Research Article

Development of Nano-Taraxacum Officinale Synbiotic and its Effects on Gut Health and Blood Physiological Indices in Laying Hens

Rasool H. Khalati[1]*, Alhusnah A. Mansoor[2] and Arshed H. Alhafadhi[3]

[1]Department of Animal Production, College of Agriculture, University of Wasit, Iraq; [2]Department of Soil and Water, Marshes Researches Center, University of Thi-Qar,Iraq; [3]Department of Medical Laboratories, College of Health and Medical Technologies, Al-Ayen University, Thi-Qar, Iraq.

Abstract | This study evaluated a host-derived nano-synbiotic formulation based on probiotic strains isolated from laying hens and incorporated into a plant-derived nano-carrier as a dietary strategy to improve gut health and physiological status. A total of 168 laying hens (40 weeks of age) were assigned to seven dietary treatments for 8 weeks, with three replicates per treatment and eight hens per replicate. The dietary treatments included a basal diet (T1), nano-*Taraxacum officinale* at 1, 2, or 3 g/kg diet (T2–T4), and nano-synbiotic supplementation at the same inclusion levels (T5–T7). At 48 weeks of age, jejunal samples were collected for microbiological and histomorphological analyses, and blood samples were obtained for physiological evaluation. Compared with the control and nano-carrier-only treatments, nano-synbiotic supplementation improved intestinal microbial balance, characterized by increased lactic acid bacteria counts, reduced coliform populations, and a higher lactic acid bacteria to coliform ratio. Intestinal morphology was also enhanced, as evidenced by increased villus height and villus height to crypt depth ratio. Favorable physiological responses were observed, including increased serum protein fractions and calcium concentration, reduced serum glucose and cholesterol levels, stable liver enzyme activities (AST and ALT), and lower heterophil to lymphocyte ratios. Among all dietary treatments, nano-synbiotic supplementation at 2 g/kg diet (T6) consistently produced the most pronounced improvements across microbiological, histological, and physiological parameters. In addition, probiotic strains exhibited high viability after nano-loading and remained stable during a 56-day storage period under field conditions. These findings indicate that the host-derived nano-synbiotic formulation represents an effective and safe antibiotic-free dietary strategy for enhancing gut functionality and physiological efficiency in laying hens.

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***Correspondence** |Rasool Hassan Khalati, Department of Animal Production, College of Agriculture, University of Wasit, Iraq; **Email:** rahassan@uowasit.edu.iq
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Introduction

The laying hen industry faces significant challenges in enhancing gastrointestinal health and improving production performance, particularly in light of increasing restrictions on the use of antibiotics as growth promoters. Recent scientific evidence has demonstrated that maintaining a balanced

gut microbiota is crucial for optimizing nutrient utilization, supporting immunity, and reducing oxidative stress in poultry, all of which positively influence egg production and quality. In this context, synbiotics and postbiotics have emerged as promising alternatives capable of achieving a functional synergy between probiotics and prebiotics, enhancing the survival of beneficial bacteria and stimulating physiological activity within the gastrointestinal tract (Abd El-Hack *et al*., 2020; Al-Salhi, 2026).

Applied studies have shown that supplementing poultry diets with synbiotics can improve production performance by increasing nutrient utilization efficiency and enhancing gut microbial balance, which is positively reflected in various production parameters compared to control groups. Recent research has indicated that the combined use of probiotics and prebiotics contributes to improved production performance, digestion, and feed conversion efficiency, in addition to supporting overall gut health (Dev *et al*., 2020). Other reviews have reported that the integration of probiotics and synbiotics enhances overall performance and reduces production losses associated with gastrointestinal disorders (Alagawany *et al*., 2021), while additional studies have demonstrated that this biological synergy improves local immunity and reduces the incidence of intestinal disturbances, thereby contributing to production stability throughout extended laying cycles (Saeed *et al*., 2023). In the same context, Abd El Latif and Omar (2023) reported that the inclusion of prebiotics, probiotics, and synbiotics significantly improved production performance, digestibility, and gut microbial balance, confirming the pivotal role of these additives in enhancing physiological efficiency and productivity in poultry. Despite these advances, there remains a need to develop functional feeding strategies that integrate the efficacy of host-specific probiotics with natural prebiotic sources, thereby overcoming the stability limitations of conventional probiotics within the gastrointestinal tract under challenging environmental conditions.

Accordingly, recent research has focused on the use of plant-based sources rich in inulin and their nanoscale delivery to maximize functional benefits, as studies have indicated that nanotechnology enhances the stability of bioactive compounds and improves the delivery of probiotics and prebiotics within the digestive tract, thereby supporting their functional effects in the gut (Dangi *et al*., 2023). In this context, the present study introduces an innovative approach based on nanosized *Taraxacum officinale* root powder loaded with *Lactobacillus* strains isolated from the laying hens themselves, aiming to evaluate the effects of this nanosynbiotic on gut health and Blood Physiological Indices , with high expectations for concurrent improvements in production parameters and egg quality.

Production performance parameters were not included in the present manuscript to maintain focus on gut health, microbial modulation, and physiological responses, and to avoid excessive manuscript length. Production-related outcomes will be reported separately in another article.

Materials and Methods

Source of bacterial strains

The lactic acid bacteria strains used in this study were obtained from bacterial isolates from the small intestine of healthy adult laying hens. These isolates were first registered globally with the National Center for Biotechnology Information (NCBI), where *Lactobacillus gasseri* strain Al-Salhi-1 was registered under number MW848596 and *Lactobacillus helveticus* strain Al-Salhi-2 under number MW848597. After isolating and identifying several lactic acid bacteria strains, these two strains were found to be the most abundant in the small intestine, with L. *helveticus* accounting for approximately 30% and L. *gasseri* for approximately 25% of the total isolated strains (Al-Salhi *et al*., 2022). Therefore, these two strains were selected for use in this study.

Activation of preserved bacterial strains

The bacterial strains preserved on de Man, Rogosa and Sharpe (MRS) agar slants were activated by transferring a number of bacterial colonies into sterile MRS broth medium, followed by incubation under anaerobic conditions at 37°C for 48 hours, to ensure the restoration of bacterial vitality and attainment of the logarithmic phase of growth, as described by Da Silva *et al*. (2018).

Preparation of wild chicory root nanopowder

Wild chicory (Taraxacum officinale) plants were collected from roadsides and grounds of Thi-Qar University and taxonomically authenticated by specialists at the Department of Horticulture and

Landscape Design, College of Agriculture and Marshes, University of Thi-Qar. The plant material was washed with tap water followed by distilled water to remove impurities. Roots were separated from aerial parts due to their higher inulin content, cut into small pieces, and dried in an oven at 40–45 °C until constant weight. The dried roots were milled and sieved to obtain a uniform fine powder (Kumar *et al.*, 2025).

This powder was subsequently converted into wild chicory root nanopowder using an appropriate nanofabrication technique reported for plant-based biomaterials (Song *et al.*, 2019). After nanoparticle preparation, the nanopowder was sterilized using a method demonstrated to preserve its physicochemical properties without adversely affecting its suitability for subsequent probiotic loading or bacterial viability (Acioli de Siqueira *et al.*, 2025). The sterilized nanopowder was stored under sterile conditions until further use.

Loading of probiotic strains and lyophilization

Two strains of Lactobacillus gasseri and Lactobacillus helveticus were loaded as a combined mixture onto inulin-rich chicory root nanopowder by gradually mixing the active liquid bacterial cultures with the sterile nanopowder under gentle stirring. This process ensured homogeneous distribution and promoted cell adhesion to the surface of the plant nanoparticles, thereby achieving a synergistic effect between probiotics and prebiotics. This synergistic interaction enhanced bacterial survival and stability against environmental stress within the carrier matrix (Laina *et al.*, 2025). A 1:5 (v/w) loading ratio was selected based on preliminary experiments, as it provided the highest post-treatment viability and activity of bacterial cells, indicating efficient utilization of inulin from the plant carrier to support probiotics.

After loading, the mixture was allowed to stand for an appropriate period to enhance bacterial adhesion, followed by freezing at −20°C for at least 12 hours. Lyophilization was then performed according to established protocols to preserve probiotic viability and minimize cell loss during processing, in line with best practices for probiotic fixation and delivery in dried formulations. This freeze-drying method effectively maintains the viability and stability of the dried bacterial cells during storage at 4°C prior to animal testing. Recent studies have highlighted the importance of selecting precise drying protocols to achieve the highest post-drying survival rates of probiotic cells (Bolla *et al.*, 2011).Figure 1 illustrates the main stages of preparing the Nano-Taraxacum officinale synbiotic, including loading of bacterial strains and lyophilization, and leading to the final product.

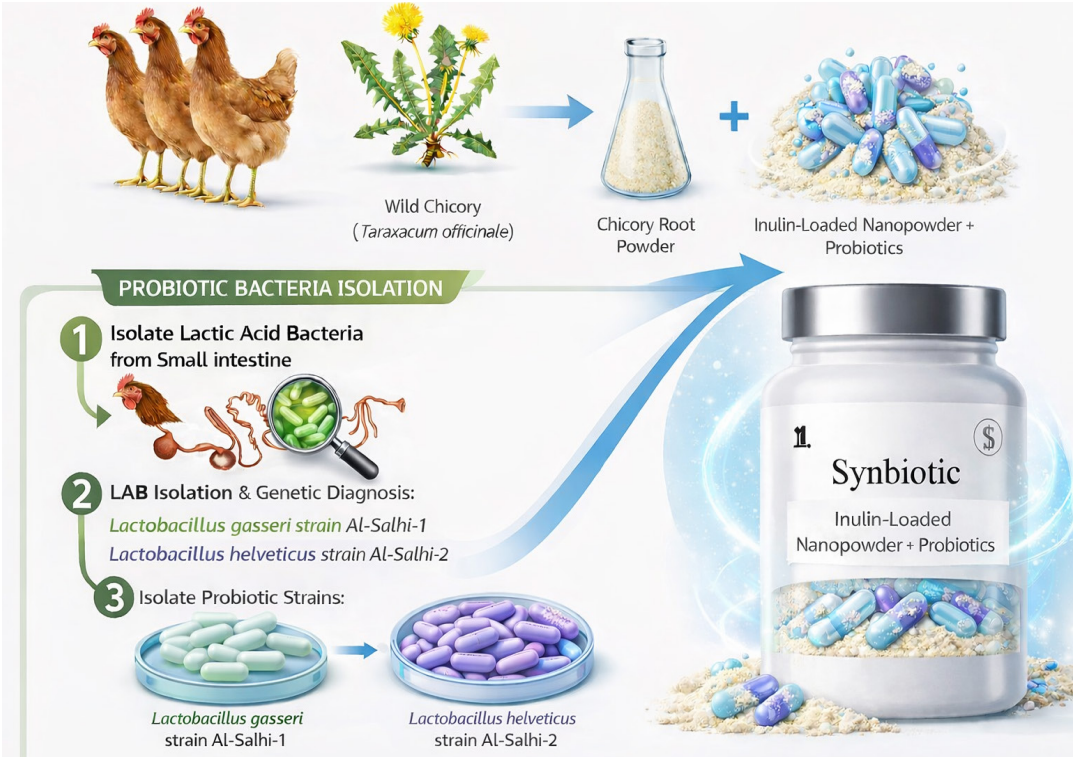

Figure 1: *Preparation of nano-taraxacum officinale synbiotic powder*

Flock management and experimental procedure

Laying hen management

The experiment was conducted on 40-week-old laying hens at a private farm in Thi Qar Governorate, southern Iraq, operating under a fully automated management system equipped with environmental sensors. A total of 168 laying hens were randomly allocated to seven dietary treatments, with three replicates per treatment and eight hens per replicate. Each replicate consisted of one cage (1 × 1 m^2 with a height of 60 cm) and was considered the experimental unit.

Table 1: *Ingredient and calculated chemical composition of the layer diet*

Ingredient	Inclusion (%)
Yellow maize (corn)	46.5
Wheat	18
Soybean meal (48% CP)	22
Vitamin–mineral premix (6%)	2
Limestone ($CaCO_3$)	9
Vegetable oil (sunflower)	1.2
Salt (NaCl)	0.3
Total	100
Calculated chemical composition	
Metabolizable Energy (kcal/kg)	2808
Crude Protein (%)	17.36
Energy : Protein Ratio	161.7
Crude Fiber (%)	3.72
Calcium (%)	4.08
Available phosphorus (%)	0.45
Lysine (%)	0.84
Methionine (%)	0.35
Methionine + Cystine (%)	0.67

The basal diet was offered at 130 g/hen/day, divided into two daily feedings at 06:00 a.m. and 04:00 p.m. using designated metal feeders for each replicate. The diet contained 17.36% crude protein and 2808 kcal/kg metabolizable energy (Table 1).The prepared Nano-*Taraxacum officinale* powder, either alone or as a synbiotic formulation with *Lactobacillus gasseri* and *Lactobacillus helveticus*, was incorporated into the basal diet at different inclusion levels, as detailed in Table 2.

Fresh drinking water was continuously supplied via nipple drinkers. The experiment lasted 8 weeks, during which environmental conditions, including temperature, ventilation, and lighting, were maintained according to Lohmann Brown management guidelines to ensure stable and suitable conditions for egg production (Lohmann Tierzucht GmbH, 2020).

The premix manufactured by the Iraqi Laymix Company, Erbil Governorate, contained 6% crude protein and 4331.57 kcal/kg of metabolizable energy. It also provided 1.50% lysine, 5.90% methionine, 5.00% methionine and cysteine, 24.05% calcium, 10.20% available phosphorus, and 0.85% threonine.

Chemical analysis was conducted according to NRC (1994) recommendations.

Table 2: *Dietary treatments applied to evaluate gut health and physiological responses of laying hens*

Treatment	Abbreviation	Composition
T1	C	1 kg diet only (Control)
T2	NTO1	1 kg diet + 1 g Nano-*T. officinale* powder
T3	NTO2	1 kg diet + 2 g Nano-*T. officinale* powder
T4	NTO3	1 kg diet + 3 g Nano-*T. officinale* powder
T5	NSY1	1 kg diet + 1 g Nano-synbiotic
T6	NSY2	1 kg diet + 2 g Nano-synbiotic
T7	NSY3	1 kg diet + 3 g Nano-synbiotic

**NTO : Nano-Taraxacum officinale .*
** NSY : Nano-synbiotic (Nano-T. officinale + Lactobacillus gasseri + Lactobacillus helveticus)*

Blood sampling and biochemical analyses

Blood samples were collected from the wing vein of laying hens every two weeks throughout the 8-week experimental period. Each sample was divided into two portions: one containing anticoagulant for hematological analysis, including packed cell volume (PCV) and heterophil-to-lymphocyte (H/L) ratio, and the other without anticoagulant for serum separation. Serum was obtained by centrifugation and stored at 4°C until biochemical analyses were performed. Biochemical tests included Total Protein, Albumin, Globulin, Calcium, Glucose, Cholesterol, and liver enzyme activities (AST and ALT), all samples were collected and analyzed following the procedures described by Al-Salhi and Al-Shatty (2023); Al-Salhi (2025).

Microbiological analysis

At 48 weeks of age, two laying hens were randomly selected from each treatment and humanely slaughtered in accordance with accepted animal

welfare and ethical guidelines. Immediately after slaughter, jejunal contents were aseptically collected for microbiological analysis. One gram of each sample was homogenized in sterile physiological saline, and serial tenfold dilutions were prepared.

