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Performance and physiological responses of *E. coli*-challenged broiler chickens to dietary *Moringa oleifera*

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Abstract: This study was conducted to examine the effects of *Moringa oleifera* (MO) seed powder on the performance and gut health of broilers challenged with *E. coli*. A total of 720 one-day-old Ross 308 broiler chicks were randomly allocated to 72 pens across two separate rooms, following a 2 × 6 factorial design. The factors per room included (*i*) challenge: no or yes, and (*ii*) feed additive: control (none), antibiotic [oxytetracycline (OTC) at 0.5 g/kg], MO 0.1%, MO 0.2%, MO 0.4%, and MO 0.8%. At 9 days old, birds in both challenged and non-challenged rooms were inoculated with 1.5 ml of *E. coli*-O157:H7 inoculant (3.8×10^8 CFU) and 1.5 ml of saline, respectively. Performance data showed that, throughout the study, unchallenged birds had better weight gain (WG) and feed conversion ratio (FCR) than challenged birds. On day 35, broilers given OTC and all MO levels showed improved WG ($P < 0.001$) and FCR ($P < 0.003$). Interactions between challenge and additives were observed on day 10 for FCR ($P = 0.031$); on days 24 and 35 for WG ($P < 0.001$); and on days 24 and 35 for FCR ($P < 0.004$ and $P = 0.002$, respectively). On day 24, challenged birds fed all MO levels exhibited higher WG and better FCR than challenged controls, while on day 35, both challenged and unchallenged MO-fed birds showed improved WG and FCR compared to control groups. *E. coli* significantly increased crypt depth (CD), jejunum muscle thickness, and caecal *E. coli* colonies. MO significantly enhanced villus height (VH), the VH : CD ratio, villous tip width, and surface area, while decreasing CD, muscle thickness, and *E. coli* colonies. Challenged birds had significantly lower serum total protein, albumin, and Newcastle Disease Virus (NDV) titres, while serum alanine aminotransferase ALT activity was higher than in non-challenged birds. Overall, Moringa at 0.4% showed comparable or better results than OTC in preventing *E. coli*-induced declines in broiler performance and gut health.

Keywords: *Escherichia coli*; gut histomorphology; meat-type chicken; phytogenics; productivity; serum biochemical

Avian colibacillosis, which is an enteric disease caused by enterotoxigenic *Escherichia coli*, is a highly contagious disease that poses a significant threat to poultry health and production worldwide. This leads to significant economic losses for the poultry industry by increasing mortality and morbidity rates, reducing weight gain, deteriorating feed efficiency, raising condemnation rates at slaughter, and elevating treatment expenses (Nawaz et al. 2024). The condition is associated with various clinical signs, including airsacculitis, pericarditis, perihepatitis, omphalitis, and cellulitis, all of which negatively affect the health and productivity of poultry (Saeed et al. 2025). *E. coli* commonly exists in the digestive tract of chickens, with some studies demonstrating high incidence

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rates in both healthy and diseased birds and proof of colonisation at counts more than 10^6 CFU/g in the caeca, especially for *E. coli* O157:H7 strains. Subsequently, high challenge doses of *E. coli* O157 are biologically pertinent and can result in intense infection and mortality in challenged poultry (Azza et al. 2018). Diseased chickens and their tainted products are also substantial holders of *E. coli*, which affects human health (Wu et al. 2021).

Typically, since the mid-20th century, in-feed non-therapeutic doses of antibiotics have been widely implemented to improve animal performance and to inhibit, cure, and prevent outbreaks of bacterial diseases in farm animals by specifically altering the gut microflora, reducing bacterial fermentation, decreasing the intestinal wall's thickness, and inhibiting bacterial catabolism (Moore et al. 1946). While these medicines can reduce mortality and enhance productivity in infected flocks, their excessive or improper use has increased the global emergence of antibiotic-resistant bacteria and led to the presence of risky residues in poultry products for human beings (Brown et al. 2017). In addition, resistance of *E. coli* to drugs has increased resistance genes such as plasmid-mediated Amp-C beta-lactamases (Amp-C) and/or extended-spectrum beta-lactamases (ESBL) (Laube et al. 2013). Consequently, since 2006, the EU has implemented a complete ban of in-feed use of antibiotics for livestock, including chickens (Anadon 2006). On the other hand, some other countries, such as the USA, Australia, Japan, China, and Canada, under strict regulations have restricted or excluded some antibiotic-derived additives in the feed of food animals (Krysiak et al. 2021; Zheng et al. 2025). Accordingly, new strategies, such as in-feed use of safe potential alternatives to antibiotics like probiotics, prebiotics, organic acids, essential oils, yeast, and phytogenic compounds, must be adopted for controlling *E. coli*, reducing its severity, and improving animals' performance and gut health (Salem et al. 2023).

Moringa oleifera, as one of the medicinal plants, has garnered significant attention in chicken nutrition. *M. oleifera* belongs to the family *Moringaceae* and is commonly known as horseradish or drumstick tree. It possesses both nutritional and therapeutic values (Gul et al. 2024). It has several minerals, vitamins, and phytochemicals. Flavonoids like quercetin, kaempferol, and rutin; alkaloids like moringine and moringinine, monounsaturated fatty acids like oleic acid, palmitic acid, and stearic acid; important amino acids like lysine, threonine, and methionine; fat- and water-soluble vitamins like vitamins A, D, E, C, B1, B2, B3, and B6; and minerals like calcium, magnesium, phosphorus, potassium and iron, and antioxidants are all found in its leaves and seeds (Srivastava et al. 2023). In accordance, in ancient medicine, fruit, leaves, seeds, bark, gum, leaf, and seed oil of the medicinal plant *M. oleifera* have been used for the curing of gastrointestinal, haematological, hepato-renal, cardiovascular, infectious, and inflammatory illnesses (Pareek et al. 2023). Numerous studies indicated that incorporating *M. oleifera* leaf powder or extract into the chickens' diet significantly enhanced their performance by increasing weight gain, improving feed conversion ratio, and enhancing carcass quality. It has been demonstrated that in-feed supplementation of *M. oleifera* improved the growth performance of broilers, resulting from enhanced gut morphology by augmenting villus height and the villus-to-crypt ratio, elevated antioxidant enzyme activity, reduced *E. coli* populations, and promoted the proliferation of beneficial gut bacteria such as *Lactobacillus* (Akib et al. 2024; Gul et al. 2024). Thus, this study aimed to determine the potential of dietary *Moringa oleifera* in improving growth performance, enhancing gut health, modulating serum biochemical indices related to liver function and metabolic status, boosting immune function, and reducing mortality rate in broiler chickens experimentally induced with *E. coli*.

MATERIAL AND METHODS

The experimental procedures were approved by the Animal Ethics Committee at the Department of Animal Production, College of Agricultural Engineering Sciences, University of Duhok (Approval No. UoD AEC17052025).

Moringa oleifera active compounds identification. Active compounds of *Moringa oleifera* seed powder were identified at the laboratories of the Directorate of Environment and Waters/Ministry of Sciences and Technology, