scientific reports

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Biological evaluation of chelated trace minerals with multi-strain probiotics and enzymes on production performance of broiler chickens

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Recent days, feed biotechnology is creating land mark differences among traditional and sustainable poultry formulations. Biotechnology is now enables us to incorporate useful additives in broilers ration like enzymes, probiotics and more absorbable mineral sources. Individual effect of each additive had been explored extensively but combined effect of chelated minerals, enzymes and probiotics was studied in this research for sustainable broiler production. The purpose of present study was to evaluate the effects of dietary supplementation of *Nutri biyo*® (Nb) on different growth and physiological parameters in broilers. A total of 240 day-old chicks were randomly divided into 4 treatments having 6 replicates each (10 birds per replicate). The treatments were T0 (basal diet), T1 (basal diet + 0.5 g Nb), T2 (basal diet + 1 g Nb) and T3 (basal diet + 1.5 g Nb). During starter, finisher and overall duration, growth performance was improved in group receiving the highest inclusion of Nb. Furthermore, total tract nutrient digestibility, immune organ index and meat quality (springiness, chewiness, adhesiveness, compression, shear force and hardness values,) were observed significant ($p < 0.05$) in birds fed with 1.5 g of Nb as compared to control group on both 21st and 35th day. Antibody titers, carcass characteristics (carcass, thigh, heart, gizzard, back and proventriculus percentages), ileal digestibility (crude protein, dry matter, and ether extract) and bone measurements (tibia length, weight, wall thickness, and structural indicators) were improved ($p < 0.05$) by T3 group. Hematological parameters such as hemoglobin, packed cell volume, lymphocyte and red blood cell counts revealed significant increases in birds fed 1.0 and 1.5 g/kg. Other hematological parameters, crude fiber (ileal) digestibility, breast, liver, wings and pancreas weights remained unchanged. These findings suggest Nutri Biyo® at 1.5 g dose level as an effective feed additive for improving overall broiler performance.

Keywords Feed biotechnology, Synergistic effect, Chelated minerals, Probiotics, Broiler, Nutri Biyo®

Feed biotechnology involves some bio-engineered advanced feed additives like different mineral sources, probiotics, enzymes, amino acids etc. to improve nutrient efficiency, growth and ultimately animal productivity. Broilers are a key source of affordable animal protein. By 2032 global meat output is projected to reach 382 million tons with poultry contributing around 20 million tons to this growth at an annual increase of 1.3%[1].

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To meet rising demand, poultry nutrition has increasingly shifted toward feed additives like trace minerals, probiotics and enzymes which support gut health, nutrient absorption and reduce environmental waste[2].

Trace minerals are critical for immunity, bone health and metabolism. While inorganic forms are poorly absorbed, chelated minerals (bound with amino/organic acids) show higher bioavailability and reduce environmental pollution[3,4]. Antibiotic growth promoters were traditionally used to boost poultry performance but their overuse has led to antibiotic-resistant bacteria prompting a shift to safer alternatives such as probiotics[5]. These live microbes enhance gut health, immunity and feed conversion by modulating the microbiota and suppressing pathogens[6]. Multi-strain formulations with species like *Lactobacillus* and *Enterococcus* outperform single strains and also improve meat quality[7]. Exogenous enzymes like xylanase and phytase help break down complex plant fibers (NSPs), reducing digesta viscosity and enhancing nutrient availability, growth and feed efficiency[8]. Phytase also improves phosphorus absorption supporting bone development and reducing mineral excretion.

Despite their individual benefits and several research trials, limited research exists on combining these additives. The *Nutri Biyo*® (Nb) is a novel formulation that integrates probiotics, enzymes and chelated minerals to boost gut health, immunity, nutrient digestibility and skeletal development. This study aims to evaluate the effects of Nb on growth performance, nutrient digestibility, hematology, immune functions, meat, carcass and bone characteristics in broilers.

Materials and methods

The experiment was conducted at the Raja Muhammad Akram Animal Nutrition Research Center, University of Agriculture, Faisalabad, Pakistan.

Dietary treatments

A total of 240-day-old Ross 308 broiler chickens were randomly allocated to four treatments with six replicates of 10 birds each. Treatments included: T0 (control), T1 (0.5 g Nb), T2 (1.0 g/kg Nb), and T3 (1.5 g/kg Nb). Birds were housed in individual pens, provided mash feed and water ad libitum, and reared for 35 days under uniform management and environmental conditions. Ingredient composition for the starter and finisher diets was consistent throughout the treatments and is given in Table 1 along with nutrient composition while composition of Nb is given in Table 2.

Broiler husbandry practices

Birds were procured from "Jadeed Hatchery Ferozwatwan, district Sheikhupura, Pakistan". Before chick placement, the shed was cleaned, disinfected, and fumigated using potassium permanganate and formaldehyde (15 g $KMnO_4$ + 30 ml formalin/m^3), followed by 24-hour sealing and ventilation. Feeders and drinkers were sanitized, and wood shavings were used as bedding at a depth of 2–3 inches. Biosecurity measures, including restricted access and disinfectant footbaths, were strictly followed. Birds were vaccinated against the viral diseases according to the vaccination schedule. Lighting and was provided, and the temperature were gradually reduced from 95 °F (35 °C) to 75 °F (24 °C) over five weeks.

Data collection

Growth performance

The body weight of chicks, feed offer and refusal were recorded after their arrival and at the end of every week from each replicate. The following formula was used to calculate FCR every week.

$$\mathrm{FCR} = \frac{\text{Feed intake in grams}}{\text{Weight gain in grams}}$$

Nutrient digestibility

Apparent nutrient digestibility of CP and EE was determined using indirect marker method. Celite® (a source of acid-insoluble ash) was added 1% to the experimental diets. An adaptation period of three days was given to birds. On the 35th day, birds were slaughtered and ileal digesta was sampled from the region between Meckel's diverticulum and around 2 cm anterior to the ileocecal junction while for total tract digestibility, fecal collection was undertaken for 24 h. Fecal and ileal digesta samples were collected in labeled zipper bags replicate-wise and stored at -10 ℃ till analyzed for acid-insoluble ash (AIA). Experimental diets were also analyzed for AIA and nutrient composition following AOAC[9] and the following relationship was used for determining coefficient of digestibility for each nutrient:

$$\text{Coefficient of digestibility } \% = 100 - \left[100 \times \frac{\text{Marker in feed (\%)}}{\text{Marker in feces (\%)}} \times \frac{\text{Nutrient in feces (\%)}}{\text{Nutrient in feed (\%)}}\right]$$

Immune response

Antibody titers against the ND virus were examined by the HI test on 35th day. For this purpose, two-fold serial dilutions of serum samples (0.025 ml) were prepared in normal saline in 96 wells plates. The ND virus antigens were added to every well of the plate in equal volume. The plate was left for 10 min at 25 ˚C and 0.05 ml of chicken red blood cells were added to each well. The plate was shaken and kept until an agglutination of blood cells emerged. The HI titers were presented as the $\log_2$ values were calculated. Antibody titers for infectious bronchitis (IB) were determined on day 35th by using commercial kits developed to detect the antibody titer of

Ingredients (%)	Starter basal diet	Finisher basal diet
Corn	51	51
Soybean meal	23	18
Canola meal	9	7
Rice broken	5.19	7.1
Rice polish	2.34	6.5
Corn gluten 60%	2.6	3.5
Fish meal	1	1
L-lysine	0.4	0.2
L-methionine	0.21	0.2
Limestone	1.44	1
Di-calcium phosphate	1.52	1.5
Oil	2.2	2
*Premix	0.1	1
Total	100	100
Nutrient composition (%)		
Protein	23	18
ME(Kcal/kg)	2950	3150
Crude fibre	3.37	4.37
Ether extract	5.11	5.11
Ash	2.57	2.97
Calcium	1.06	1.06
Available Phosphorus	0.45	0.45
Lysine	1.13	1.13
Methionine	0.52	0.52
Threonine	0.85	0.85
Linoleic acid	1.18	1.18

Table 1. Ingredient and nutrient composition of basal starter and finisher diets. *Vitamin-mineral premix contains the following: Vitamin A, 20,000 K/IU; Vitamin D_3, 5400 KIU; Vitamin E, 48,000 mg; Vitamin B_{12}, 20 mg; Niacin, 60,000 mg; Pantothenic acid, 20,000 mg; Folic acid, 1600 mg; Biotin, 200 mg, Iron, 10,000 mg; Zinc, 120,000 mg; Manganese, 140,000 mg; Copper, 12,000 mg; Iodine, 1800 mg; Cobalt, 400 mg; Selenium, 360 mg.

Minerals	
Amino acid-Fe chelate	250 mg/kg
Amino acid-Se chelate	5 mg/kg
Amino acid-Zn chelate	500 mg/kg
Amino acid-Mn chelate	500 mg/kg
Amino acid-Cu chelate	150 mg/kg
Enzymes	
Xylanase	10 U/kg
Cellulase	500 U/kg
Glucanase	1.5 U/kg
Lipase	1 U/kg
Mannanase	1 U/kg
Phytase	1 U/kg
Probiotics	
Bacillus subtilis	10×10^{10} CFU/kg
Lactobacillus farciminis	12×10^{10} CFU/kg
Lactobacillus acidophilus	10×10^{10} CFU/kg
Lactobacillus plantarum	3×10^{10} CFU/kg
Lactobacillus casei	3×10^{10} CFU/kg
Bacillus amyloliquefaciens	30×10^{10} CFU/kg

Table 2. Key ingredient composition of *Nutri Biyo*®.

the vaccine before and after vaccination. At the 21st and 35th day, two birds per replicate were selected randomly and slaughtered. After dissection, the immune organs such as the bursa, thymus and spleen were excised from the slaughtered birds. After carefully removing the fats and connective tissues, the organ weight was determined with the digital weighing balance. The immune organ index will be calculated as stated in Zhao et al.[10].

$$\text{Immune organ index } \% \ = \text{Weight of organ } (\text{g}) \,/\, (\text{Live weight befor slaughter } (\text{g}) \ \times \ 100$$

Hematology

At the 35th day, around 1 mL of blood was drawn from the brachial wing vein of two birds per replicate and immediately transferred to the ethylenediaminetetraacetic acid (EDTA) coated tubes to prevent clotting. Blood was stored in a cool box and processed within an hour. To determine the hematological parameters, WBCs and RBC count, Hb, MCV, MCHC, MCH and total leukocyte count were identified using a hemocytometer under a light microscope. Packed cell volume was determined by the hematocrit method by centrifugation.

Tibia morphometry and mineralization

Tibia bones were obtained from 2 birds per replicate on the 35th day. After boiling the bones for ten minutes, the muscles, fat and connective tissues were removed. Bone weight was calculated with the help of a digital weighing machine. With the help of a vernier caliper, the tibia length, diaphysis diameter, lateral wall thickness and medial wall thickness were calculated. The robusticity index[11], medullary canal diameter[12], Bone weight/length index[13] and Tibiotarsal index[14] were calculated as follows,

$$\text{Robusticity index } \ = \text{bone length/cube root of bone weight}$$

$$\text{Medullary canal diameter} = \text{Diaphysis diameter } - \ (\text{Thickness of medial wall } + \ \text{lateral wall})$$

$$\text{Bone weight/length index } \ = \text{Tibia weight } / \text{ Tibia length}$$

$$\text{Tibiotarsal index} = [(\text{ Diaphysis diameter } - \ \text{Medullary canal diameter}) \,/\, \text{Diaphysis diameter}] \ \times \ 100$$

Meat tenderness

At the 21st and 35th day, two birds per replicate were selected randomly for measuring meat tenderness. They were slaughtered and samples from the pectoralis major (breast) muscle were collected. Penetrometry of breast muscles was done to determine the shear force using a texture analyzer (Texture Analyzer TX-700; LAMY Rheology, Champagne au Mont d'Or, France). Breast muscles were kept perpendicular to the blade and the following parameters were set: maximum speed, 2 mm/s; measure time, 20 s; force to start, 5 g; wait position, 10 mm; force set, 10 N and maximum distance, 5 mm.

Carcass characteristics

Two birds from each replicate were selected randomly as a sample for determining carcass characteristics by slaughtering at 35th day. Anesthesia was given as Ketamine 20 mg per kg of body weight. All the visceral organs, head, shanks and feathers were removed and carcass yield percentage was calculated. Dressing, breast, thigh meat, heart, liver and gizzard percentages and were calculated by dividing their weights with live weight and multiplying with 100.

Statistical analysis

The data under each treatment were pooled and examined using analysis of variance under completely randomized design and means were compared using Tukey's Test[15].

Results

Effect of *Nutri Biyo*® supplementation on broilers growth performance

The results of Nb supplementation on broilers growth performance during the starter (1–21 days), finisher (22–35 days) and overall (1–35 days) phases are shown in Table 3. Significant differences ($p < 0.05$) were observed among the treatment groups for feed intake, body weight gain and feed conversion ratio during all phases with the inclusion of 1.5 g Nb. During starter phase, feed intake (1282 vs. 1328 g), weight gain (897 vs. 849 g) and FCR (1.47 vs. 1.50) progressively improved by T3. Similar pattern of results had been observed during finisher phase, distinct superscripts among treatments confirmed statistical differences regarding feed intake (2176 vs. 2151 g), weight gain (1444 vs. 1391 g) and FCR (1.50 vs. 1.54) with T3 treatment.

Significant differences ($p < 0.05$) were observed among all treatment groups for feed intake, body weight gain and feed conversion ratio during overall experimental trial (Table 3). Feed intake increased progressively with the supplementation levels, ranging from 3433.50 g in the control group to 3504.15 g in the group receiving 1.5 g/kg. Similarly, body weight gain was improved significantly in T3 group (2342 vs. 2241 g). The most efficient FCR was observed in T3 (1.49), followed by T1 and T2 at 1.52, while the least efficient conversion was noted in T0 (1.53).

Effect of *Nutri Biyo*® supplementation on nutrient digestibility in broiler chickens

Table 4 contained the outcomes of the Nb supplementation on nutrient digestibility in broiler chickens. The highest total tract digestibility values for dry matter, ether extract, crude protein, crude fiber and ash were 85.91, 90.15, 81.02, 74.51 and 62.81%, respectively by the birds fed diet contained 1.5 g/kg Nb at the end of starter phase. Similarly, total tract nutrient digestibility at 35th day was also improved at same dose level and the highest

Parameters	Experimental treatments				SEM	*P*-value
	T0	T1	T2	T3		
Starter phase (1–21 days)						
Feed intake (g)	1282.09^{d}	1297.95^{c}	1315.30^{b}	1328.10^{a}	7.761	0.026
Body weight gain (g)	849.54^{d}	854.12^{c}	871.80^{b}	897.69^{a}	7.741	0.019
Feed conversion ratio	1.50^{b}	1.51^{a}	1.50^{b}	1.47^{c}	0.004	0.015
Finisher phase (22–35 days)						
Feed intake (g)	2151.41^{d}	2153.84^{c}	2163.42^{b}	2176.05^{a}	1.215	0.027
Body weight gain (g)	1391.89^{d}	1404.83^{c}	1414.33^{b}	1444.77^{a}	4.750	0.029
Feed conversion ratio	1.54^{d}	1.53^{c}	1.52^{b}	1.50^{a}	0.003	0.019
Overall (1–35 days)						
Feed intake (g)	3433.50^{d}	3451.79^{c}	3478.72^{b}	3504.15^{a}	15.55	0.048
Body weight gain (g)	2241.43^{d}	2264.15^{c}	2286.13^{b}	2342.46^{a}	21.29	0.029
Feed conversion ratio	1.53^{a}	1.52^{b}	1.52^{b}	1.49^{d}	0.007	0.017

Table 3. Effect of *Nutri biyo*® supplementation on growth performance of broiler chickens. $^{a, b,c, d}$Means with different superscripts within a row differ significantly ($p < 0.05$).

Digestibility (%)	Experimental treatments				SEM	*P*-value
	T0	T1	T2	T3		
Total tract digestibility (21st day)						
Dry matter	80.01^{d}	81.45^{c}	83.28^{b}	85.91^{a}	0.313	0.002
Ether extraction	85.93^{d}	86.87^{c}	88.32^{b}	90.15^{a}	0.025	0.001
Crude protein	75.94^{d}	77.71^{c}	78.16^{b}	81.02^{a}	0.034	0.033
Crude fiber	70.74^{d}	71.39^{c}	72.12^{b}	74.51^{a}	0.031	0.022
Ash	58.10^{d}	59.25^{c}	60.93^{b}	62.81^{a}	0.033	0.013
Total tract digestibility (35th day)						
Dry matter	82.75^{d}	83.25^{c}	85.50^{b}	88.94^{a}	0.314	0.002
Ether extraction	86.64^{d}	87.76^{c}	89.27^{b}	91.80^{a}	0.024	0.023
Crude protein	77.22^{d}	79.03^{c}	80.57^{b}	83.26^{a}	0.031	0.001
Crude fiber	72.06^{d}	73.37^{c}	74.78^{b}	77.34^{a}	0.032	0.002
Ash	60.88^{d}	61.13^{c}	62.05^{b}	64.85^{a}	0.023	0.014
Ileal digestibility (35th day)						
Dry Matter	68.5^{c}	71.3^{b}	74.2^{ab}	75.8^{a}	1.0	0.021
Ether Extract	75.1^{b}	77.3^{b}	80.5^{a}	81.2^{a}	1.4	0.034
Crude Protein	71.2^{c}	73.6^{bc}	76.8^{b}	79.1^{a}	0.95	0.011
Crude Fiber	41.8	42.9	45.0	45.6	1.5	0.108

Table 4. Effect of *Nutri biyo*® supplementation on nutrient digestibility in broiler chickens. $^{a, b,c, d}$Means with different superscripts within a row differ significantly ($p < 0.05$).

values are 82.75, 91.80, 83.26, 77.34 and 60.88% for dry matter, ether extract, crude protein, crude fiber and ash digestibility, respectively.

The influence of different levels of Nb (0.5, 1.0 and 1.5 g/kg) on ileal nutrients digestibility in broiler chickens at 35th day is presented in Table 4. The DM and CP digestibility were significantly enhanced ($p < 0.05$) in all supplemented groups compared to the control. Birds in the T3 group, exhibited the highest CP (79.1%), DM (75.8%) and EE (81.2%) digestibility. The CF digestibility was remained unaffected but numerically higher digestibility showed by T3 (45.6%).

Effect of *Nutri Biyo*® supplementation on immune functions in broiler chickens

The effect of Nb supplementation on the immune response of broiler chickens is presented in the Table 5. Significant differences ($p < 0.05$) were observed among the treatment groups for both Newcastle Disease (ND) titers and Infectious Bronchitis (IB) antibody levels. The ND $\log_2$ titers increased progressively from 6.83 in the control group (T0) to 10.00 in the highest supplemented group (T3) while IB S/P ratios improved significantly, ranging from 0.47 in T0 to 1.02 in T3 ($p = 0.031$), indicating a dose-dependent improvement in humoral immunity ($p = 0.015$).

The effects of Nb supplementation at different dietary inclusion levels (0.5, 1.0 and 1.5 g/kg) on the relative weights of lymphoid organs such as the bursa of fabricius, spleen and thymus in broiler chickens at the 21st and 35th day are presented in Table 5. The highest bursa weight was found as 0.58 and 0.67% at 21st and 35th day,

Immune response	Experimental treatments				SEM	*P*-value
	T0	T1	T2	T3		
Immune organs index (21st day)						
Bursa	0.39^{c}	0.46^{b}	0.53^{ab}	0.58^{a}	0.02	0.021
Spleen	0.24^{b}	0.28^{b}	0.31^{ab}	0.34^{a}	0.015	0.036
Thymus	0.61^{b}	0.68^{b}	0.74^{ab}	0.79^{a}	0.03	0.033
Immune organs index (35th day)						
Bursa	0.45^{c}	0.55^{b}	0.62^{ab}	0.67^{a}	0.02	0.019
Spleen	0.29^{b}	0.31^{b}	0.34^{ab}	0.37^{a}	0.015	0.041
Thymus	0.77^{b}	0.82^{b}	0.88^{ab}	0.91^{a}	0.03	0.035
Antibody titers (35th day)						
ND Log_2	6.83^{c}	8.63^{b}	9.66^{ab}	10.00^{a}	0.582	0.015
IB S/P	0.47^{c}	0.92^{b}	0.94^{ab}	1.02^{a}	0.102	0.031

Table 5. Effect of *Nutri biyo*® supplementation on immune functions in broilers. S/P = Sample to positive control ratio. $^{a, b,c}$Means with different superscripts within a row differ significantly ($p < 0.05$).

Parameters	Experimental treatments				SEM	*P*-value
	T0	T1	T2	T3		
Hb (g/dL)	9.8^{c}	10.2^{bc}	10.9^{ab}	11.3^{a}	0.22	0.016
PCV (%)	29.5^{c}	30.9^{bc}	32.7^{b}	33.9^{a}	0.85	0.020
RBC ($\times 10^6$/mm^3)	2.75^{c}	2.88^{bc}	3.05^{b}	3.15^{a}	0.07	0.014
WBC ($\times 10^6$/mm^3)	18.6	19.4	21.0	21.8	0.95	0.072
Lymphocytes (%)	60.2^{c}	62.3^{bc}	64.8^{b}	66.0^{a}	1.3	0.038
Monocytes (%)	5.8	6.1	6.4	6.5	0.28	0.301
Heterophils (%)	31.5^{a}	29.2^{ab}	26.8^{bc}	25.5^{c}	1.2	0.025
Basophils (%)	0.8	0.8	0.9	0.9	0.05	0.487
Eosinophils (%)	1.7	1.6	1.5	1.4	0.10	0.261
MCV (fL)	107.3	107.3	107.2	107.6	1.5	0.991
MCH (pg)	35.6	35.4	35.7	35.9	0.85	0.842
MCHC (%)	33.2	33.4	33.5	33.6	0.75	0.926

Table 6. Dietary effect of *Nutri Biyo*® on hematological parameters in broiler chickens. $^{a, b,c}$Means with different superscripts within a row differ significantly ($p < 0.05$).

respectively by T3 group, showing a dose-dependent enhancement. Similar results were obtained from same treatment group regarding spleen (0.34% at 21st day, 0.37% at 35th day) and thymus (0.79% at 21st day, 0.91% at 35th day) weights.

Effect of *Nutri Biyo*® supplementation on hematological responses in broiler chickens

The effects of dietary supplementation of Nb at different inclusion levels (0.5 g/kg, 1.0 g/kg and 1.5 g/kg) on the hematological parameters of broiler chickens are summarized in Table 6. The Hb (10.9 g/dL) and PCV (32.7%) values were significantly increased ($p < 0.05$) in T2 group as compared to control. The T3 group showed highest RBC counts (3.15×10^6/mm^3) and lymphocyte percentages (66.0%) than other groups while WBC counts was not influenced by dietary treatments. In contrast, heterophil percentages were significantly reduced ($p < 0.05$) from 31.5% in T1 to 25.5% in T3, indicating a potential enhancement in immune modulation. Other leukocyte types, including monocytes, basophils and eosinophils, were not significantly affected ($p > 0.05$) by Nb supplementation. Similarly, erythrocyte indices such as MCV, MCH and MCHC remained statistically unchanged, suggesting that Nb did not adversely impact red blood cell integrity or hemoglobin content.

Effect of *Nutri Biyo*® supplementation on tibia morphology and mineralization in broiler chickens

The effect of Nb supplementation on tibia morphometry and mineralization is presented in Table 7. The highest tibia length (10.6 cm) and weight (9.4 g) were recorded in T3, compared to 9.5 cm and 7.8 g, respectively, in T0. Lateral (0.51 cm) and medial (0.49 cm) wall thicknesses were also significantly increased ($p < 0.05$) in T3 group. Diaphysis diameter and medullary canal diameter showed a non-significant difference across the treatment groups. The tibiotarsal, robusticity and bone weight/length index were significantly elevated in the higher supplementation groups (T2 and T3).

Significant differences ($p < 0.05$) were observed in both calcium and phosphorus content. Calcium and phosphorous contents were highest in T3 as 34 and 15.2%, respectively.

Tibia character	Experimental treatments				SEM	*P*-value
	T0	T1	T2	T3		
Tibia morphometry						
Tibia Length (cm)	9.5^{c}	9.9^{b}	10.3^{ab}	10.6^{a}	0.15	0.017
Tibia Weight (g)	7.8^{c}	8.4^{b}	9.1^{ab}	9.4^{a}	0.25	0.022
Lateral Wall Thickness (cm)	0.42^{b}	0.44^{b}	0.49^{a}	0.51^{a}	0.02	0.031
Medial Wall Thickness (cm)	0.40^{b}	0.43^{b}	0.47^{a}	0.49^{a}	0.02	0.026
Diaphysis Diameter (cm)	0.74	0.76	0.78	0.80	0.03	0.119
Medullary Canal Diameter (cm)	0.42	0.44	0.46	0.47	0.02	0.153
Tibiotarsal Index	0.51^{b}	0.53^{b}	0.56^{a}	0.57^{a}	0.01	0.018
Robusticity Index	0.82^{b}	0.84^{b}	0.88^{a}	0.89^{a}	0.02	0.022
Bone Index	0.82^{b}	0.85^{b}	0.89^{a}	0.91^{a}	0.03	0.019
Tibia mineralization						
Calcium	19.2^{c}	23^{b}	25^{ab}	34^{a}	0.963	0.032
Phosphorus	10.8^{c}	14.2^{b}	13.9^{ab}	15.2^{a}	0.121	0.015

Table 7. Effect of *Nutri biyo*® supplementation bone mineralization in broiler on 35th day. [a,b,c]Means with different superscripts within a row differ significantly (p<0.05).

Parameters	Experimental treatments				SEM	*P*-value
	T0	T1	T2	T3		
Meat tenderness at the 21st day						
Shear Force (N)	27.5^{a}	24.8^{b}	22.3^{c}	20.6^{c}	0.75	0.009
Hardness (N)	26.1^{a}	23.9^{b}	21.5^{c}	19.7^{c}	0.8	0.011
Springiness (mm)	0.49^{b}	0.52^{ab}	0.55^{a}	0.56^{a}	0.02	0.039
Cohesiveness	0.38	0.39	0.40	0.41	0.01	0.084
Adhesiveness (N s)	0.21^{a}	0.18^{b}	0.15^{c}	0.13^{c}	0.01	0.015
Chewiness (N mm)	9.6^{a}	8.1^{b}	7.0^{c}	6.4^{c}	0.35	0.012
Compression Value (N)	29.0^{a}	26.5^{b}	24.2^{c}	22.0^{c}	0.8	0.007
Meat tenderness at the 35th day						
Shear Force (N)	25.8^{a}	22.9^{b}	20.7^{c}	19.1^{c}	0.68	0.008
Hardness (N)	24.9^{a}	22.3^{b}	19.8^{c}	18.2^{c}	0.75	0.010
Springiness (mm)	0.51^{b}	0.54^{ab}	0.57^{a}	0.58^{a}	0.02	0.031
Cohesiveness	0.39	0.40	0.41	0.42	0.01	0.078
Adhesiveness (N s)	0.20^{a}	0.17^{b}	0.14^{c}	0.12^{c}	0.01	0.012
Chewiness (N·mm)	8.7^{a}	7.3^{b}	6.2^{c}	5.7^{c}	0.32	0.010
Compression Value (N)	27.2^{a}	24.4^{b}	22.1^{c}	20.0^{c}	0.70	0.006

Table 8. Dietary effect of *Nutri Biyo*® on the meat tenderness. [a, b,c]Means with different superscripts within a row differ significantly ($p < 0.05$).

Effect of *Nutri Biyo*® supplementation on meat tenderness in broiler chickens

The results regarding the effect of Nb supplementation on the meat tenderness on broiler breast meat at the 21st and 35th day are presented in Table 8. Shear force decreased significantly ($p < 0.05$) across the treatment groups at 21st day. The T0 recorded the highest shear force (27.5 N). In comparison, the lowest value was seen in T3 (20.6 N), indicating softer and more tender meat with increasing Nb inclusion. In line with shear force, the hardness, chewiness, adhesiveness and compression values significantly decreased ($p < 0.05$) with higher Nb inclusion. Springiness, which refers to the ability of the meat to return to its original shape after compression, increased significantly ($p < 0.05$) with Nb supplementation. The highest value (0.56 mm) was observed in T3, compared to 0.49 mm in T0, indicating improved resilience and elasticity of meat texture. However, cohesiveness showed a non-significant trend ($p > 0.05$), it increased slightly with higher levels of Nb, from 0.38 in T1 to 0.41 in T3 at 21st day.

While shear force at 35th day was significantly reduced ($p = 0.008$) with increasing Nb levels, the lowest values were recorded in T2 and T3. Hardness was significantly decreased, T1 meat samples recorded highest (24.9 N) hardness, while meat from birds in T3 had a much softer texture (18.2 N). Springiness was also significantly improved ($p = 0.031$), increasing from 0.51 mm in T0 to 0.58 mm in T3. Adhesiveness, chewiness and compression value were significantly decreased ($p = 0.012$) with increasing Nb levels. Cohesiveness showed a slight, non-significant increase from 0.39 to 0.42 at 35th day.

Parameters (%)	Experimental treatments				SEM	*P*-value
	T0	T1	T2	T3		
Carcass	55.37^{c}	56.22^{b}	59.44^{ab}	60.52^{a}	0.425	0.019
Breast	27.29	28.99	30.46	28.93	0.030	0.621
Thigh	20.72^{c}	22.06^{b}	23.69^{ab}	24.57^{a}	0.440	0.021
Heart	0.41^{c}	0.46^{b}	0.51^{ab}	0.58^{a}	0.025	0.035
Liver	1.24	1.21	1.30	1.31	0.005	0.781
Gizzard	1.58^{c}	1.73^{b}	2.10^{ab}	2.49^{a}	0.075	0.008
Wings	8.46	8.32	8.98	9.11	0.065	0.541
Back	15.42^{c}	16.82^{b}	17.01^{ab}	17.99^{a}	0.095	0.020
Pancreas	0.05	0.06	0.90	0.81	0.005	0.926
Proventriculus	0.50^{c}	0.52^{b}	0.67^{ab}	1.0^{a}	0.010	0.011

Table 9. Effect of *Nutri biyo*® supplementation on carcass characteristics in broiler. $^{a, b,c}$Means with different superscripts within a row differ significantly ($p < 0.05$).

Effect of *Nutri Biyo*® supplementation on carcass characteristics in broiler chickens

The effect of Nb supplementation on carcass characteristics and visceral organ development in broilers at day 35 is presented in Table 9. Carcass yield (60.52 vs. 55.37%), thigh percentages (24.57 vs. 20.72%) heart (0.58 vs. 0.41%), proventriculus (1.0 vs. 0.50%), gizzard (2.49 vs. 1.58%) and back portion (17.99 vs. 15.42%) were improved by the highest supplemented group (T3). On the other hand, no significant differences were found in breast, liver, wings, and pancreas weights ($p > 0.05$).

Discussion

Growth performance

In the present study, dietary supplementation with Nb significantly improved feed intake, weight gain and FCR during all phases in broiler chickens, with the highest consumption recorded in the T3 group (1.5 g/kg feed). Improved FCR is typically linked to higher digestibility, reduced maintenance energy requirements, and diminished immunological stress. These findings are supported by Kiarie et al.[16], who reported similar feed intake improvements in broilers fed enzyme-probiotic blends. Similar effects were reported by Biswas et al.[17], who observed improved broiler performance with enzyme-supplemented diets. No significant differences in body weight gain were observed among the treatment groups during the first week of the trial, likely due to the immature digestive and microbial systems of neonatal chicks[18]. However, as the gastrointestinal tract matured, improved enzyme activity and microbial stability facilitated greater nutrient interaction and uptake. This delayed but progressive growth response aligns with findings by Yaseen et al.[19] and Vaarst et al.[20], who noted that dietary supplements exert more pronounced effects after gut development. Manafi et al.[21] similarly reported improved FCR with probiotics due to better microbial balance and reduced intestinal inflammation. The observed week-wise improvement in FCR indicates a time-dependent response, consistent with the progressive development of the gut and its increasing capacity to respond to dietary supplementation. This enhanced growth performance is attributed to better nutrient digestion and absorption facilitated by the synergistic effects of probiotics, enzymes, and chelated minerals, especially at the highest supplementation level. Moreover, the presence of amino acid-chelated trace minerals in Nb may have enhanced feed palatability and mineral bioavailability, contributing to optimal metabolic function and appetite regulation[22]. Furthermore, the inclusion of chelated trace minerals such as zinc, copper, and selenium likely supported metabolic efficiency by acting as cofactors in key physiological processes, including protein synthesis and enzymatic regulation[23]. Their higher bioavailability compared to inorganic forms ensured adequate mineral support for optimal skeletal and muscular development[24]. The inclusion of multi-strain probiotics such as *Bacillus subtilis* and *Lactobacillus plantarum* helped stabilize gut microflora, reduce pathogenic bacteria, and promote a favorable microbial environment, thereby enhancing digestion and stimulating voluntary feed intake[21,25]. Additionally, the enzymatic blend of Nb, including xylanase, cellulase, and phytase, also contributed by breaking down complex feed components. While xylanase and cellulase targeted fibrous structures to release nutrients bound within cell walls, phytase enhanced the bioavailability of essential minerals like calcium and phosphorus by hydrolyzing phytate complexes[26]. These enzymatic actions improved nutrient release and absorption, thereby supporting lean muscle growth.

Total tract nutrient digestibility

The present study revealed that dietary supplementation with Nb significantly improved total tract nutrient digestibility in broilers, with the T3 group (1.5 g/kg feed) showing the most pronounced effects. Birds in this group exhibited higher digestibility coefficients for dry matter, crude protein, ether extract, crude fiber, and total ash, indicating enhanced gastrointestinal efficiency and more effective nutrient assimilation. The results of present study are consistent with findings of Mallo et al.[27], who observed enhanced energy and nutrient availability with enzyme supplementation targeting NSPs and phytates. The dose-dependent improvement in digestibility, with the T3 group achieving the highest values, suggests that the efficacy of Nb increases with higher inclusion rates. This trend aligns with the meta-analysis by Sugiharto et al.[28] and Nollet et al.[29], which confirmed the dose-responsive benefits of combined probiotic and enzyme supplementation. While nitrogen retention

was not directly measured, the enhanced protein digestibility observed suggests lower fecal nitrogen output and improved nitrogen utilization for muscle accretion. This physiological efficiency supports better growth uniformity and feed conversion across the flock when digestive capacity is optimized. These improvements were driven by the synergistic actions of amino acid-chelated trace minerals, multi-strain probiotics and exogenous enzymes, and present in the Nb formulation. Greater ash digestibility reflected improved mineral absorption, facilitated by the high bioavailability of amino acid-chelated trace minerals. Unlike inorganic salts, these chelated forms bypass antagonistic interactions during digestion and are absorbed via amino acid transport pathways[23]. Enhanced dry matter and crude protein digestibility likely resulted from improved enzymatic degradation and microbial modulation within the gut. Enzymes such as xylanase, cellulase, and phytase played critical roles in breaking down complex polysaccharides and phytate structures common in plant-based diets, thereby releasing encapsulated nutrients. Xylanase and cellulase facilitated the breakdown of fibrous feed matrices, while phytase liberated phosphorus and calcium from phytate complexes, improving mineral and amino acid availability essential for growth and metabolic function[26]. Improvements in ether extract and crude fiber digestibility further support the role of enzyme action in nutrient release. Since dietary fats are often embedded in fibrous matrices, their breakdown enhanced lipid absorption. Reduced digesta viscosity and improved intestinal transit contributed to greater nutrient uptake efficiency,

Ileal nutrient digestibility

Ileal nutrient digestibility reflects how effectively the broiler's small intestine absorbs critical nutrients such as crude protein, dry matter and ether extract prior to microbial fermentation in the hindgut. This parameter is vital in poultry nutrition as it serves as a direct indicator of dietary efficiency and gut health. These findings are consistent with a growing body of literature that recognizes the benefits of integrated feed additives in replacing or complementing traditional growth promoters. Zaghari et al.[30] found that dietary inclusion of *Bacillus subtilis* and *Bacillus licheniformis* enhanced ileal digestibility of CP and calcium with a more pronounced effect observed in the *B. licheniformis* group. Similarly, Yaqoob et al.[31] observed that a multi-enzyme and probiotic combination improved ileal CP and fiber digestibility particularly in energy-reduced diets. These outcomes align with our findings suggesting that microbial and enzymatic support can overcome the digestive limitations of plant-based feed ingredients. Dimas et al.[32] reported enhanced digestibility of phosphorus and calcium with increasing enzyme inclusion particularly when combined with phytase showing that these enzymes synergistically optimize nutrient release. Kim et al.[33] also demonstrated that combining phytase with a multi-enzyme blend significantly boosted ileal digestibility of CP, DM and minerals like phosphorus and calcium. Hashemi et al.[34] found that zinc-methionine supplementation at 120 and 180 mg/kg significantly improved ileal CP digestibility compared to non-supplemented controls. Their findings reinforce the idea that mineral bioavailability when optimized supports enzymatic activity and gut function which is essential for efficient protein digestion and amino acid absorption. The CF digestibility did not improve significantly in our trial. This may be due to the structural rigidity of fibrous components like cellulose and lignin which require cellulolytic enzymes and longer fermentation times to break down—conditions not prevalent in the ileum. Luo et al.[35] observed that even with enzyme and probiotic inclusion, birds fed high-ANF diets showed limited improvement in fiber digestibility underscoring the ileum's enzymatic constraints in processing fibrous material. Rodriguez-Soriano et al.[36] and Khan et al.[37] used different exogenous enzymes and reported significant results on ileal digestibility. Yaqoob et al.[31] and Leeson[38], emphasized that enzyme and mineral interactions are highly dose-sensitive and exceeding or underdelivering these additives may result in suboptimal outcomes. Improved digestibility translates to lower feed costs and higher production efficiency. Better CP and EE utilization reduces the dietary requirement for high-cost ingredients like soybean meal and oil. Moreover, improved DM digestibility correlates with lower fecal output contributing to better litter quality and reduced environmental nitrogen and phosphorus excretion. These sustainability benefits are increasingly important in modern poultry systems where feed costs represent over 60% of total production expenses. The enhancement in digestibility can be attributed to the synergistic functions of Nb's components — probiotics, multi-enzymes and chelated trace minerals. Each plays a complementary role: enzymes break down complex feed molecules, this mechanism increases nutrient bioavailability and decreases digesta viscosity resulting in improved transit and absorption, while probiotics balance the gut microbiota and chelated minerals support enzymatic and metabolic processes by acting as cofactors in enzymatic reactions and improving tissue development in the digestive tract. Together they create a conducive environment for optimal nutrient assimilation in the ileum. Probiotics improve digestibility by modulating gut microbial populations, reducing pathogenic bacteria and producing short-chain fatty acids that support enterocyte health.

Immune functions

The findings of present study suggested a more robust humoral immune response and improved vaccine efficacy in birds receiving higher levels of supplementation, highlighting the immunostimulatory potential of the additive. The beneficial microbes promote a balanced intestinal microbiota and inhibit the colonization of enteric pathogens through competitive exclusion. This microbial equilibrium supports mucosal integrity and stimulates local immune responses, thereby enhancing antigen recognition and systemic antibody production[39]. The elevated NDV antibody titers in the supplemented groups likely result from probiotic-mediated modulation of immune signaling. By upregulating cytokine expression and promoting the proliferation and differentiation of B lymphocytes, probiotics stimulate immunoglobulin synthesis, enhancing vaccine responsiveness. Furthermore, the probiotic-induced reduction of intestinal pathogens likely minimized nutrient competition between microbes and host cells. In a healthier gut environment, more nutrients are allocated toward immune development and antibody synthesis rather than microbial defense. Manafi et al.[21] similarly reported increased antibody titers in broilers supplemented with multi-strain probiotics, attributing the effect to improved intestinal immune status and efficient antigen presentation. In parallel, the inclusion of amino acid-chelated trace minerals

such as zinc and selenium further strengthened immune performance. Zinc is essential for thymic hormone production and T-cell activity, while selenium supports antioxidant defenses in immune cells via glutathione peroxidase, minimizing oxidative damage during immune activation[40]. Ciurescu et al.[23] reported similar enhancements in humoral immunity in broilers supplemented with chelated mineral complexes, underscoring their critical role in antibody generation and immune regulation. This trend aligns with findings by Qiu et al.[40], who observed progressive immunostimulant in broilers receiving increasing levels of probiotic.