Lactic acid bacteria were enumerated on de Man, Rogosa and Sharpe (MRS) agar following anaerobic incubation at 37°C for 48 h, while coliform bacteria were counted on MacConkey agar after aerobic incubation at 37°C for 24 h. Microbial counts were expressed as $\log_{10}$ CFU/g of jejunal content. The LAB/Coliform ratio was calculated based on $\log_{10}$ values as an indicator of intestinal microbial balance, according to Da Silva *et al.* (2018).

Microbial viability before and after nano-loading and storage stability

The viability of Lactobacillus gasseri and Lactobacillus helveticus was determined before and after loading onto the nano-synbiotic carrier. Bacterial counts were assessed by serial dilution and plate count methods, and results were expressed as CFU/g. To evaluate the storage stability of the nano-synbiotic under practical conditions, three sealed packages of the final product were placed at different locations within a commercial poultry house. Viable counts were measured at the initial time (day 0) and subsequently at 14-day intervals up to 56 days. At each sampling point, representative samples were collected aseptically, and microbial viability was determined using standard microbiological procedures. Data were expressed as $\log_{10}$ CFU/g (Mean ± SE), and differences among storage periods were analyzed statistically to assess potential changes in bacterial viability over time.

Intestinal histomorphology

At 48 weeks of age, two laying hens from each treatment were humanely slaughtered following accepted animal welfare guidelines. Ileal samples were collected from the same anatomical location in all birds to ensure sample uniformity. Approximately 2cm segments were excised, gently flushed with saline, and fixed in 10% neutral buffered formalin for histological evaluation.

Fixed samples were processed using standard histological procedures, sectioned, and stained with hematoxylin and eosin. Villus height and crypt depth were measured under light microscopy, and the villus height to crypt depth ratio was calculated as an indicator of intestinal absorptive capacity, according to Uni *et al.* (1998).

Statistical analysis

All data were analyzed using SPSS (2018). One-way analysis of variance (ANOVA) was performed to evaluate differences among treatment means, and significant differences were separated using Duncan's Multiple Range Test ($p < 0.05$).

Results and Discussion

Effect of nano-carrier loading on probiotic cell viability

Data presented in Table 3 indicate that loading Lactobacillus gasseri and Lactobacillus helveticus onto the nano-carrier derived from Taraxacum officinale roots did not result in a significant reduction in bacterial viability. High viable cell counts were maintained after nano-loading, exceeding 10^8 CFU/g for each individual strain, while the combined preparation reached approximately 4.8×10^9 CFU/g. Moreover, the logarithmic values of viable counts after nano-loading demonstrated remarkable stability, reflecting the efficiency of the nano-carrier in preserving probiotic cell viability during the loading process.

Table 3: *Viability of probiotic strains before and after nano-loading (Mean±SE)*

Strain	Before loading (CFU/g)	After loading with nano-carrier (CFU/g)	$\log_{10}$ CFU/g
Lactobacillus gasseri	2.5×10^8	2.3×10^8	8.36±0.02
Lactobacillus helveticus	3.0×10^8	2.8×10^8	8.45±0.04
L. gasseri + *L. helveticus*	5.0×10^9	4.8×10^9	9.68±0.03

Effect of storage duration on nano-synbiotic viability

Table 4 summarizes the effect of storage duration (0–56 days) on the viability of the developed nano-synbiotic. The results demonstrate that viable counts remained consistently high throughout the entire storage period, with only minimal numerical variations observed over time. No significant differences were detected in either total viable counts or logarithmic values among storage intervals, indicating that probiotic populations were effectively preserved during storage under practical field conditions.

The preserved probiotic viability observed immediately

after nano-loading (Table 3) and throughout the extended storage period (Table 4) is consistent with previous reports demonstrating the stabilizing role of nano-enabled synbiotic systems (Dangi *et al*., 2023; Laina *et al*., 2025). This dual stability highlights the functional efficiency of the developed plant-based nano-synbiotic system. It can be primarily attributed to the inulin-rich nano-carrier derived from Taraxacum officinale, which provides both nutritional support and physical protection to probiotic cells. The prebiotic matrix likely sustained basal metabolic activity, while the nano-scale structure reduced cellular exposure to adverse environmental factors such as oxygen, humidity, and temperature fluctuations. In addition, the strong adhesion of bacterial cells to the nanoparticle surface may have contributed to minimizing structural damage during processing and storage. Collectively, these mechanisms support the enhanced probiotic robustness and shelf stability observed in the present study, in agreement with recent nano-synbiotic research (Dangi *et al*., 2023; Laina *et al*., 2025).

Table 4: *Effect of storage duration (0–56 days) on nano-synbiotic viability (Mean±SE)*

Storage period (days)	Viable count (CFU/g)	log_{10} CFU/g
0 (Initial)	4.8×10^9	9.68 ± 0.03
14	4.7×10^9	9.67 ± 0.05
28	4.6×10^9	9.66 ± 0.03
42	4.6×10^9	9.66 ± 0.05
56	4.5×10^9	9.65 ± 0.06
Significance	N.S	N.S

N.S: No significant differences between treatment means.

Effect of nano-synbiotic supplementation on intestinal microbial balance

The data presented in Table 5 indicate that supplementation with Nano-Taraxacum officinale powder alone or within the nano-synbiotic formulation led to a progressive and significant improvement in the intestinal microbial balance of laying hen gut microbiota compared with the control group. Treatments supplemented with the nano-carrier alone (T2–T4) showed a marked increase in lactic acid bacteria (LAB) counts accompanied by a gradual reduction in coliform counts, resulting in an elevated LAB/Coliform ratio dependent on the level of inclusion. However, this improvement was more pronounced in the nano-synbiotic-supplemented treatments (T5–T7), which exhibited the highest significant LAB counts and the lowest coliform counts, particularly in T6 and T7, with T6 achieving the highest LAB/Coliform ratio overall.

Table 5: *Effect of Nano-Taraxacum officinale synbiotic on LAB, Coliform and LAB / Coliform ratio of laying hens (Mean±SE)*

Treatments	LAB (log_{10} CFU/g)	Coliform (log_{10} CFU/g)	LAB / Coliform Ratio
T1	6.20 ± 0.08 ^{d}	5.85 ± 0.07 ^{a}	1.06 ± 0.04 ^{d}
T2	6.55 ± 0.09^{cd}	5.60 ± 0.08 ^{ab}	1.17 ± 0.05 ^{cd}
T3	6.90 ± 0.07^{bc}	5.30 ± 0.06 b	1.30 ± 0.06 ^{bc}
T4	7.15 ± 0.06 ^{b}	5.05 ± 0.07 ^{bc}	1.42 ± 0.05 ^{b}
T5	7.40 ± 0.05 ^{b}	4.80 ± 0.06 ^{cd}	1.54 ± 0.04 ^{b}
T6	7.85 ± 0.04 ^{a}	**4.35 ± 0.05** ^{d}	**1.81 ± 0.03** ^{a}
T7	8.05 ± 0.05 ^{a}	4.45 ± 0.05 ^{cd}	1.81 ± 0.04 ^{a}
Significance	*	*	*

**Different letters in a column indicate significant differences (P ≤ 0.05).*

This improvement in intestinal microbial balance can be attributed to the effectiveness of the nano-synbiotic formulation in enhancing the survival and activity of probiotic strains within the gastrointestinal tract. The Taraxacum officinale root powder, rich in inulin, provides a selective prebiotic substrate that supports the proliferation of LAB while inhibiting the growth of pathogenic bacteria, consistent with the demonstrated role of prebiotics in promoting beneficial microbiota composition in poultry (Hashemitabar and Hosseinian, 2024). Additionally, nano-encapsulation of probiotics likely improved the delivery and viability of live bacterial cells through harsh gastric conditions, enhancing their ability to colonize and competitively exclude undesirable microbes, as described in recent poultry gut health studies (Naeem and Bourassa, 2025; Idowu *et al*., 2025). The notable increase in the LAB/Coliform ratio serves as a functional indicator of improved gut health and microbial stability, which is expected to positively affect nutrient digestion and absorption, thereby providing a physiological basis for the subsequent improvements observed in intestinal histomorphology and hematological parameters, particularly at the inclusion level of 2 g/kg feed (T6).

The reduction in coliform counts observed in the present study may also be partially explained by improved microbial control and hygienic status within the intestinal environment. It is well established that

natural bioactive compounds with antimicrobial properties can contribute to limiting the proliferation of pathogenic bacteria. Previous studies have demonstrated that biologically derived antibacterial agents and natural disinfectants can effectively reduce microbial load and improve sanitary conditions in poultry-related environments (Al-Salhi *et al*., 2025; Naser *et al*., 2025). Such effects may indirectly support the establishment of beneficial microbiota and enhance gut microbial balance.

Effect of nano-synbiotic supplementation on ileal histo-morphology of laying hens

The results presented in Table 6 demonstrate that supplementation with Nano-Taraxacum officinale, particularly when administered in the nano-synbiotic form, induced a significant improvement in the histomorphological characteristics of the ileum compared with the control treatment. A progressive and significant increase in villus height was observed with increasing inclusion levels, whereas no significant differences were detected in crypt depth among the experimental treatments. Consequently, a marked increase in the villus height-to-crypt depth ratio was recorded, especially in treatments T6 and T7, with T6 exhibiting the highest value for this parameter.

Table 6: *Effect of synbiotic on ileal villus height, crypt depth, and villus height to crypt depth ratio of laying hens at 48 weeks of age (Mean ± SE)*

Treat-ments	Villus height (µm)	Crypt depth (µm)	Villus height /crypt depth
T1	860.38 ± 25.11 ^{d}	195.23 ± 8.22	4.41 ± 0.18 ^{d}
T2	950 .06± 28.29^{cd}	200.65 ± 7.25	4.75 ± 0.17 ^{cd}
T3	1035.87 ±30.37^{bc}	205.40 ± 7.14	5.05 ± 0.20 ^{bc}
T4	1120.65 ± 28.95^{b}	210.27 ± 6.33	5.33 ± 0.19 ^{b}
T5	1210.32 ± 26.55^{b}	215.11 ± 6.17	5.63 ± 0.18 ^{b}
T6	1340.44 ± 24.88^{a}	220.35 ± 5.68	6.09 ± 0.16 ^{a}
T7	1300.64 ± 27.26^{a}	222.41 ± 6.21	5.86 ± 0.17 ^{ab}
Signifi-cance	*	N. S	*

