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REGULAR ARTICLES

Effects of dietary methionine, betaine, and turmeric extract on growth performance, myogenic gene expression, serum protein profile, and intestinal histomorphology in broiler chickens

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Abstract

Poultry production increasingly relies on nutritional strategies to improve growth efficiency and health, with growing interest in phytobiotics and methyl-donor nutrients that modulate physiological and molecular pathways. This study investigated the integrated physiological, molecular, and intestinal responses of broiler chickens to dietary supplementation with DL-methionine, betaine, and turmeric extract. A total of 108 one-day-old Hubbard broiler chicks were randomly allocated to four dietary treatments, each comprising three replicates of nine birds: a basal control diet and diets supplemented with DL-methionine (1 g/kg), betaine (1 g/kg), or turmeric extract (200 mg/kg). Growth performance was recorded throughout the experimental period, and blood, breast muscle, and intestinal samples were collected at 35 days of age for biochemical, gene expression, and histomorphological analyses. Dietary supplementation significantly improved final body weight, body weight gain, and feed conversion ratio ($P<0.05$), with the most pronounced responses observed in the methionine- and betaine-supplemented groups. Serum biochemical assessment showed significantly higher concentrations of total protein, albumin, and globulin in supplemented birds ($P<0.05$), indicating enhanced systemic protein metabolism. At the molecular level, Myogenic differentiation factor (MyoD) expression was not affected by dietary treatments ($P>0.05$), while Myogenin (MyoG) was significantly upregulated and Myostatin (MSTN) was significantly downregulated, particularly in birds receiving turmeric extract ($P<0.05$).Collectively, these findings demonstrate that phytobiotic and methyl-donor supplementation enhances broiler growth efficiency through coordinated modulation of protein metabolism, intestinal morphology, and myogenic regulatory pathways.

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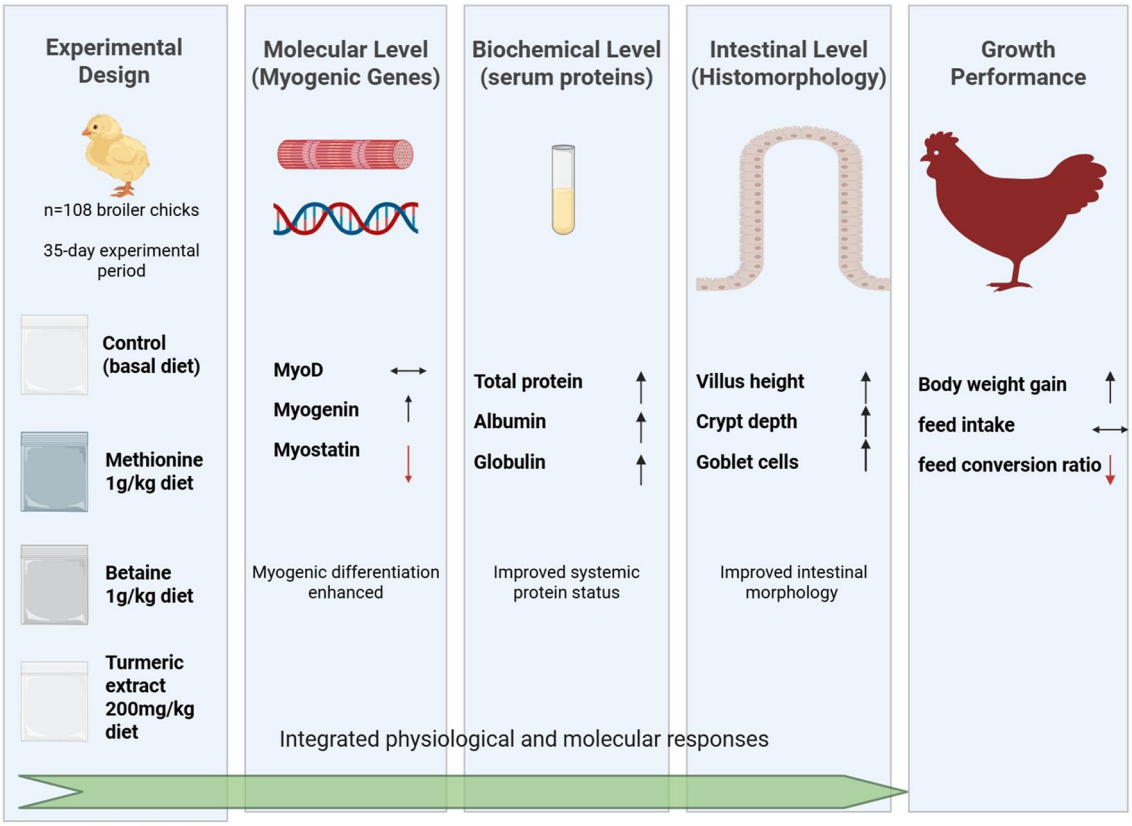
Graphical Abstract



Keywords Intestinal integrity · Methyl donors · Muscle differentiation · Nutrigenomic regulation · Phytogenic feed additives · Poultry productivity

Introduction

The poultry production industry faces increasing pressure to enhance productivity and meat quality while operating under constraints related to feed efficiency and regulatory limitations on antimicrobial use (Aidara-Kane et al. 2018; Korver 2023; Bist et al. 2024). Antibiotic growth promoters (AGPs) were widely used to improve growth performance; however, their extensive application has been associated with disruption of intestinal microbiota, antimicrobial resistance, and antibiotic residues in poultry products, raising concerns for animal and public health (Muaz et al. 2018; Rafiq et al. 2022). Consequently, restrictions on AGP use have intensified the demand for safe and sustainable nutritional alternatives (Barreto et al. 2008; Rizzo et al. 2008; Rafiq et al. 2022).

Phytobiotics have gained attention as natural feed-based alternatives due to their broad antimicrobial, antioxidant, immunomodulatory, and growth-promoting activities. These effects are largely attributed to their ability to maintain intestinal morphology, strengthen epithelial barrier function, and shape the gut microbial ecosystem, thereby improving nutrient utilization and absorption efficiency (Aljumaah et al. 2020a, b; Gilani et al. 2021; Abdul Kari et al. 2022; Obianwuna et al. 2024). In this context, supplementation with medicinal plant extracts such as *Phyllanthus amarus* has been reported to enhance growth performance in broiler chickens through modulation of intestinal microbiota, stimulation of digestive enzyme activity, and improved nutrient utilization efficiency (Mba Anthonia Nkiru et al. 2025). In addition, plant-derived essential oils exhibit antimicrobial activity by disrupting bacterial cell membrane integrity, impairing metabolic activity, and reducing ATP production, which ultimately leads to bacterial cell death and limits the development of antimicrobial resistance (Mustafa et al. 2025). Moreover, phytonutrient-rich medicinal plant extracts containing polyphenols, flavonoids, condensed tannins, and saponins have been shown to modulate microbial fermentation patterns, enhance nutrient degradability, increase volatile fatty acid production, and reduce methane

emissions through their regulatory effects on microbial populations (Phupaboon et al. 2025).

Among nutritional and phytogenic additives, methionine, betaine, and turmeric extract have attracted increasing interest because they exert distinct yet complementary biological functions in poultry. Methionine is an essential sulfur-containing amino acid that supports protein synthesis and acts as a precursor for S-adenosylmethionine (SAM), thereby contributing to muscle development and tissue growth (Niculescu and Zeisel 2002; Willke 2014; Romanet et al. 2021; Babazadeh and Ahmadi Simab 2022). Betaine serves as both a methyl donor and an osmoprotectant, supporting cellular hydration, intestinal epithelial integrity, and protein metabolism, particularly under physiological and environmental stress conditions (Abd El-Ghany and Babazadeh 2022; Wang et al. 2022; Bhatt et al. 2024). Turmeric extract, derived from *Curcuma longa* and enriched in curcuminoids, exhibits pronounced antioxidant and anti-inflammatory activities and has been investigated for its capacity to influence immune responsiveness, redox balance, and gastrointestinal functionality in poultry (Galli et al. 2020; Yadav et al. 2020). Despite their distinct mechanisms, these additives converge on shared metabolic and intestinal pathways that collectively shape muscle growth and efficiency of nutrient utilization.

Efficient growth in broiler chickens is closely associated with the structural and functional status of the gastrointestinal tract, as the intestinal epithelium constitutes the principal interface for nutrient uptake and host–microbial interactions (Wang et al. 2020). Alterations in villus architecture, crypt dynamics, and goblet cell populations directly influence absorptive capacity and barrier function, thereby affecting systemic metabolism and muscle development (Broom 2018; Gilani et al. 2021; Peng et al. 2023).

Recent advances in poultry nutrition have emphasized integrative and nutrigenomic approaches linking dietary bioactive compounds with molecular responses, intestinal functionality, and growth performance. However, most studies have examined methionine, betaine, or turmeric supplementation in isolation, with limited emphasis on the coordinated relationship between myogenic gene expression, systemic protein metabolism, and intestinal histomorphology within a unified, multi-level framework (Gilani et al. 2021; Alagawany et al. 2022; Naeem and Fatima 2025).

Accordingly, the present study examines the combined influence of dietary methionine, betaine, and turmeric extract on myogenesis and growth-associated gene expression in broiler chickens. Skeletal muscle development is orchestrated by a network of myogenic regulatory factors (MRFs), including myogenic differentiation factor (MyoD), Myf5, Myogenin (MyoG), and MRF4, which govern successive stages of myogenic lineage commitment, differentiation, and myofiber maturation. Primary MRFs (MyoD and Myf5) govern early myogenic commitment, whereas secondary MRFs (MyoG and MRF4) regulate terminal differentiation and myotube formation (Zhang et al. 1995; Berkes and Tapscott 2005; Wen et al. 2014; Allymehr et al. 2024). In contrast, Myostatin (MSTN), a member of the transforming growth factor-β (TGF-β) superfamily, functions as a central inhibitory regulator of skeletal muscle development by limiting myogenic differentiation and muscle fiber hypertrophy (Sakr et al. 2020; Omidizadeh et al. 2021). Despite this established role, limited information is available on how dietary phytobiotics and methyl-donor nutrients coordinately modulate these gene pathways alongside systemic protein metabolism and intestinal histomorphology in broiler chickens (Naeem and Fatima 2025).

Therefore, this study investigated the effects of dietary supplementation with methionine, betaine, and turmeric extract on growth performance, serum protein profile, myogenic gene expression, and intestinal histomorphology in broiler chickens, with the aim of providing an integrated mechanistic perspective on how these additives influence gut function, protein metabolism, and muscle development to improve overall production efficiency.

Materials and methods

Ethical approval

All procedures involving live birds were reviewed and approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Benha University, Egypt, and were conducted in compliance with internationally recognized standards for the care and use of laboratory animals, including the ARRIVE guidelines and applicable institutional regulations (Approval No. BUFVTM 60-12-24).

Birds, housing, and management

A total of 108 one-day-old Hubbard broiler chicks were sourced from Pyramids Poultry Company **(Al-Haram, Giza, Egypt)**. The trial was carried out from April 29 to June 3, 2024, corresponding to a 35-day rearing period. Birds were housed in floor pens lined with wood-shavings litter at the experimental facilities of the Faculty of Veterinary Medicine, Benha University, Egypt. Chicks received a starter diet from day 0 to 11, a grower diet from day 11 to 23, and a finisher diet from day 23 to 35, with feed and water provided ad libitum throughout the study.

A 23-h daily lighting program was implemented. Brooding temperature was maintained at 35–32 °C during the first week and gradually reduced on a weekly basis to reach

approximately 24 °C by the end of the experimental period. Relative humidity was kept within 65–75%, and ventilation was ensured using natural airflow and low-pressure fans. Birds were vaccinated in accordance with the recommended immunization schedule against Newcastle disease (ND), avian influenza (AI), and infectious bronchitis (IB), following the protocol outlined by (Ayoub et al. 2019).

Experimental design and dietary treatments

Experimental diets were formulated to satisfy the nutrient requirements of broiler chickens as outlined by the National Research Council (NRC 1994**)**. The 108 chicks were randomly assigned to four dietary treatment groups, each consisting of three replicates with nine birds per replicate (27 birds per treatment).

Group 1 Basal diet (control).

Group 2 Basal diet formulated with DL-methionine (1 g/kg ration) (Pokoo-Aikins et al. 2021).

Group 3 Basal diet formulated with betaine anhydrous (1 g/kg ration) (Chen et al. 2022).

Group 4 Basal diet formulated with turmeric extract (200 mg/kg ration) (Utami et al. 2020; Sureshbabu et al. 2023).

The inclusion levels of DL-methionine (1 g/kg diet) and betaine anhydrous (1 g/kg diet) were selected based on previously reported effective supplementation levels in broiler nutrition studies evaluating growth performance, carcass traits, and antioxidant status (Pokoo-aikins et al. 2021; Chen et al. 2022). The inclusion level of turmeric extract (200 mg/kg diet) was selected as a moderate supplementation level within the effective range previously reported in broiler chickens and has been shown to improve antioxidant status, growth-related gene expression, and carcass characteristics (Utami et al. 2020; Sureshbabu et al. 2023).

DL-methionine was obtained from Al-Wadi 3 M Factory **(Qalyubia**, **Egypt)**, while betaine anhydrous and turmeric extract were obtained from AB Chem Pharmaceutical Raw Materials Company **(Dakahlia**, **Egypt)**. The ingredient composition and calculated nutrient profile of the experimental diets are presented in Table 1. The purity and physical form of each supplement complied with the manufacturers' specifications for feed-grade additives.

At the end of the experimental period (day 35), twelve birds (three birds per treatment) were randomly selected for sampling. The birds were humanely euthanized via manual cervical dislocation performed by a trained professional to

Table 1 Ingredients and calculated chemical composition of the experimental diets

Ingredients	Starter (%) (0–11 d)	Grower (%) (11–23 d)	Finisher (%) (23–35 d)
Yellow corn grain	53.4	58.18	64.8
Soybean meal (46% CP)	37.5	31	24.3
Corn gluten meal	3.5	4	4.5
Vegetable oil	0.7	1.6	2.5
Limestone	1.8	1.6	1.3
Monocalcium phosphate	1.5	1.35	1
L-Lysine	0.25	0.25	0.27
DL-methionine	0.21	0.23	0.21
Vitamin–mineral premix [a]	0.3	0.3	0.3
Sodium chloride	0.25	0.25	0.25
Sodium bicarbonate	0.17	0.17	0.17
L-threonine	0.04	0.06	0.07
Anticoccidial	0.25	0.25	0.25
Antimycotoxin	0.1	0.1	0.1
Choline chloride	0.05	0.05	0.05
Nutrients(%,unless otherwise stated)			
Dry Matter	86.58	86.57	86.63
Metabolizable energy (Mcal/kg)	2.88	3.01	3.15
Crude protein	22.79	20.75	18.62
Crude fiber	3.17	2.93	2.68
Calcium	1.02	0.91	0.74
Available phosphorus	0.44	0.41	0.35
Sodium	0.17	0.17	0.17
Chloride	0.24	0.24	0.25
Choline	2.26 mg/g	2.16 mg/g	2.05 mg/g
Folate	0.90 mg/g	0.88 mg/g	0.85 mg/g
Digestible Lysine	1.25	1.11	0.97
Digestible Methionine	0.58	0.58	0.54
Digestible Methionine + cysteine	1.02	0.97	0.89
Digestible Threonine	0.75	0.71	0.64
Digestible tryptophan	0.23	0.20	0.17
Digestible arginine	1.41	1.24	1.07
Digestible valine	1.07	0.97	0.87
Lysine	1.46	1.29	1.12
Methionine	0.61	0.61	0.57
Threonine	0.93	0.87	0.78
Arginine	1.44	1.27	1.10
Potassium	0.92	0.81	0.70
Linoleic acid	1.33	1.42	1.53
Acidity and alkalinity	0.00	0.00	0.00
Total valine	1.10	1.00	0.89

Table 1 (continued)

Ingredients	Starter (%) (0–11 d)	Grower (%) (11–23 d)	Finisher (%) (23–35 d)
Tryptophan	0.33	0.29	0.25
Methionine +cysteine	0.99	0.96	0.89

[a]Vitamin–mineral premix (code 102; Misr Feed Additives Company, Egypt) was used in the experimental diets. Each 3 kg of premix contained 12,000,000 IU of vitamin A, 5,000,000 IU of vitamin D_3, 15,000 mg of vitamin E, 3,000 mg of vitamin K_3, 2,000 mg of vitamin B_1 (thiamine), 6,000 mg of vitamin B_2 (riboflavin), 3,000 mg of vitamin B_6 (pyridoxine), and 15 mg of vitamin B_{12} (cyanocobalamin). It also provided 10,000 mg of pantothenic acid, 40,000 mg of niacin, 1,500 mg of folic acid, and 75 mg of biotin. The mineral mixture supplied 600 g of choline chloride, 100 g of manganese, 80 g of zinc, 40 g of iron, 10 g of copper, 1 g of iodine, and 0.3 g of selenium. Calcium carbonate was used as a carrier up to 3 kg. The premix was included in the diet at a rate of 3 kg per metric ton of feed. The premix was added to all experimental diets to meet the birds' vitamin and mineral requirements according to NRC (1994)

ensure rapid loss of consciousness and minimal distress. This method was applied in accordance with the AVMA Guidelines for the Euthanasia of Animals (2020) and was selected to avoid the use of chemical anesthetics that could potentially interfere with physiological, biochemical, and molecular parameters. All animal handling and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, Benha University, and were conducted in accordance with the approved institutional ethical standards.

Growth performance measurements

Body weight was measured at day 0 and at the completion of each feeding phase (days 11, 23, and 35), and feed intake was recorded at the corresponding time points in accordance with a phase-based growth assessment approach (Ahsan and Cengiz 2020; Kuter and Önol 2021). Feed intake for each replicate was determined as the difference between the amount of feed offered and feed residuals.

Body weight gain (BWG) was calculated as the difference between final and initial body weights, while average daily gain (ADG) was obtained by dividing BWG by the number of experimental days per phase. Feed conversion ratio (FCR) was computed as feed intake per unit of BWG and used as an indicator of feed efficiency. Relative growth rate (RGR) was determined following the method of (Brody and Lardy 1946) and expressed as the proportional increase in body weight relative to mean body weight.

All growth performance parameters were calculated on a replicate basis using standard equations as previously described by (Lambert et al. 1936) and (Brody and Lardy 1946). Mortality was recorded daily and used to adjust FCR calculations when applicable.

Serum biochemical analysis

On day 35, blood (2 mL) was collected from jugular vein of 12 birds (three birds per treatment) using sterile syringes and transferred into plain tubes (without anticoagulant). The samples were allowed to clot and then centrifuged at 2,500 × g for 15 min to separate serum, which was subsequently stored at −20 °C for later analysis. Serum total protein and albumin concentrations were quantified using commercial colorimetric assay kits based on the Biuret and bromocresol green methods, respectively (Weichselbaum 1946; Doumas et al. 1971). Globulin concentration was derived as the difference between total protein and albumin values (Bovo et al. 2015).

Gene expression analysis

Sample collection

Immediately following euthanasia at day 35, pectoralis major muscle samples were collected from three birds per treatment ($n=3$ per group). Tissue samples were harvested within 1–2 min to ensure maximal RNA stability. The target muscle tissues were precisely excised using sterile, RNase-free surgical tools and immediately immersed in RNAlater stabilization solution to preserve RNA integrity. Samples were maintained at 4 °C for short-term stabilization and subsequently transferred to an ultra-low temperature freezer (−80 °C) for long-term storage until total RNA extraction. This rapid sampling protocol was strictly followed to minimize RNA degradation and prevent stress-induced alterations in gene expression profiles.

RNA extraction and cDNA synthesis

Total RNA was isolated using the GF-1 Total RNA Extraction Kit **(Vivantis, Malaysia)** in accordance with the manufacturer's protocol. RNA purity and concentration were assessed spectrophotometrically, and integrity was confirmed by agarose gel electrophoresis. Samples exhibiting A260/A280 ratios between 1.8 and 2.0 were considered suitable for downstream analyses. First-strand cDNA was synthesized from 1 µg of total RNA using the COSMO cDNA PLUS Synthesis Kit **(Willowfort, UK)**.

Quantitative real-time PCR

Quantitative real-time PCR was conducted using the HERA SYBR qPCR Kit **(Willowfort, UK)** on a Real-Time PCR

System **(Applied Biosystems**, **USA)**. Relative mRNA expression levels of MyoD, Myogenin, and Myostatin were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001), with β-actin serving as the reference gene. Primer sequences are listed in Table 2, and all reactions were performed in technical triplicates.

Intestinal histomorphology

On day 35, intestinal segments from the duodenum, jejunum, and ileum were collected from three birds per treatment, and approximately 2.5 cm from each segment was excised and trimmed. Tissues were immediately fixed in 10% neutral buffered formalin for 72 h. Following fixation, samples were dehydrated through a graded ethanol series and embedded in paraffin. Serial longitudinal Sect. (5 µm) were prepared using a rotary microtome **(RM 2145**, **Leica Microsystems**, **Wetzlar**, **Germany)** for subsequent histological evaluation. Sections were mounted on glass slides and routinely stained with hematoxylin and eosin (H&E). Histomorphometric evaluation was conducted using ImageJ software **(National Institutes of Health**, **MD**, **USA)**. Villus height was determined from the villus tip to the villus–crypt junction, villus width was measured at the mid-point of the villus, and crypt depth was assessed from the crypt–villus junction to the base of the crypt, following the criteria described by (Baurhoo et al. 2007; Belote et al. 2023). Goblet cell density was calculated as the number of goblet cells per unit surface area (mm^2).

Statistical analysis

Data were processed using IBM SPSS Statistics for Windows **(Version 25.0; IBM Corp.**, **Armonk**, **NY**, **USA)**. Treatment effects were evaluated by one-way analysis of variance (ANOVA), and mean separation was performed using Duncan's multiple range test (DMRT). Results are presented as means ± standard error (SE), and statistical significance was set at $P<0.05$. Prior to ANOVA, assumptions of normality and homogeneity of variance were verified. Graphical outputs were generated using GraphPad Prism **(Version 10.0; GraphPad Software**, **San Diego**, **CA**, **USA)**.

Results

Growth performance

As presented in Table 3, final body weight, total body weight gain, average daily gain, and relative growth rate were significantly influenced by dietary treatments ($P<0.01$). The methionine- and betaine-treated groups consistently exhibited the highest values compared with the control and turmeric-supplemented groups.

Cumulative feed conversion ratio (FCR) was significantly reduced ($P<0.05$) in birds receiving methionine and betaine, whereas total feed intake did not differ among treatments ($P>0.05$).

Total serum protein, albumin and globulin

As shown in Table 4, serum concentrations of total protein, albumin, and globulin were significantly affected by dietary supplementation ($P<0.05$). The methionine- and betaine-treated groups recorded the highest values across all parameters, while the lowest concentrations were consistently observed in the control and turmeric-treated birds.

Growth- related genes expression

As illustrated in Fig. 1A–C, the relative expression of myogenic differentiation factor (MyoD; Fig. 1A) did not differ significantly among dietary treatments ($P>0.05$). In contrast, myogenin (MyoG; Fig. 1B) was significantly upregulated, and myostatin (MSTN; Fig. 1C) was significantly downregulated, particularly in birds receiving the turmeric-based diet ($P<0.05$).

Intestinal histomorphology: duodenum, jejunum, and ileum

The quantitative and qualitative assessments of intestinal histomorphology are presented in Tables 5, 6 and 7 and illustrated in Figs. 2A–C and 3, and 4, with representative photomicrographs shown in Figs. 5, 6 and 7.

Across all intestinal segments (duodenum, jejunum, and ileum), villus height showed a highly significant treatment

Table 2 Primers used for quantitative real-time PCR

Gene	Primer sequence(5`-3`)	product size(bp)	Reference
B-actin	**Forward**: ACCCCAAAGCCAACAGA **Reverse**: CCAGAGTCCATCACAATACC	**136**	(Mohammed et al. 2021)
MyoD	**Forward**: GATTTCCACAGACAACTCCACAT **Reverse**: GAATCTGGGCTCCACTGTCACT	**116**	(Fergany et al. 2017)
MyoG	**Forward**: GTGGGATGGTGATGCTGGAA **Reverse**: TTGGAGAGGAGTGGGAAAGGA	**109**	(Fergany et al. 2017)
MSTN	**Forward**: GCAAAAGCTAGCAGTCTATG **Reverse**: TCCGTCTTTTTCAGCGTTCT	**119**	(Dushyanth et al. 2016; Sakr et al. 2022)

Table 3 Effect of dietary treatments on growth performance in broiler chickens

Parameter	Control	Methionine	Betaine	Turmeric extract	*P*-value
Initial BW(g)	46.85±0.61	46.30±0.57	46.30±0.69	45.93±0.66	o.778
Starter BW(g)	290.74±5.77	300.19±5.70	285.85±6.96	281.11±6.16	0.162
Grower BW(g)	947.59±20.54^{b}	1044.26±18.88^{a}	1011.48±12.98^{a}	956.3±18.75^{b}	<0.001
Final BW(g)	1759.81±32.16^{b}	1892.78±29.26^{a}	1876.85±25.11^{a}	1758.48±43.41^{b}	0.004
BWG starter(g)	243.89±5.92	253.89±5.59	239.56±6.99	235.19±6.09	0.176
BWG grower(g)	656.85±17.46^{b}	744.07±14.13^{a}	725.63±9.42^{a}	675.19±14.86^{b}	<0.001
BWG finisher(g)	812.22±17.49	848.52±20.9	865.37±18.12	802.19±30.37	0.155
Total BWG(g)	1712.96±32.05^{b}	1846.48±29.33^{a}	1830.56±25.17^{a}	1712.56±43.35^{b}	0.003
FI starter (g)	270.22±0.32^{c}	289.04±2.06^{b}	284.39±2.04^{b}	299.56±2.67^{a}	<0.001
FI grower(g)	1014.33±9.08^{c}	1066.03±4.60^{a}	1035.17±5.26^{b}	1032.14±7.55^{bc}	<0.001
FI finisher(g)	1506.37±11.98	1466.18±8.95	1478.65±3.01	1469.44±19.52	0.096
Total FI each chick (g)	2790.92±19.26	2821.25±15.35	2798.22±3.34	2801.15±29.48	0.716
FCR starter	1.13.00±0.03^{b}	1.15.00±0.03^{b}	1.22.00±0.05^{ab}	1.30±0.04^{a}	0.007
FCR grower	1.59.00±0.07^{a}	1.45.00±0.03^{b}	1.43.00±0.02^{b}	1.55±0.04^{ab}	0.021
FCR finisher	1.88.00±0.05	1.76.00±0.06	1.73.00±0.04	1.91±0.09	0.098
Cumulative FCR	1.65.00±0.04^{a}	1.54.00±0.02^{b}	1.54.00±0.02^{b}	1.66±0.05^{a}	0.011
RGR starter (%)	144.00±1.33	146.25±0.93	143.48±1.70	143.36±1.27	0.388
RGR grower (%)	105.78±1.45^{b}	110.68±0.64^{a}	112.13±1.32^{a}	109.10±1.12^{a}	0.002
RGR finisher (%)	60.16±1.01	57.83±1.25	59.88±0.91	58.69±1.43	0.465
Total RGR (%)	189.54±0.22^{c}	190.39±0.19^{a}	190.33±0.20^{ab}	189.65±0.33^{bc}	0.002
ADG starter(g/day)	22.17±0.54	23.08±0.51	21.78±0.64	21.38±0.55	0.176
ADG grower (g/day)	54.74±1.45^{b}	62.01±1.18^{a}	60.47±0.78^{a}	56.27±1.24^{b}	<0.001
ADG finisher (g/day)	67.69±1.46	70.71±1.74	72.11±1.51	66.85±2.53	0.155
Total ADG (g/day)	48.94±0.92^{b}	52.76±0.84^{a}	52.30±0.72^{a}	48.93±1.24^{b}	0.003

Values are expressed as means±SE. Means in the same row with different superscript letters (a, b, c) differ significantly ($P<0.05$). BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; RGR (,relative growth rate; ADG, average daily gain

Table 4 Effect of dietary treatments on serum biochemical parameters in broiler chickens

parameter	Control	Methionine	Betaine	Turmeric extract	*P*-value
Total Protein (g/dL^{-1})	3.22±0.12^{b}	5.34±0.80^{a}	5.31±0.17^{a}	2.99±0.09^{b}	0.005
Albumin (g/dL^{-1})	1.07±0.07^{b}	1.60±0.15^{a}	1.46±0.13^{a}	1.09±0.06^{b}	0.019
Globulin (g/dL^{-1})	2.15±0.09^{b}	3.75±0.91^{a}	3.85±0.05^{a}	1.90±0.14^{b}	0.032

Values are expressed as means±SE. Means in the same row with different superscript letters (a, b) differ significantly ($P<0.05$)

effect ($P<0.001$), with methionine-fed birds consistently exhibiting the greatest values, followed by betaine and turmeric extract, whereas the control group recorded the lowest values. Crypt depth also differed significantly among treatments ($P<0.001$), with methionine and/or betaine generally producing deeper crypts compared with the control across all segments.

Goblet cell density was significantly affected by dietary supplementation ($P<0.001$), reaching the highest levels in the methionine group, followed by betaine and turmeric extract, while the lowest counts were observed in the control birds throughout the small intestine. In contrast, villus width and the villus height-to-crypt depth ratio did not show significant differences among treatments in most intestinal regions ($P>0.05$).

Discussion

This study demonstrates that dietary supplementation with methionine, betaine, and turmeric extract exerts complementary yet mechanistically distinct effects on broiler growth performance, systemic protein metabolism, molecular regulation of myogenesis, and intestinal mucosal architecture, reflecting an integrated nutrigenomic response linking dietary inputs and physiological adaptation.

The observed enhancements in growth indices, including body weight and feed efficiency, may be attributed to improved nutrient utilization and enhanced growth efficiency in birds receiving dietary additives, consistent with previous reports (Gilani et al. 2021).

The positive growth response associated with methionine supplementation can be primarily explained by its fundamental involvement in protein synthesis and muscle tissue accretion, in addition to its role as a precursor for cysteine

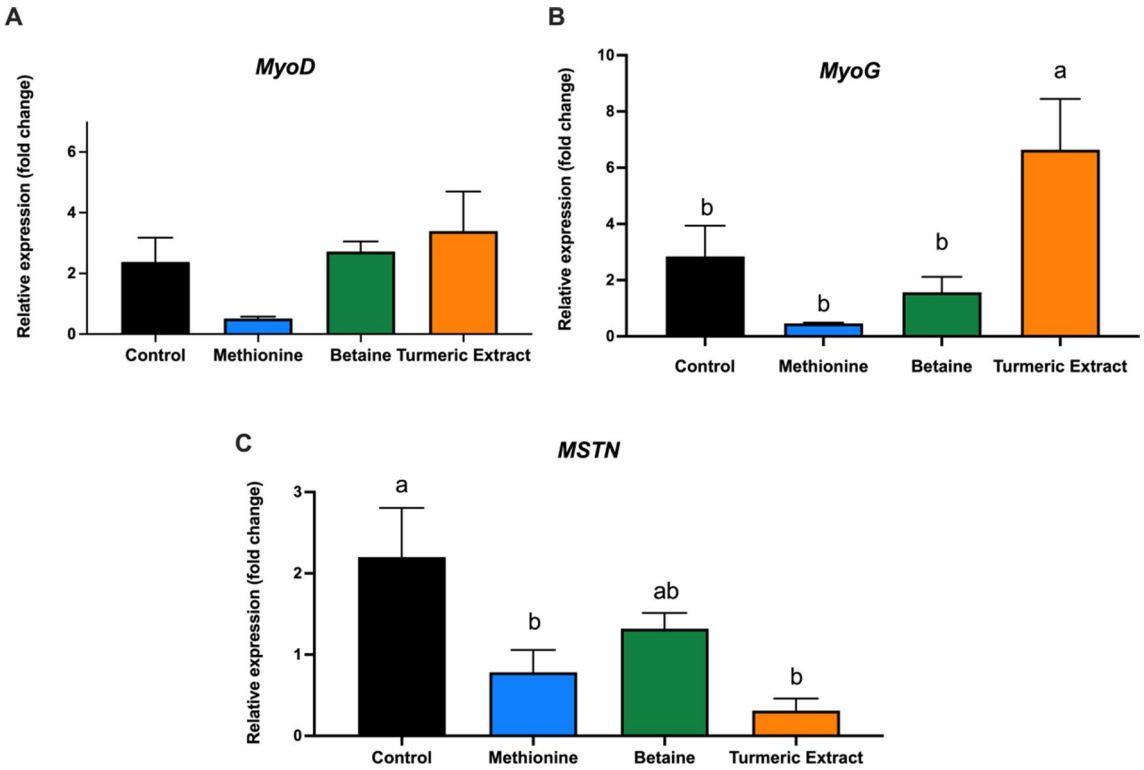


Fig. 1 Relative gene expression of MyoD (**A**), MyoG (**B**), and MSTN (**C**) in broiler chickens among the experimental groups. Data are presented as means ± SE. Bars with different superscript letters differ significantly among treatments, whereas bars sharing the same letter do not differ significantly ($P < 0.05$)

Table 5 Effect of dietary treatments on duodenal histomorphological parameters of broiler chickens

Parameter	Control	Methionine	Betaine	Turmeric extract	*P*-value
Villus height (µm)	195.96 ± 13.55^{d}	501.69 ± 1.41a	398.38 ± 3.94^{b}	292.62 ± 8.44^{c}	< 0.001
Villus width (µm)	47.85 ± 1.54^{b}	53.13 ± 0.96^{b}	67.43 ± 3.04^{a}	49.84 ± 4.10b	0.004
Crypt depth (µm)	36.85 ± 6.63^{c}	127.56 ± 5.49^{a}	104.79 ± 1.94^{b}	107.11 ± 3.66^{b}	< 0.001
Goblet cell count (no/mm²)	115.67 ± 6.89^{c}	218.00 ± 5.69^{a}	154.33 ± 6.69^{b}	144.00 ± 8.19^{b}	< 0.001
Villous height/Crypt depth ratio	5.91 ± 1.65	3.95 ± 0.15	3.81 ± 0.10	2.74 ± 0.15	0.133

Values are expressed as means ± SE. Means in the same row with different superscript letters (a, b, c, d) differ significantly ($P < 0.05$)

Table 6 Effect of dietary treatments on jejunal histomorphological parameters of broiler chickens

Parameter	Control	Methionine	Betaine	Turmeric extract	*P*-value
Villus height (µm)	383.61 ± 32.46^{c}	713.86 ± 11.55^{a}	671.80 ± 5.37^{a}	522.82 ± 8.61^{b}	< 0.001
Villus width (µm)	48.41 ± 5.01	58.60 ± 4.68	48.23 ± 6.96	56.80 ± 3.49	0.400
Crypt depth (µm)	80.15 ± 5.85^{c}	175.19 ± 6.80^{a}	156.20 ± 6.32^{a}	114.89 ± 4.84^{b}	< 0.001
Goblet cell count (no/mm²)	330.33 ± 18.41^{d}	556.33 ± 23.98^{a}	477.67 ± 12.55^{b}	406.33 ± 4.37^{c}	< 0.001
Villous height/Crypt depth ratio	4.88 ± 0.69	4.08 ± 0.09	4.31 ± 0.14	4.56 ± 0.17	0.484

Values are expressed as means ± SE. Means in the same row with different superscript letters (a, b, c, d) differ significantly ($P < 0.05$)

and creatine biosynthesis. These metabolic processes rely heavily on S-adenosylmethionine–dependent methyl group transfer, linking methionine availability to anabolic capacity and growth performance (Brosnan et al. 2007; Willke 2014; Babazadeh and Ahmadi Simab 2022). In agreement with these mechanisms, (Pokoo-Aikins et al. 2021) this study demonstrates that dietary inclusion of DL-methionine at 1 g/kg resulted in significant improvements in feed conversion efficiency and average daily gain.

The growth-enhancing effects of betaine are largely attributed to its dual physiological function as both a methyl donor and an osmoprotective compound. By maintaining intestinal epithelial integrity and supporting one-carbon metabolism, betaine facilitates nutrient absorption while conserving methionine for protein synthesis, thereby

Table 7 Effect of dietary treatments on ileal histomorphological parameters of broiler chickens

Parameter	Control	Methionine	Betaine	Turmeric extract	*P*-value
Villus height (µm)	165.33 ± 16.12^{c}	343.23 ± 19.93^{a}	320.49 ± 14.51^{ab}	265.11 ± 27.05^{b}	0.001
Villus width (µm)	59.72 ± 5.21	49.54 ± 1.93	52.28 ± 4.55	52.13 ± 3.46	0.367
Crypt depth (µm)	26.52 ± 3.29^{b}	62.05 ± 4.28^{a}	65.26 ± 6.56^{a}	59.77 ± 4.14^{a}	0.001
Goblet cell count (no/mm²)	60.00 ± 8.39^{c}	181.00 ± 10.12^{a}	143.33 ± 5.24^{b}	121.33 ± 4.06^{b}	<0.001
Villous height/Crypt depth ratio	6.46 ± 1.14	5.63 ± 0.72	5.07 ± 0.81	4.41 ± 0.18	0.371

Values are expressed as means ± SE. Means in the same row with different superscript letters (a, b, c, d) differ significantly ($P < 0.05$)

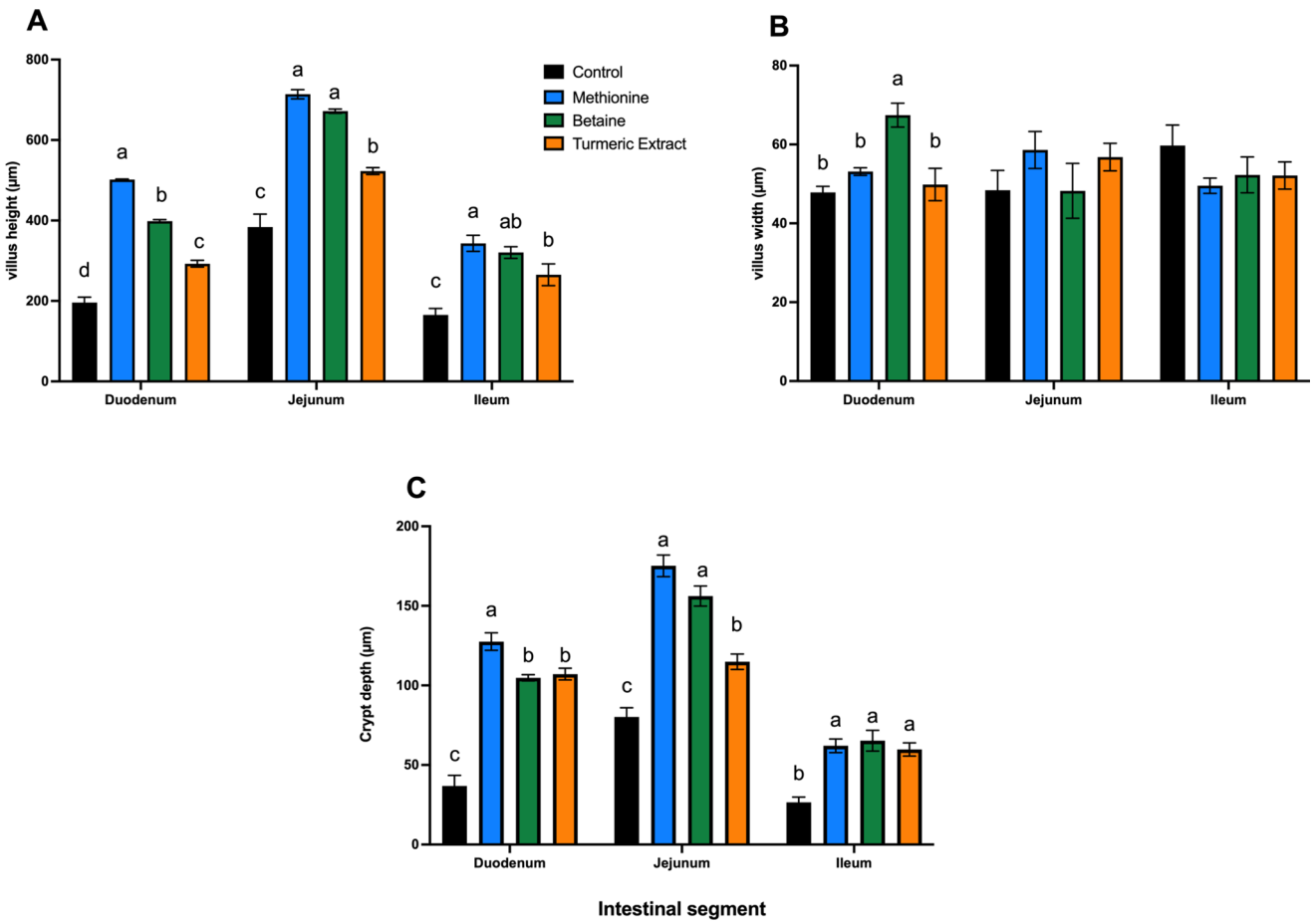


Fig. 2 Effects of dietary methionine, betaine, and turmeric extract on intestinal histomorphological parameters of broiler chickens at 35 days of age. (**A**) Villus height (µm), (**B**) villus width (µm), and (**C**) crypt depth (µm) in the duodenum, jejunum, and ileum. Data are presented as means ± SE. Bars with different superscript letters within the same intestinal segment indicate significant differences among treatments, whereas bars with the same letter or without superscript letters indicate no significant difference. Statistical significance was considered at ($P < 0.05$)

promoting muscle deposition and feed efficiency (Honarbakhsh et al. 2007; Ratriyanto et al. 2010; Sayed and Downing 2011; Abd El-Ghany and Babazadeh 2022). Supporting this concept, (Chen et al. 2022) reported that dietary anhydrous betaine at 1 g/kg significantly enhanced average daily gain, feed conversion ratio, and breast muscle yield, effects that were associated with activation of Nrf2 and HO-1 signaling pathways and improved antioxidant status. Similarly, (Chen et al. 2018) demonstrated that dietary betaine supplementation at 1,000 mg/kg elicited dose-dependent enhancements in body weight gain and feed efficiency, effects that were associated with stimulation of the IGF-1/mTOR signaling axis and a concomitant increase in skeletal muscle anabolic activity.

The comparatively moderate growth response observed in the turmeric extract–treated group aligns with the well-documented antioxidant and anti-inflammatory properties of curcuminoids. Curcumin has been shown to activate

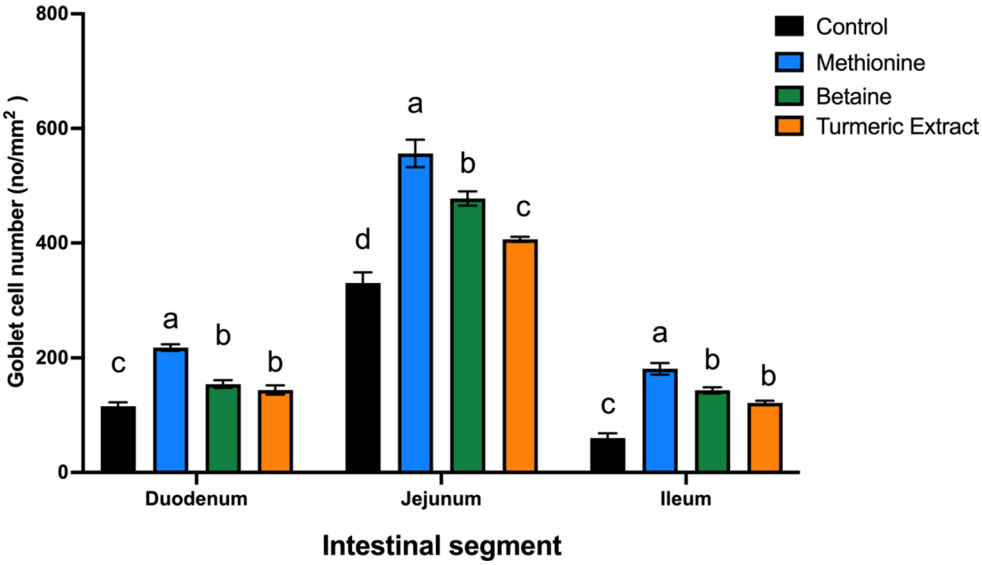


Fig. 3 Effect of dietary treatment on goblet cell density (number of goblet cells per mm^2 of intestinal mucosa) in the duodenum, jejunum, and ileum of broiler chickens at 35 days of age. Values are expressed as means ± SE. Bars with different superscript letters within the same intestinal segment indicate significant differences among treatments, whereas bars with the same letter or without superscript letters indicate no significant difference. Statistical significance was considered at ($P < 0.05$)

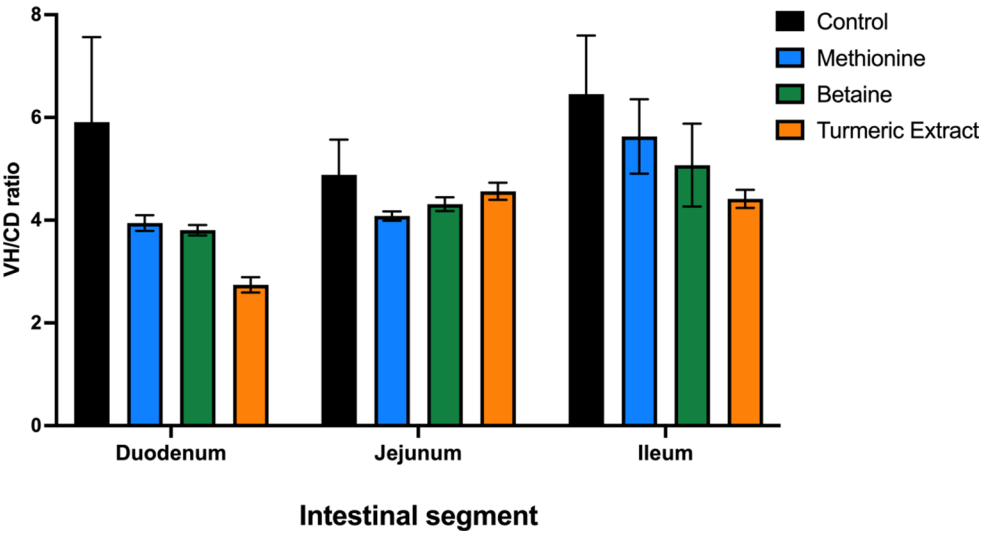


Fig. 4 Effect of dietary treatments on villus height to crypt depth (VL/CD) ratio in different intestinal segments (duodenum, jejunum, ileum) of broiler chickens at 35 days of age. Values are expressed as means ± SE. No significant differences were observed among dietary treatments within the same intestinal segment ($P > 0.05$)

Nrf2-mediated cytoprotective pathways while suppressing NF-κB–dependent inflammatory signaling, thereby reducing oxidative and inflammatory stress in metabolically active tissues such as the intestine and liver (Shahcheraghi et al. 2021; Sureshbabu et al. 2023). (Rajput et al. 2013) demonstrated that dietary turmeric incorporation at 150–200 mg/kg was associated with enhanced body weight and feed conversion efficiency, supporting the growth-promoting potential observed in the present study, whereas (Wang et al. 2015) observed improvements in feed efficiency without significant increases in final body weight, particularly at moderate doses.

Similarly, combinations of plant-derived bioactive compounds such as clove leaf extract, mangosteen peel extract, and coconut shell liquid smoke have been evaluated as alternatives to antibiotic growth promoters in poultry nutrition, where bioactive mixtures showed trends toward improving feed conversion efficiency despite the absence of significant changes in growth performance parameters, supporting their potential functional role as phytogenic feed additives (Haryati et al. 2024). These findings are consistent with recent evidence demonstrating that supplementation with essential oil blends significantly improves body weight and feed conversion ratio in broiler chickens, likely through enhanced nutrient utilization efficiency and modulation of gut microbial populations (Yu et al. 2024).

These improvements in growth performance appear to be closely associated with the concurrent enhancement of intestinal absorptive capacity, systemic protein metabolism, and regulation of muscle-related gene expression observed in the present study, suggesting that the growth response reflects coordinated nutrigenomic adaptations rather than isolated nutritional effects, consistent with recent evidence highlighting the role of dietary methyl donors and phytobiotics in regulating growth-related metabolic and transcriptional pathways in poultry (Naeem and Fatima 2025).

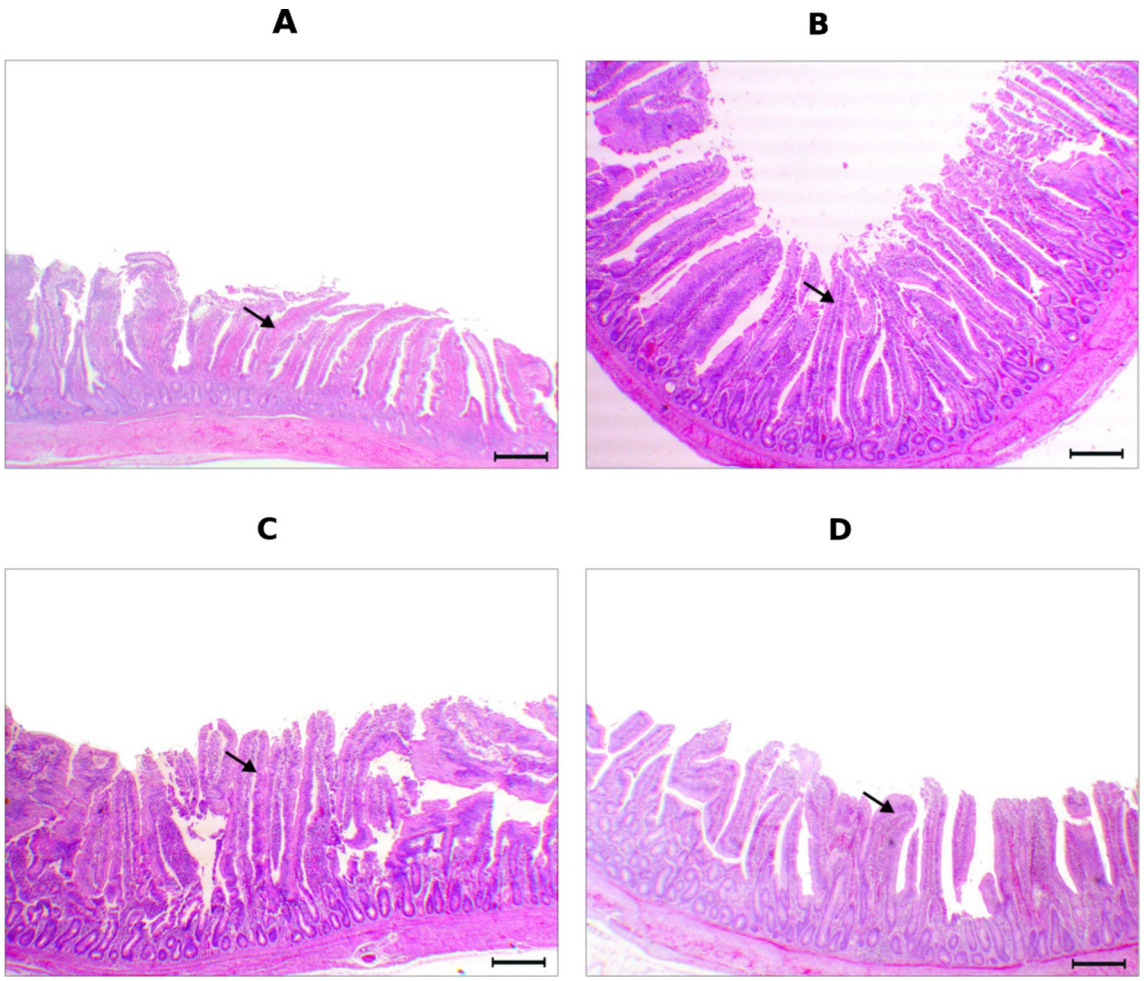


Fig. 5 Representative photomicrographs of the duodenum of broiler chickens illustrating the effects of dietary treatments on intestinal histomorphology. (**A**) Control group showing normal duodenal villi (arrow). (**B**) Methionine-supplemented group showing a marked increase in duodenal villus length (arrow). (**C**) Betaine-supplemented group showing a pronounced increase in duodenal villus length (arrow). (**D**) Turmeric extract-supplemented group showing a mild increase in duodenal villus length (arrow). Sections were stained with hematoxylin and eosin (H&E). Scale bar = 100 µm; magnification X 50

Collectively, these findings indicate that dietary supplementation with methionine, betaine, and turmeric extract improves broiler growth efficiency, with the most pronounced responses observed in the methionine- and betaine-supplemented groups, in agreement with the overall biochemical, molecular, and intestinal outcomes.

Alterations in serum biochemical indices observed in the present study indicate enhanced systemic protein metabolism and immune competence in birds receiving dietary supplementation, as evidenced by increased concentrations of total protein, albumin, and globulin.

The elevation of serum total protein and albumin in methionine- and betaine-fed birds may reflect improved hepatic protein synthesis and amino acid utilization, given the pivotal role of methionine as a limiting amino acid for albumin production. In addition, betaine contributes to homocysteine remethylation via the betaine–homocysteine methyltransferase (BHMT) pathway, thereby sustaining S-adenosylmethionine availability and supporting hepatic metabolic function (Alirezaei et al. 2011; Willke 2014; Abd El-Ghany and Babazadeh 2022; Babazadeh and Ahmadi Simab 2022).

In birds receiving turmeric extract, the observed increases in serum protein fractions are consistent with the hepatoprotective, antioxidant, and immunomodulatory effects of curcumin. These effects are largely mediated through activation of Nrf2-dependent antioxidant signaling and inhibition of NF-κB–driven inflammatory pathways, mechanisms that collectively support hepatic function, humoral immunity, and protein synthesis efficiency (Shahcheraghi et al. 2021; Sureshbabu et al. 2023; Obianwuna et al. 2024). These improvements in circulating protein fractions may also reflect enhanced intestinal absorptive efficiency and improved availability of dietary amino acids observed in the

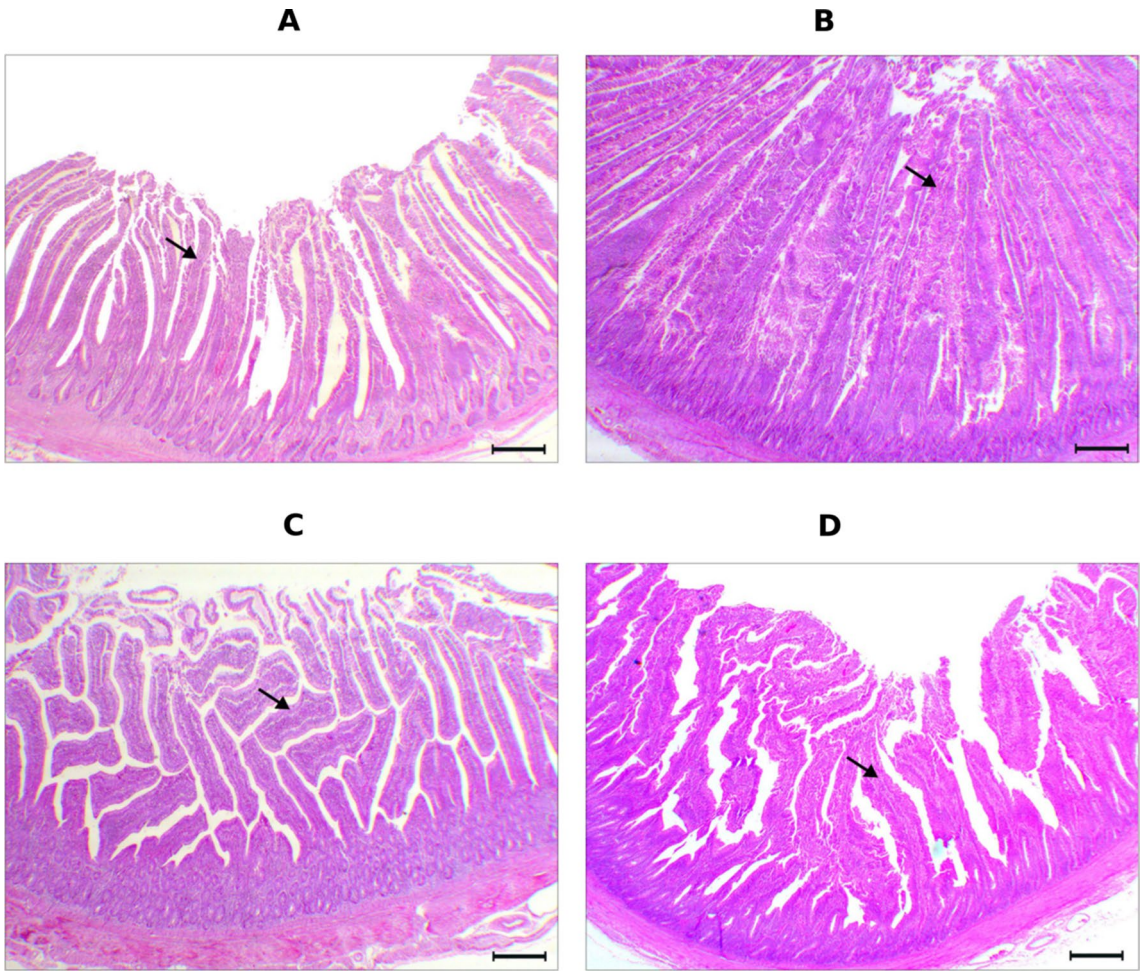


Fig. 6 Representative photomicrographs of the jejunum of broiler chickens illustrating the effects of dietary treatments on intestinal histomorphology. (**A**) Control group showing normal long thin jejunal villi (arrow). (**B**) Methionine-supplemented group showing a marked increase in jejunal villus length (arrow). (**C**) Betaine-supplemented group showing a pronounced increase in jejunal villus length (arrow). (**D**) Turmeric extract-supplemented group showing a mild increase in jejunal villus length (arrow). Sections were stained with hematoxylin and eosin (H&E). Scale bar = 100 µm; magnification X50

present study, further supporting the role of methyl donors and phytogenic compounds in coordinating hepatic protein metabolism with intestinal functional capacity (Naeem and Fatima 2025).

Taken together, the serum protein profile observed in this study closely parallels the improvements in growth performance and intestinal morphology, reinforcing a coordinated relationship between hepatic function, immune status, and nutrient utilization in broiler chickens receiving dietary methionine, betaine, or turmeric extract.

In the present investigation, dietary treatments did not significantly influence MyoD expression, a finding that aligns with the established role of MyoD as a primary regulator of early myogenic commitment rather than late-stage hypertrophic growth. At 35 days of age, broiler muscle development is predominantly characterized by myofiber hypertrophy, during which the transcriptional control exerted by early myogenic regulatory factors becomes less pronounced, while post-transcriptional, metabolic, and signaling mechanisms play a greater role in muscle accretion (Miyake et al. 2007; Bentzinger et al. 2012; Sławińska et al. 2013; Saleh et al. 2024).

In contrast, Myogenin expression showed a clear treatment response, with turmeric extract producing the highest expression, followed by the control group, while methionine and betaine exhibited lower MyoG mRNA abundance. As Myogenin is a key regulator of terminal myogenic differentiation and myofiber maturation, this pattern suggests that turmeric extract may preferentially enhance late-stage differentiation and myofiber fusion. In contrast, methionine and betaine may promote muscle growth through mechanisms less dependent on MyoG transcriptional activation (Berkes and Tapscott 2005; Fergany et al. 2017).

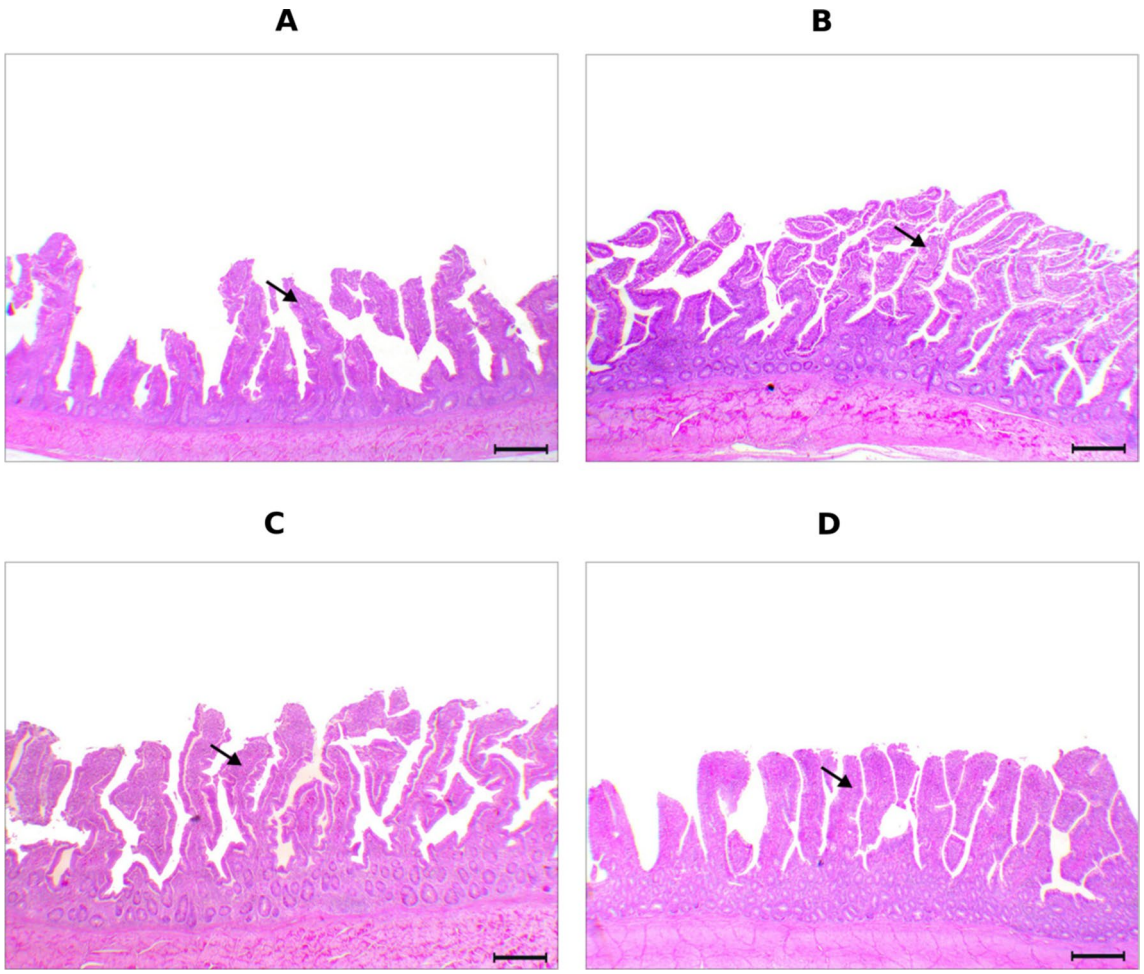


Fig. 7 Representative photomicrographs of the ileum of broiler chickens illustrating the effects of dietary treatments on intestinal histomorphology. (**A**) Control group showing normal blunted ileal villi (arrow). (**B**) Methionine-supplemented group showing a marked increase in ileal villus length (arrow). (**C**) Betaine-supplemented group showing a pronounced increase in ileal villus length (arrow). (**D**) Turmeric extract-supplemented group showing a substantial increase in ileal villus length (arrow). Sections were stained with hematoxylin and eosin (**H&E**). Scale bar = 100 µm. magnification X50

Myostatin (MSTN), a member of the TGF-β superfamily and a well-recognized negative regulator of skeletal muscle growth, was significantly downregulated in all supplemented groups relative to the control. The most pronounced suppression was observed in turmeric-fed birds, followed by methionine and betaine treatments. Reduced MSTN expression is indicative of attenuated inhibitory signaling on muscle hypertrophy and anabolic balance, which is consistent with the enhanced growth performance recorded in supplemented birds (Sakr et al. 2020; Omidizadeh et al. 2021). Previous work in broilers has shown that MSTN expression tends to be elevated in low body-weight phenotypes, whereas MyoD and MyoG expression is higher in birds exhibiting superior growth, underscoring the close association between myogenic regulation and performance traits (Fergany et al. 2017).

Dietary methyl donors such as methionine and betaine regulate muscle-related gene expression through one-carbon metabolism by modulating DNA methylation and histone modifications, thereby influencing transcriptional programs of growth-associated loci including MSTN and IGF-1, which may link nutrient availability directly to myogenic and anabolic pathways in poultry (Naeem and Fatima 2025).

Supporting this concept, (Liu et al. 2010) demonstrated that enhanced methylation of MSTN exon 1 was inversely associated with MSTN mRNA abundance in broiler skeletal muscle, reporting that methionine supplementation (0.61% vs. 0.21% during 4–8 weeks) markedly downregulated MSTN expression. Similarly, (Wen et al. 2014, 2017) observed that high-methionine diets did not significantly alter MyoD or MyoG expression but substantially suppressed MSTN transcription, accompanied by activation of ERK and mTOR signaling pathways. Further support was provided by (Le Quynh Chau et al. 2021), who showed that elevated SID methionine reduced MSTN expression

while enhancing Myf5 and MEF2B expression, resulting in increased breast muscle yield and reduced fat deposition.

However, previous studies have reported that methionine supplementation can increase MyoD expression in broiler skeletal muscle, suggesting enhanced activation of early myogenic regulatory pathways under specific nutritional conditions (Ahmad et al. 2023). The absence of a similar response in the present study may reflect differences in dietary composition, methionine inclusion level, or the developmental stage at sampling. Collectively, these studies support the concept that methionine primarily promotes muscle accretion by relieving myostatin-mediated inhibition and facilitating anabolic signaling rather than directly regulating early myogenic commitment.

The regulatory effects of betaine on myogenic gene expression appear to be context-dependent. (Chen et al. 2018) observed that dietary betaine at 1,000 mg/kg upregulated MyoD and MyoG expression via enhanced MEF2B transcription and IGF-1/mTOR pathway activation, while MSTN expression remained unchanged. In contrast, the present findings showed no stimulation of MyoD and a moderate downregulation of MSTN. Supporting this variability, transcriptomic and in vitro studies have demonstrated that betaine can promote myogenic differentiation through epigenetic modulation and activation of Jak–STAT signaling (Wang et al. 2022), resulting in increased expression of MyoD, Myf5, and MyoG in murine C2C12 cells (Senesi et al. 2013). Moreover, (Chen et al. 2019) observed in Cherry Valley ducks that increasing betaine levels linearly decreased MSTN expression and enhanced mTOR phosphorylation, potentially associated with increased intramuscular methionine availability. These findings suggest that betaine responsiveness may depend on species, tissue specificity, dosage, and developmental stage.

Although direct evidence for turmeric-mediated regulation of myogenic genes in broilers remains limited, its pronounced antioxidant and anti-inflammatory properties provide a plausible mechanistic basis for the observed upregulation of myogenin and suppression of myostatin. Curcumin activates the Nrf2/Keap1/ARE signaling pathway, thereby enhancing cytoprotective enzymes such as HO-1, SOD, and GPx, while concurrently inhibiting NF-κB–dependent inflammatory signaling. These combined effects reduce pro-inflammatory cytokine production and create a cellular environment that favors anabolic myogenic processes (Shahcheraghi et al. 2021; Sureshbabu et al. 2023). Supporting this interpretation, (Hafez et al. 2022) demonstrated that curcumin supplementation downregulated MSTN and upregulated growth-related genes (GHR and IGF-1) in broilers reared under high stocking density. Evidence from mammalian models further indicates that curcumin can enhance MyoD and MyoG expression and promote myotube formation via Wnt5a/Ca^{2+}-dependent signaling (Wang et al. 2024), suggesting conserved regulatory pathways across species.

At the systems level, nutrient-responsive signaling pathways, particularly mTOR and Nrf2, integrate epigenetic regulation with metabolic and antioxidant networks, providing a mechanistic framework through which dietary phytobiotics and methyl donors may translate molecular gene modulation into improved growth performance and physiological resilience in broilers (Naeem and Fatima 2025).

Overall, the combined pattern of unchanged MyoD, altered Myogenin, and downregulated Myostatin suggests that the phytobiotics predominantly acted on later stages of myogenesis and on the balance between anabolic and catabolic signaling pathways rather than on early myogenic commitment.

Quantitative parameters including villus height, crypt depth, and the villus height-to-crypt depth (VH/CD) ratio are widely recognized as reliable indicators of intestinal health and functional integrity in poultry (Ducatelle et al. 2018; Xu et al. 2024).

Across all intestinal segments, methionine and betaine supplementation consistently increased villus height and crypt depth relative to the control, while turmeric extract produced intermediate improvements. Goblet cell density was highest in the methionine group, followed by betaine and turmeric, indicating enhanced mucosal protection and barrier function, which is consistent with evidence that phytobiotics and antioxidant-mediated pathways promote mucin production and reinforce epithelial integrity (Obianwuna et al. 2024). The absence of significant differences in villus width and (VH/CD) ratio suggests that the primary response to phytobiotics was associated with villus elongation and increased crypt activity rather than alterations in villus morphology. When increased crypt depth is accompanied by elongated villi, this pattern is generally interpreted as an indicator of active epithelial renewal and improved absorptive capacity rather than compromised gut integrity (Ducatelle et al. 2018; Gilani et al. 2021).

The improvements observed in methionine-supplemented birds can be attributed to its essential role in protein synthesis and cellular proliferation, which supports enterocyte turnover, epithelial renewal, and maintenance of mucosal structure (Willke 2014; Babazadeh and Ahmadi Simab 2022). Methionine supplementation has also been reported to regulate intestinal epithelial renewal through activation of signaling pathways such as mTOR and Wnt/β-catenin, which play essential roles in maintaining intestinal stem cell proliferation and mucosal integrity. In addition, methionine contributes to antioxidant defense through its metabolic

association with glutathione synthesis, supporting epithelial protection and intestinal functional stability (Liu et al. 2024).

In contrast, the beneficial effects of betaine are primarily attributed to its osmoprotective properties, which help stabilize epithelial cell volume, regulate intracellular osmotic pressure, preserve cellular hydration status, and maintain mucosal integrity under physiological stress conditions, thereby supporting intestinal functional stability and enhancing nutrient absorption efficiency (Eklund et al. 2005; Honarbakhsh et al. 2007; Ratriyanto et al. 2009, 2010; Abd El-Ghany and Babazadeh 2022; Yang et al. 2025).

The moderate but consistent improvements observed with turmeric extract are likely attributable to the antioxidant and anti-inflammatory properties of curcuminoids. Curcumin stimulates Nrf2-dependent cytoprotective pathways while inhibiting NF-κB–mediated inflammatory signaling, thereby maintaining epithelial structure, preserving villus integrity, and enhancing mucosal defense. (Shahcheraghi et al. 2021; Sureshbabu et al. 2023). These interpretations are supported by recent meta-analytic evidence demonstrating increased villus height and reduced crypt depth in broilers receiving curcumin supplementation (Hernández-García et al. 2025).Similarly, dietary supplementation with essential oil blends has been reported to significantly increase jejunal villus height and improve intestinal structural integrity in broiler chickens, supporting enhanced absorptive capacity and growth performance (Yu et al. 2024).

These improvements in intestinal architecture likely contributed to the enhanced nutrient utilization efficiency and systemic metabolic responses observed in the present study, further supporting the coordinated relationship between intestinal function, muscle-related gene expression, and growth performance in supplemented broiler chickens.

Based on the present findings, future investigations may further explore the synergistic interactions between methionine, betaine, and turmeric extract at different inclusion levels and under varying environmental conditions such as heat stress or disease challenge models. Additional studies focusing on epigenetic regulation, muscle development–related gene networks, and intestinal health–associated molecular pathways are warranted to further clarify the mechanisms underlying the observed improvements in growth performance, serum protein profile, and intestinal morphology. Such approaches may contribute to the development of more precise nutritional strategies aimed at improving broiler productivity and health while supporting sustainable antibiotic-free poultry production systems.

Overall, the current study highlights that dietary supplementation with methionine, betaine, and turmeric extract enhances growth performance, serum protein profile, and small-intestinal morphology in broiler chickens. These additives modulate myogenic gene expression by upregulating myogenin and downregulating myostatin, suggesting stimulation of late-stage myogenic differentiation. Among the tested supplements, methionine produced the most consistent and practically relevant benefits under the experimental conditions of this study. However, because the present study relied primarily on mRNA-level measurements without corresponding protein-level validation, interpretation of the functional consequences of gene expression changes should be approached with caution and therefore requires further validation under different production systems, genetic backgrounds, and environmental stress conditions (e.g., heat stress or high stocking density).

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Author contributions Gehad M. Abdelzaher conducted the experiment, performed data analysis, prepared tables and figures, carried out the literature review, and drafted the initial version of the manuscript. Olla A. Khalifa conceptualized and designed the study, developed the experimental protocol, supervised the research, and critically reviewed and edited the manuscript. Samar H. Baloza supervised the study and provided critical revision and editorial input to the manuscript. All authors have read and approved the final version of the manuscript.

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Data availability The datasets generated and analyzed during the present study are available from the corresponding author upon reasonable request.

Declarations

Ethical standards All experimental procedures involving animals were approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Benha University, Egypt (Approval No. BUFVTM.60-12-24) and were conducted in accordance with internationally recognized guidelines for the care and use of laboratory animals.

Consent to publish Not applicable.

Consent to participate Not applicable.

Competing of interests The authors declare no competing interests.

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