Immune organ index

The immune organ index, represented by the relative weights of key lymphoid tissues including the bursa of Fabricius, spleen, and thymus, is a recognized biomarker of immune development and systemic health in broilers. In the present study, the highest inclusion rate (1.5 g/kg) resulted in the most pronounced increases: bursa (0.67%), spleen (0.37%), and thymus (0.91%) at day 35, indicating enhanced immune tissue growth and immunocompetence. These enhancements can be attributed to the synergistic action of Nb's bioactive constituent like probiotics, digestive enzymes, and chelated trace minerals. Probiotics such as *Bacillus* and *Lactobacillus* species improve gut microbial balance, enhance mucosal immunity, and reduce intestinal inflammation, indirectly fostering the proliferation of immune cells and lymphoid tissue development. Concurrently, digestive enzymes improve nutrient availability by breaking down complex feed components, releasing essential amino acids, vitamins, and minerals vital for immune organogenesis. Chelated minerals such as zinc and selenium serve as cofactors in numerous immunoregulatory enzymes, thus supporting structural and functional development of immune tissues. These findings align with those of El-Kelawy[41], and Attia et al.[42] who observed increased bursal weights in broilers receiving enzyme-probiotic combinations, attributing improvements to enhanced gut health and oxidative balance. Histological studies provide further insight. Zhyla et al.[43] reported that probiotic-fed broilers had improved lymphoid cell density and structural integrity in spleen and bursa tissues, even when changes in gross organ size were modest. This suggests that dietary interventions with probiotics yield deeper immunological enhancements beyond physical metrics alone. Supporting this, Sjofjan et al.[44], in a meta-analysis of 93 studies, confirmed consistent improvements in the development of all three immune organs across various probiotic types and rearing conditions. The observed immune organ enhancement also appears to benefit from the synergistic effects of probiotics and minerals. Hashemitabar and Hosseinian[45], Hossain et al.[46], Dibamehr et al.[47] and Mangisah et al.[48] showed that different strains, alone or in combination, significantly increased lymphoid organ weights and immune cells under different managemental conditions, demonstrating their resilience-enhancing role. Although most literature supports these findings, however, Nunez et al.[49] reported no significant improvement in lymphoid organ weights with organic minerals alone. They speculated that immune stimulation, such as mild pathogen exposure or stress, may be necessary to reveal the mineral's immunological benefits. Given the commercial trial conditions of the present study, natural environmental challenges may have contributed to the observed positive responses. Physiologically, increases in immune organ weight imply elevated lymphopoiesis and immune readiness, often linked to enhanced antibody production and improved disease resistance. Though this study did not evaluate functional immune outputs beyond antibody titers, Zhao et al.[10] confirmed that enzyme-probiotic complexes improve both organ weight and IgA titers, affirming the functional significance of such structural enhancements. Economically, improvements in immune organ development offer potential for reduced antibiotic use and improved flock resilience, particularly in stress-prone environments.

Hematology

Hematological parameters offer vital insight into the physiological and immunological responses of broiler chickens to dietary interventions. Variables such as Hb, PCV, RBC and differential leukocyte counts serve as indicators of oxygen transport capacity, immune status and overall health. In the current study, Nb supplementation significantly influenced several hematological indices, suggesting enhanced erythropoiesis and improved physiological performance. Probiotics can modulate the gut microbiota indirectly stimulating hematopoiesis by enhancing nutrient absorption and reducing systemic inflammation. Enzymes meanwhile improve the availability of amino acids and vitamins essential for red blood cell synthesis. Finally, trace minerals such as iron, zinc and copper are integral components of hemoglobin and enzymes involved in hematopoietic processes. Supporting these findings, Islam et al.[50], Younas et al.[51], El-Sayed et al.[52] and Attia et al.[42] found that broilers supplemented with probiotics and enzymes exhibited significantly hematological responses compared control groups which they attributed to improved gut health and nutrient bioavailability. In contrast, studies like that of Hidayat et al.[53] found no significant effects of probiotic supplementation on hematological parameters indicating that results may vary depending on the probiotic strain and dosage used. In the present study, the increase in lymphocyte count and reduction in heterophils at higher Nb levels indicate enhanced immune regulation. This leukocyte pattern has been associated with reduced stress and infection risk. Chelated minerals likely played a contributory role in supporting hematological improvements particularly by enhancing the bioavailability of iron and copper which are vital for hemoglobin synthesis. Biabani et al.[3] and Ayoola et al.[54] showed that supplementation with chelated trace minerals improved hematological values including RBC and Hb levels particularly under stress conditions. From a mechanistic perspective, the improvements observed may stem from enhanced protein and iron metabolism facilitated by better nutrient digestibility. Studies by Khan et al.[37] noted that ginger-derived phyto-protease enhanced CP digestibility which in turn supported hematological values due to improved amino acid availability for red blood cell synthesis. Moreover, probiotics may secrete metabolites that promote hematopoiesis directly by interacting with gut-associated lymphoid tissue (GALT) which plays a central role in immune and blood cell development. The impact of enzymes on hematology, though indirect, is supported by their role in improving nutrient extraction. The improvements in Hb, PCV, RBC and lymphocyte counts suggest better oxygen-carrying capacity and immune resilience likely driven by enhanced nutrient utilization and microbial modulation. These findings are well-supported by recent literature

and reinforce the importance of integrated feed strategies in modern poultry production. Despite positive findings across many studies, some authors reported inconsistent results. For example, Mahmoud et al.[55] found that while phytase inclusion improved growth performance and carcass traits, hematological values remained largely unaffected. This discrepancy may arise from differences in experimental conditions, baseline diet quality or the physiological status of the broilers.

Tibia characteristics

The present study demonstrated that dietary supplementation with Nb significantly improved tibia characters in broiler chickens, mainly by T3 group supplemented at 1.5 g/kg of feed. These findings suggest enhanced mineral retention and skeletal development, which are critical for supporting structural integrity in rapidly growing broilers. Phytate is a major anti-nutritional factor in poultry diets that binds essential minerals such as calcium and phosphorus, rendering them unavailable for absorption. The action of phytase in degrading these complexes increases the bioavailability of bound minerals, facilitating their absorption in the small intestine and subsequent deposition in bone tissue[26]. These results are consistent with findings by Abdilahi et al.[56], who reported improved tibial ash content and mineral concentrations in broilers fed diets supplemented with enzyme complexes containing phytase. The observed enhancements in bone mineralization may also be influenced by the presence of specific probiotic strains such as Bacillus subtilis, which can indirectly support mineral uptake by modulating intestinal microbiota and improving gut barrier function. Minerals such as zinc, manganese and copper play vital roles in bone metabolism, acting as cofactors for enzymes involved in collagen synthesis, bone matrix formation and oxidative stress regulation. Chelated minerals are absorbed through amino acid pathways, which enhances their stability in the digestive tract and minimizes antagonistic interactions that often occur with inorganic mineral salts[23]. Their higher absorption efficiency ensures adequate delivery to mineralizing tissues, supporting the structural maturation of the skeletal system. Zinc is critical for collagen cross-linking and alkaline phosphatase activity, both essential for mineral deposition and matrix integrity. Manganese contributes to proteoglycan synthesis, while copper is required for the enzymatic formation of elastin and collagen. Together, these trace minerals strengthen the bone matrix and enhance mechanical stability. Studies by Bao et al.[57] and Leeson and Summers[24] also support the superior efficacy of organic trace minerals in improving bone mineral density and reducing skeletal deformities in broilers. The physiological improvements observed in tibial mineralization are particularly relevant in the context of broiler production, where rapid muscle growth often outpaces skeletal development. In such cases, inadequate mineralization can lead to leg disorders and compromised locomotion. These findings confirm the functional role of Nb in promoting skeletal mineral deposition and structural bone development through improved nutrient availability and utilization.

