabolic efficiency and could approach a regulatory equilibrium state.

The significant reductions in AST and ALT activities observed in supplemented treatments indicate improved hepatic integrity and reduced metabolic strain. Lower transaminase activity is generally interpreted as a marker of enhanced liver health and reduced cellular leakage, which aligns with improved nutrient processing capacity. The pronounced reduction in these enzymes in T4 further confirms that this dosage supported metabolic homeostasis more effectively than the higher inclusion level.

These findings are consistent with previous studies. Dev *et al.* (2020) reported that synbiotic supplementation improves serum biochemical profiles and enhances antioxidant status in broilers. Abdel Hafeez *et al.* (2017) demonstrated that optimal synbiotic levels elevate protein fractions while reducing stress-related biochemical markers. Similarly, Khalil *et al.* (2021) observed significant reductions in cholesterol levels and improvements in protein metabolism following synbiotic supplementation, which they linked to enhanced growth performance.

The observed biochemical improvements are physiologically interconnected with the enhanced productive performance reported in Table 2. Improved protein synthesis capacity, better lipid regulation, and reduced hepatic stress collectively contribute to more efficient nutrient partitioning toward muscle accretion. Therefore, the metabolic optimization reflected in Table 3 and Table 4 provides mechanistic support for the superior growth performance and feed efficiency observed at the 3 g/kg inclusion level. In summary, nano-synbiotic supplementation positively modulated metabolic and hepatic indicators, with the 3 g/kg level achieving the most balanced and pronounced physiological response. These biochemical adaptations likely played a central role in supporting the improved productive performance and economic efficiency recorded in this study.

Effect of nano-synbiotic supplementation on cecal microbial population

As illustrated in Figure 3, nano-synbiotic supplementation significantly affected cecal microbial counts (P≤0.05).

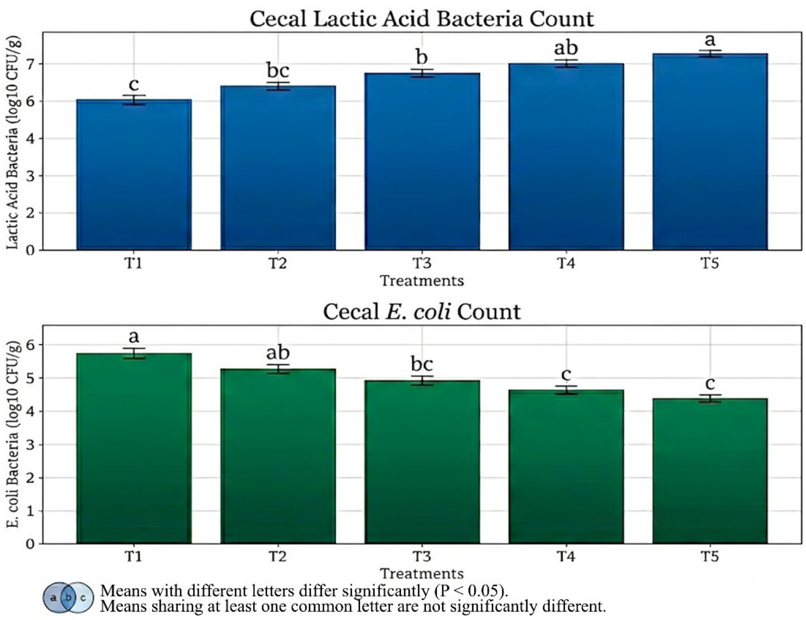

Figure 3: *Effect of nano-synbiotic supplementation on cecal lactic acid bacteria and Escherichia coli.*

Lactic acid bacteria populations were significantly increased (P≤0.05) in all supplemented treatments compared with the control, with the highest count observed in T4, followed by the other treatments according to supplementation level, indicating a graded response. Conversely, Escherichia coli counts were significantly reduced ($P \leq 0.05$) in all experimental treatments relative to the control. The greatest reduction was recorded in T4, with intermediate values observed in the remaining supplemented groups. Overall, all supplemented treatments demonstrated a significant improvement in intestinal microbial balance compared with the control, with T4 showing the most pronounced effect.