**Different letters in a column indicate significant differences (P≤0.05). N.S: No significant differences between treatment means.*

These improvements in intestinal histomorphology reflect a functional enhancement in the absorptive capacity of the intestinal mucosa, as increased villus height and a higher villus height-to-crypt depth ratio are widely recognized indicators of improved digestive efficiency, nutrient absorption, and epithelial cell turnover stability (Uni *et al*., 1998). This positive response can be attributed to the integrated action of the nano-synbiotic in promoting intestinal microbial balance, as evidenced by the results in Table 5, where increased lactic acid bacteria populations and reduced pathogenic bacteria likely contributed to reduced local inflammation and improved epithelial integrity. Similar relationships between synbiotic supplementation, microbial modulation, and enhanced intestinal morphology have been reported in laying hens and broilers (Dev *et al*., 2020; Alagawany *et al*., 2021).

In addition, the inulin content of *Taraxacum officinale* root powder serves as a selective energy source for beneficial microbiota, supporting the production of short-chain fatty acids that play a key role in stimulating villus development and maintaining crypt architecture. Moreover, nano-loading of probiotic strains enhances the delivery and persistence of viable bacterial cells at the site of action within the intestine, prolonging their biological activity and explaining the superior response observed in nano-synbiotic treatments compared with the nano-carrier alone. The pronounced improvement in intestinal histomorphology, particularly at the inclusion level of 2 g/kg diet (T6), indicates the achievement of an optimal balance between microbial modulation and tissue-level response, which is consistent with the subsequent improvements observed in hematological and physiological parameters reported in the following tables.

Table 7: *Effect of nano-taraxacum officinale synbiotic on PCV% and H/L of laying hens (Mean±SE)*

Treat-ments	PCV%		H/L	
	44 weeks	48 weeks	44 weeks	48 weeks
T1	27.00±1.00^{d}	28.10±0.9 ^{d}	0.46±0.04^{a}	0.44 ± 0.03 ^{a}
T2	27.30 ±0.8^{cd}	29.40±0.9^{cd}	0.45±0.03^{a}	0.43 ± 0.04 ^{a}
T3	28.50 ±0.7^{bc}	30.00±0.8^{bc}	0.42±0.04^{ab}	0.41 ± 0.03^{ab}
T4	30.10±1.00^{b}	31.5 ± 0.9 ^{b}	0.41±0.03^{ab}	0.40 ± 0.04^{ab}
T5	31.80 ±0.8 ^{b}	32.6 ± 0.7 ^{b}	0.39±0.03^{ab}	0.38 ± 0.03^{ab}
T6	33.0 ± 0.6 ^{a}	35.1 ± 0.5 ^{a}	0.35 ±0.02^{b}	0.34 ± 0.02 ^{b}
T7	32.4 ± 0.9 ^{a}	34.5 ± 0.9 ^{a}	0.36±0.03^{ab}	0.35 ± 0.02^{ab}
Signif-icance	*	*	*	*

**Different letters in a column indicate significant differences (P ≤ 0.05).*

Effect of nano-synbiotic supplementation on packed cell volume and heterophil-to-lymphocyte ratio

The results presented in Table 7 show that dietary

supplementation with Nano-*Taraxacum officinale* synbiotic significantly influenced packed cell volume (PCV%) and the heterophil-to-lymphocyte (H/L) ratio of laying hens at both 44 and 48 weeks of age. A progressive and significant increase in PCV% was observed with increasing supplementation levels, particularly in the nano-synbiotic treatments (T5–T7), with T6 exhibiting the highest PCV values at both sampling periods. In contrast, the H/L ratio showed a significant and consistent reduction in the nano-synbiotic–supplemented groups compared with the control, indicating a lower physiological stress response, with the most pronounced effect again recorded in T6.

The elevation in PCV% observed in nano-synbiotic–fed hens reflects an improvement in hematopoietic activity and overall physiological status, as PCV is widely recognized as a sensitive indicator of oxygen-carrying capacity and metabolic efficiency in poultry. Concurrently, the reduction in the H/L ratio suggests enhanced immune balance and reduced stress load, as this ratio is considered a reliable biomarker of chronic stress and immunological challenge in birds (Gross and Siegel, 1983). The observed improvements in both parameters can be mechanistically linked to the enhanced intestinal health and absorptive capacity demonstrated in Table 5 and Table 6, where improved microbial balance and intestinal morphology likely facilitated more efficient nutrient assimilation and reduced systemic inflammatory pressure.

Furthermore, the nano-synbiotic formulation may have contributed to improved immune homeostasis through sustained probiotic activity and prebiotic-mediated support of beneficial gut microbiota, thereby modulating the gut–immune axis. Enhanced availability of nutrients and bioactive metabolites, including short-chain fatty acids, can positively influence leukocyte differentiation and erythropoiesis, ultimately reflected in improved PCV values and a reduced H/L ratio. Similar reductions in H/L ratio and improvements in hematological indices following synbiotic supplementation have been reported in laying hens and broilers, confirming the role of synbiotics in mitigating stress and supporting immune competence (Al-Salhi and Al-Shatty, 2022; Saeed *et al*., 2023). The superior response observed at the inclusion level of 2 g/kg diet (T6) indicates that this level provides an optimal balance between physiological stimulation and systemic adaptation, aligning with the overall performance and health responses recorded throughout the study.