The enhancements in bone morphometry can be attributed to improved mineral bioavailability and nutrient utilization, facilitated by the synergistic effects of probiotics, enzymes and chelated trace minerals in Nb. Multienzymes help release bound nutrients from feed matrices, while probiotics modulate intestinal microbiota and improve mineral absorption. Chelated minerals, especially zinc, manganese and phosphorus, directly support bone mineralization and structural development. Consistent with our findings, Mohammadizad et al.[58]. and Sohail et al.[59]. demonstrated that advanced chelated trace minerals improved tibial mineralization and structural parameters including bone ash, phosphorus and calcium contents. The observed improvement in tibiotarsal and robusticity indices further supports the structural integrity of bone. These indices relate bone length and diameter to overall strength and resilience. Lee et al.[60]. similarly reported that fermented Bacillus subtilis-based supplements improved the tibiotarsal index and mineral retention in tibiae, supporting our interpretation that microbial and enzymatic action enhances mineral utilization. Probiotic supplementation alone has also been shown to affect skeletal development. Guo et al.[61]. confirmed that the co-administration of vitamin K_3 and Bacillus subtilis resulted in synergistic improvements in tibial strength and ash content. These findings validate the probiotic-mineral synergy observed in the current study. Moreover, changes in wall thickness were substantial. According to Sahraei et al.[62]. , such enhancements are indicative of greater calcium deposition and mechanical strength in the cortical bone layer. These patterns were also reported by Shah et al.[63]. , who found that combined probiotic and zinc supplementation reduced medullary canal diameter and improved the tibiotarsal index. Contrastingly, Steczny and Kokoszynski[64] and Hajimohammadi et al.[65]. found no significant impact of probiotics on tibia bone morphometry. This discrepancy could be due to differences in probiotic strains, dosage, or genetic lines used. The resulting improved bone matrix formation and mineralization ensure better leg strength and lower incidence of skeletal disorders. Economically, stronger bones reduce losses from culling, lameness and carcass downgrades, contributing to higher profitability and improved welfare standards. Additionally, improved mineral absorption leads to reduced fecal mineral excretion, supporting environmental sustainability in poultry operations.

Meat tenderness

Meat tenderness is a key indicator of broiler meat quality and consumer acceptability, representing the integrity and structure of muscle fibers post-slaughter. In this study, tenderness-related parameters such as shear force, hardness, chewiness and compression value were significantly improved in birds supplemented with Nb, especially at 1.0 and 1.5 g/kg inclusion levels. These results are supported by Wang et al.[66], Liu et al.[67] and Tang et al.[68], who found that dietary inclusion of different probiotics strains improved meat quality parameters and reduced cooking losses and shear force values in broilers, enhancing juiciness and meat tenderness. As noted by Yibar and Uzabaci[69], probiotics consistently improved broiler meat quality across multiple studies especially in tenderness, water retention and color metrics. Similarly, Li et al.[70], and Soni et al.[4] reported similar results on meat quality by demonstrating different sources of minerals especially with organic forms enhances collagen and elastin network development directly impacting texture properties such as springiness and resilience. Zamani et al.[71] observed improved tenderness when birds were fed crude enzymes derived from fermented

palm kernel cake likely due to improved hydrolysis of anti-nutritional compounds and enhanced energy delivery to muscle tissues. However, Suryadi et al.[72] found that probiotic inclusion improved protein content and reduced fat content but had no significant effect on tenderness. Similarly, Lu et al.[73] reported no significant changes in shear force with protease supplementation alone highlighting that additive formulation and bioavailability are crucial for achieving desired results. In terms of physiological mechanism, better digestibility and nutrient uptake support muscle fiber maturation and protein deposition while the antioxidative functions of probiotics and trace minerals reduce proteolytic degradation. Together, these effects lead to improved postmortem meat characteristics such as reduced drip loss, lower cooking loss and higher structural integrity.

Carcass characteristics

Dietary supplementation with Nb at 1.5 g/kg (T3) significantly enhanced carcass characteristics in broilers, evidenced by increased dressing percentage and heavier weights of key organs including the heart, gizzard, proventriculus, and thigh muscles. These improvements reflect superior nutrient utilization and effective partitioning of energy and protein toward muscle development and organ maturation, key indicators of production efficiency in broiler systems. These effects promote better nutrient absorption and reduce metabolic losses associated with inflammation or microbial imbalance, ultimately allowing more nutrients to be directed toward carcass and organ development[21]. The increased weights of metabolically active organs like the gizzard and proventriculus suggest enhanced mechanical and enzymatic digestion, while greater heart weight implies improved cardiovascular efficiency and oxygen transport—both essential for sustaining rapid growth. These findings mirror those of Harun-Ar-Rashid et al.[74], who also reported improved visceral development in broilers receiving functional feed additives. Similar enhancements in carcass traits with enzyme-enriched diets were also reported by Abdilahi et al.[56], attributing them to improved nutrient liberation and assimilation. Increased thigh muscle mass in the T3 group further supports the muscle accretive effects of Nb. Thigh muscles, with their high metabolic demands, are particularly responsive to dietary energy and protein. Their development signifies effective nutrient conversion into lean tissue mass, an outcome indicative of efficient feed utilization and superior growth. In conclusion, Nb supplementation at 1.5 g/kg improved carcass yield and organ development through optimized nutrient digestion, microbial stability, and enhanced mineral utilization. The findings point to a dose-dependent response, with the T3 group exhibiting the most favorable carcass traits. These results affirm the functional efficacy of Nb as a performance-enhancing feed additive for improving carcass characteristics in broiler production.

Conclusion

In conclusion, the findings of this study provide compelling evidence that *Nutri Biyo*® is an effective multifunctional feed additive capable of enhancing broiler health, productivity and welfare. When supplemented at 1.5 g/kg, *Nutri Biyo*® improved different physiological indicators from growth to meat quality, the end product. These improvements are attributed to the synergistic action of probiotics, enzymes and chelated trace minerals, which optimize gut function, enhance nutrient utilization, support hematopoiesis and promote structural tissue development. Its beneficial effects became more pronounced with age suggesting its efficacy increases as the digestive and immune systems mature. The *Nutri Biyo*® presents a practical and sustainable solution for improving broiler performance under intensive rearing systems. Future studies should explore its use under varied environmental conditions and in synergy with other functional feed additives to further validate its potential in commercial poultry production.

Data availability

All data generated or analyzed during this study are included in this article and supplementary file.

Received: 28 October 2025; Accepted: 10 December 2025
Published online: 23 December 2025

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Acknowledgements

We would like to express our gratitude to all who participated and make this happen and especially to those who improved this manuscript by their valuable suggestions.

Author contributions

MN: Investigation, Data curation. A: Investigation, Data curation. SH: Writing original draft, review, editing & correspondence. MM: Statistical analysis. FA: Conceptualization. MKB: Conceptualization & supervision. WA: Analysis, reviewing & editing. MMAH: analysis, reviewing & editing. MA: Reviewing & editing. UK: Investigation. MR: Investigation. MU: Investigation. MS: Conceptualization, resource management, supervision the research, reviewing, editing & correspondence. All authors read and approved the final manuscript.

Funding

Not applicable to this manuscript because no funding agency was involved.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

All experimental procedures were approved conducted in accordance with the Pakistan Biosafety Rules 2005

and operates within the Office of Research, Innovation and Commercialization, approved by Graduate Study and Research Board at University of Agriculture, Faisalabad (DGS No.7929-32), furthermore, The study was conducted in accordance with ARRIVE guidelines.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-32495-9.

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