Discussion of cecal microbial population

The modulation of cecal microbial populations observed in the present study reflects a substantive restructuring of the intestinal ecosystem induced by nano-synbiotic supplementation. The marked increase in lactic acid bacteria (LAB) concurrent with the reduction in Escherichia coli indicates a shift toward a more stable and functionally advantageous microbial environment.

These changes should be interpreted within the ecological framework of competitive exclusion and microbial homeostasis rather than as isolated fluctuations. Enrichment of LAB populations enhances short-chain fatty acid production, reduces luminal pH, and strengthens mucosal barrier integrity. Collectively, these effects create conditions unfavorable for opportunistic pathogens, thereby limiting their proliferation. The graded response across supplementation levels suggests dose-dependent microbial modulation; however, the 3 g/kg inclusion level achieved the most balanced configuration, maximizing beneficial bacteria without evidence of ecological saturation.

The comparatively moderated response at 4 g/kg may indicate that once microbial dominance of beneficial taxa is established, further substrate availability does not proportionally expand populations. Instead, the intestinal ecosystem approaches a dynamic equilibrium constrained by microbial carrying capacity and host–microbe regulatory interactions, which are shaped by nutrient supply, immune surveillance, and mucosal architecture.

Previous investigations support these interpretations. Mookiah *et al.* (2014) demonstrated that synbiotic supplementation enhances LAB populations while suppressing coliform bacteria via competitive exclusion. Wang *et al.* (2023) reported that host-adapted lactic acid bacteria improve microbial balance and growth performance by reinforcing ecosystem stability and nutrient metabolism. Likewise, Younis *et al.* (2024) associated increased LAB counts with improved intestinal histomorphology and feed efficiency, underscoring the functional integration between microbial ecology and intestinal structure.

The microbial shifts documented herein provide a plausible mechanistic basis for the enhanced growth performance and biochemical stability observed in supplemented groups. A stabilized microbiota can attenuate inflammatory load, reduce nutrient competition between host and pathogens, and improve digestive efficiency, thereby reallocating metabolic resources toward productive growth.

Table 5: *Effect of nano-synbiotic supplementation on ileal histomorphology of broiler chickens at 35 days (Mean ± SE)*

Treatment	Villus height (µm)	Crypt depth (µm)	VH/CD Ratio
T1	962.4 ± 18.6 d	198.3 ± 4.73 a	4.85 ± 0.11 e
T2	1048.7 ± 20.2 c	186.1 ± 4.22 b	5.63 ± 0.13 d
T3	1152.6 ± 19.5 b	171.4 ± 3.81 c	6.72 ± 0.15 c
T4	1328.9 ± 22.3 a	148.6 ± 3.10 e	8.94 ± 0.19 a
T5	1241.5 ± 21.7 ab	160.3 ± 3.40 d	7.74 ± 0.17 b
Sig.	*	*	*

**Different letters within the same column indicate significant differences (P ≤ 0.05).*

Overall, nano-synbiotic supplementation effectively redirected the cecal microbiota toward a more resilient and functionally efficient state, with 3 g/kg representing the optimal inclusion level. From a production standpoint, sustaining such microbial benefits requires integration of nutritional strategies with effective environmental management. Elevated environmental microbial pressure may disrupt colonization dynamics and reduce probiotic efficacy. Accordingly, hygienic rearing conditions play a supportive role in maintaining microbial resilience. Recent evidence indicates that eco-friendly detergents derived from natural organic waste can significantly reduce pathogenic load within poultry facilities (Al-Salhi *et al*., 2025; Nasser *et al*., 2025), thereby complementing dietary synbiotic interventions and contributing to sustained intestinal microbial stability.