Table 8: *Effect of nano-taraxacum officinale synbiotic on total protein, albumin, globulin and calcium of laying hens (Mean±SE)*

Treatments	Total Protein		Albumin	
	44 weeks	48 weeks	44 weeks	48 weeks
T1	4.10 ±0.08^{c}	4.25 ±0.07^{c}	2.30 ±0.05^{c}	2.35 ±0.04^{c}
T2	4.28 ±0.09^{c}	4.40 ±0.08^{c}	2.40±0.04^{bc}	2.45±0.05^{bc}
T3	4.50±0.07^{bc}	4.65±0.09^{bc}	2.48±0.05^{bc}	2.55±0.04^{bc}
T4	4.62 ±0.08^{b}	4.78 ±0.07^{b}	2.55 ±0.04^{b}	2.62 ±0.05^{b}
T5	4.75 ±0.09^{b}	4.95 ±0.08^{b}	2.60 ±0.05^{b}	2.68 ±0.04^{b}
T6	5.05 ±0.07^{a}	5.28 ±0.06^{a}	2.75 ±0.04^{a}	2.85 ±0.03^{a}
T7	4.90±0.08^{ab}	5.10±0.07^{ab}	2.68±0.05^{ab}	2.78±0.04^{ab}
Significance	*	*	*	*
Treatments	Globulin		Calcium	
	44 weeks	48 weeks	44 weeks	48 weeks
T1	1.80 ±0.04^{c}	1.90 ±0.05^{c}	8.20 ±0.25^{c}	8.45 ±0.20^{c}
T2	1.88 ±0.05^{c}	1.95 ±0.04^{c}	8.50±0.20^{bc}	8.70±0.18^{bc}
T3	2.02±0.04^{bc}	2.10±0.05^{bc}	8.75 ±0.22^{b}	9.00 ±0.20^{b}
T4	2.07 ±0.05^{b}	2.16 ±0.04^{b}	8.80 ±0.20^{b}	9.05 ±0.22^{b}
T5	2.15 ±0.04^{b}	2.27 ±0.05^{b}	9.05 ±0.18^{b}	9.30 ±0.20^{b}
T6	2.30 ±0.03^{a}	2.43 ±0.04^{a}	9.45 ±0.15^{a}	9.75 ±0.18^{a}
T7	2.22±0.04^{ab}	2.35±0.05^{ab}	9.30 ±0.20^{a}	9.60 ±0.20^{a}
Significance	*	*	*	*

**Different letters in a column indicate significant differences (P ≤ 0.05).*

Effect of nano-synbiotic supplementation on serum protein fractions and calcium concentration

The results presented in Table 8 demonstrate that dietary supplementation with Nano-*Taraxacum officinale* synbiotic significantly affected serum protein fractions, including total protein, albumin, and globulin, as well as calcium concentration in laying hens. Nano-synbiotic–supplemented groups exhibited a significant and progressive increase in total protein levels compared with the control, with the highest values consistently observed in treatment T6. This increase was accompanied by a significant elevation in serum globulin concentration, whereas albumin levels showed only minor numerical changes and, in most cases, did not differ significantly among treatments.

The increase in total protein was therefore primarily driven by the rise in globulin concentration, which resulted in a moderate reduction in the albumin-to-

globulin (A/G) ratio in nano-synbiotic treatments. Elevated globulin levels are commonly associated with improved immune status and enhanced synthesis of immunologically active proteins, indicating a positive modulation of systemic immunity. These findings suggest that nano-synbiotic supplementation may enhance immune-related protein synthesis rather than merely altering hepatic albumin production. Similar increases in serum globulin concentration following synbiotic or probiotic supplementation have been reported in laying hens and broilers and are often linked to improved gut health and immune responsiveness (Alagawany *et al*., 2021; Saeed *et al*., 2023).

Serum calcium concentration was also significantly increased in nano-synbiotic–supplemented hens compared with the control group, with the most pronounced improvement recorded in treatment T6. This enhancement in calcium status is physiologically relevant in laying hens, reflecting improved mineral absorption and utilization. The observed increase may be attributed to improved intestinal morphology and microbial fermentation activity, as demonstrated in previous tables, which can enhance calcium solubility and uptake through the production of short-chain fatty acids and optimization of intestinal absorptive surface area. Comparable improvements in calcium metabolism associated with synbiotic supplementation have been documented in laying hens, highlighting the close interaction between gut health and mineral homeostasis (Świątkiewicz *et al*., 2014; Al-Sagan *et al*., 2020).

Overall, the coordinated improvement in serum globulin and calcium concentrations, together with the increase in total protein, indicates that nano-synbiotic supplementation particularly at an inclusion level of 2 g/kg diet (T6) supports both immune competence and mineral metabolism without disrupting protein homeostasis. These outcomes are consistent with the observed enhancements in intestinal morphology and hematological indicators reported in the preceding tables, reinforcing the integrative physiological benefits of the nano-synbiotic formulation.

Effect of Nano-synbiotic supplementation on serum glucose, cholesterol, and liver enzyme activities in laying hens

The results presented in Table 9 demonstrate that dietary supplementation with Nano-*Taraxacum officinale* synbiotic significantly affected serum glucose and cholesterol concentrations, as well as the activities of liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), in laying hens. Nano-synbiotic–supplemented groups exhibited a significant reduction in serum glucose and total cholesterol levels compared with the control treatment, with the most pronounced decreases consistently observed in treatment T6. These findings indicate an improvement in carbohydrate and lipid metabolic regulation associated with nano-synbiotic supplementation.