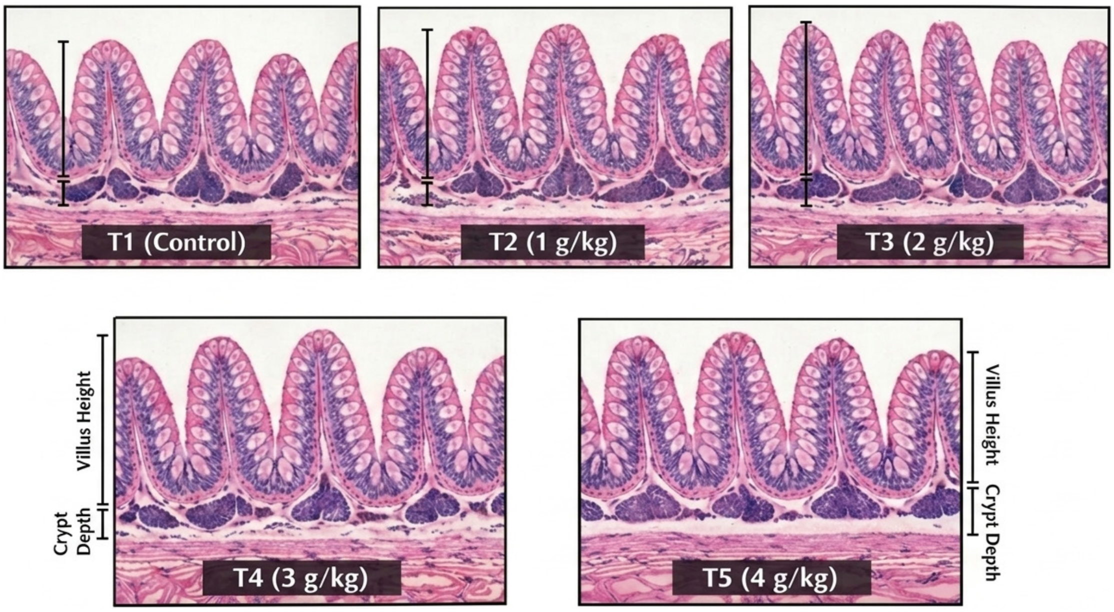

Figure 4: *Histological sections of the ileum (H&E staining) of broiler chickens at 35days*

Effect of nano-synbiotic supplementation on ileal histomorphology

Table 5 and Figure 4 demonstrate that nano-synbiotic supplementation significantly improved ileal histomorphological parameters at 35 days of age compared with the control treatment (P≤0.05).All experimental treatments showed a significant increase (P≤0.05) in villus height relative to the control, with significant differences also observed among the supplemented treatments. Villus height increased progressively according to supplementation level, with T4 exhibiting the most pronounced response. Similarly, crypt depth was significantly reduced (P≤0.05) in all supplemented groups compared with the control, with a graded response among treatments and a clear superiority of T4 over the remaining groups. Consequently, the villus height to crypt depth ratio was significantly improved (P≤0.05) in all supplemented treatments compared with the control, with T4 recording the highest value. The histological sections presented in Figure 4 are consistent with the quantitative findings shown in Table 5, revealing more developed and organized villi in the supplemented treatments relative to the control.

Discussion of Ileal histomorphology

The significant enhancement in ileal histomorphological parameters observed in Table 5 and illustrated in Figure 4 provides structural evidence supporting the functional improvements recorded in growth performance and metabolic indices. The marked increase in villus height in supplemented treatments, particularly at the 3 g/kg inclusion level, indicates an expansion of the absorptive surface area of the small intestine. Taller villi are directly associated with increased epithelial surface exposure, greater expression of digestive enzymes, and enhanced nutrient transport capacity. Consequently, this structural adaptation facilitates more efficient assimilation of amino acids, glucose, and lipids, which likely contributed to the superior body weight gain and feed conversion efficiency observed in the same treatment.