Table 9: *Effect of nano-taraxacum officinale synbiotic on glucose, cholesterol, AST and ALT of laying hens (Mean±SE)*

Treatments	Glucose (mg \100 ml)		Cholesterol (mg/100 ml)	
	44 weeks	48 weeks	44 weeks	48 weeks
T1	235 ± 13.00c	240 ±16.00^{c}	198 ±5.22^{a}	200 ±6.00^{a}
T2	242 ±12.00^{bc}	247±15.00^{bc}	192±6.32^{ab}	195±5.24^{ab}
T3	250 ±15.00^{bc}	255±16.00^{bc}	188±5.54^{b}	190 ±5.47^{b}
T4	253 ± 11.00^{b}	258 ±13.00^{b}	185 ±6.36^{b}	188 ±5.69^{b}
T5	257 ± 19.00^{b}	263 ±15.00^{b}	182±5.45^{bc}	185±5.87^{bc}
T6	265 ± 10.00^{a}	272 ±11.00^{a}	175 ±4.24^{c}	178 ±3.87^{c}
T7	263 ±16.00^{ab}	270 ±13.00^{a}	176 ±5.41^{c}	179 ±5.35^{c}
Significance	*	*	*	*
Treatments	AST (U/L)		ALT(U/L)	
	44 weeks	48 weeks	44 weeks	48 weeks
T1	145 ± 7.00	147 ± 6.00	18 ± 2.00	18 ± 2.00
T2	142 ± 6 .00	144 ± 5.00	17 ± 2.00	17 ± 2.00
T3	140 ± 5 .00	142 ± 5.00	17 ± 1.00	16 ± 1.00
T4	138 ± 5 .00	140 ± 4.00	16 ± 1.00	16 ± 1.00
T5	136 ± 5.00	138 ± 4.00	16 ± 1.00	15 ± 1.00
T6	134 ± 4 .00	136 ± 4.00	15 ± 1.00	15 ± 1.00
T7	135 ± 4.00	137 ± 4.00	15 ± 1.00	15 ± 1.00
Significance	N. S	N. S	N. S	N. S

**Different letters in a column indicate significant differences (P ≤ 0.05).*
N.S: No significant differences between treatment means.

The reduction in serum glucose concentration suggests enhanced glucose utilization and improved metabolic efficiency, which may be attributed to improved intestinal absorptive function and modulation of gut microbiota, as previously demonstrated in Tables 5 and Table 6. Probiotic and synbiotic supplementation has been reported to improve glucose homeostasis by enhancing insulin sensitivity and reducing intestinal

glucose absorption through microbial-mediated metabolic pathways. The concurrent decrease in serum cholesterol further supports the role of nano-synbiotics in regulating energy metabolism and hepatic lipid synthesis.

Serum activities of AST and ALT were not significantly elevated in nano-synbiotic–supplemented hens compared with the control group and, in some treatments, showed a numerical reduction, particularly in T6. The absence of enzyme elevation indicates that nano-synbiotic supplementation did not exert hepatotoxic effects and may contribute to maintaining hepatic cellular integrity. AST and ALT are widely used indicators of liver function in poultry, and their stability reflects normal hepatocellular membrane permeability and metabolic homeostasis.

The observed improvements in serum glucose and cholesterol concentrations, together with the maintenance of normal AST and ALT activities, suggest that nano-synbiotic supplementation supports metabolic health without inducing hepatic stress. The superior response observed at an inclusion level of 2 g/kg diet (T6) indicates that this level provides an optimal balance between metabolic modulation and physiological safety. These findings are consistent with the overall improvements observed in intestinal morphology, serum protein profile, mineral metabolism, and hematological parameters reported in the preceding tables, confirming the integrative physiological benefits of the nano-synbiotic formulation in laying hens.

Conclusions and Recommendations

This study demonstrates that supplementation with the host-derived nano-synbiotic at 2 g/kg diet (T6) provided the most favorable outcomes in laying hens by promoting a balanced intestinal microbiota, improving intestinal morphology, and supporting overall physiological efficiency. These improvements were reflected in enhanced gut functionality and metabolic status, as evidenced by improved serum glucose and cholesterol profiles without adverse effects on liver enzyme activities. In addition, the probiotic strains maintained high viability throughout the storage period under field conditions, supporting the practical applicability of the developed formulation. Accordingly, the nano-synbiotic represents an effective and safe antibiotic-free nutritional strategy for improving gut health, metabolic regulation, and physiological performance in laying hens.

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Novelty Statement

This study presents a novel antibiotic-free feeding strategy based on a host-derived nanocomposite combining probiotic strains extracted from laying hens with a plant-based nanocarrier. This work provides a robust and practical scientific approach that contributes to the advancement of poultry nutrition and gut health management, aligning with global efforts to reduce antibiotic use in laying hen production.

Author's Contribution

Rasool H. Khalati: Manuscript writing, data analysis, and overall supervision.
Alhusnah A. Mansoor: Study design and scientific review.
Arshed H. Alhafadhi: Sample collection and laboratory analyses.

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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