Simultaneously, the significant reduction in crypt depth in supplemented groups reflects decreased epithelial turnover demand and reduced intestinal stress. Crypts are regions of active cellular proliferation, and excessive crypt depth is often associated with inflammatory stimulation or compensatory hyperplasia due to mucosal damage. Therefore, the shallower crypts recorded in the supplemented treatments suggest improved mucosal stability and lower epithelial regeneration pressure. This indicates that the nano-synbiotic supported intestinal integrity, reducing the need for accelerated cell replacement and allowing more energy to be directed toward productive growth rather than tissue repair (Al-Salhi, 2026).

The villus height to crypt depth (VH/CD) ratio, considered one of the most reliable indicators of intestinal functional efficiency, was markedly improved, particularly in the 3 g/kg group. An elevated VH/CD ratio reflects a favorable balance between absorptive capacity and cellular turnover, signifying optimal digestive functionality. The pronounced superiority of the fourth treatment suggests that this inclusion level achieved the most effective structural adaptation of the intestinal mucosa.

Although the 4 g/kg treatment maintained significant improvement compared with the control, its histomorphological response was comparatively lower than that of the 3 g/kg group. This pattern mirrors the productive and biochemical findings and reinforces the concept of an optimal inclusion level. Once structural development reaches a functional plateau, further increases in supplementation may not proportionally enhance villus elongation and could reflect a biological ceiling effect governed by genetic growth potential and intestinal architectural limits.

These findings are consistent with previous studies. Uni *et al*. (1998) demonstrated that villus development is closely linked to post-hatch functional maturation and nutrient absorption efficiency. Mookiah *et al*. (2014) reported that synbiotic supplementation increases villus height and improves intestinal morphology, contributing to better feed efficiency. Similarly, Younis *et al*. (2024) observed that improvements in VH/CD ratio were directly associated with enhanced growth performance in broilers receiving synbiotic supplementation.

Importantly, the structural improvements documented in the present study provide a mechanistic bridge linking microbial modulation, metabolic stabilization, and productive enhancement. Enhanced beneficial microbial populations likely reduced mucosal inflammation and supported epithelial integrity, which in turn promoted villus development and optimized nutrient uptake. This integrated gut structural adaptation explains the concurrent improvements in feed conversion ratio, serum protein fractions, and economic efficiency observed at the 3 g/kg inclusion level.

In summary, nano-synbiotic supplementation induced significant structural remodeling of the ileal mucosa, characterized by increased villus height, reduced crypt depth, and improved VH/CD ratio. The 3 g/kg inclusion level achieved the most pronounced and functionally advantageous intestinal architecture, supporting the superior productive and physiological outcomes recorded in this study.

Conclusions and Recommendations

Nano-synbiotic supplementation positively influenced growth performance, physiological status, intestinal microbial balance, and ileal histomorphology in broiler chickens. All supplemented treatments outperformed the control group in productive traits, with significant improvements (P≤0.05) in body weight gain and feed conversion ratio without affecting feed intake. Economic efficiency was correspondingly enhanced, particularly in T4. Supplemented birds exhibited improved blood biochemical profiles, including increased protein fractions and reduced liver enzyme activities, alongside a significant increase in lactic acid bacteria and reduction in Escherichia coli populations (P≤0.05). Overall, the 3 g/kg inclusion level (T4) demonstrated the most pronounced and consistent beneficial effects under the conditions of this study.

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Novelty Statement

This study is among the first to evaluate the effects of host-derived nano-biological synbiotic on growth performance, physiological traits, and gut microbial balance in broiler chickens under practical rearing conditions. It provides an integrated assessment linking productive performance with biochemical parameters and intestinal histomorphology. Furthermore, it identifies the optimal supplementation level (3 g/kg) for achieving superior productive and economic efficiency.

Author's Contribution

Teeb Adel AlMaamouri: Study design, supervision, and manuscript writing
Hiba Sh. Hussein: Experimental work and data collection
Teeb A. Altabtabaai: Methodology and statistical analysis
Saif Sh. Kamel: Scientific review and final editing.

Generative AI and AI-assisted technology statement
The authors declare that no generative AI and AI

assisted technology was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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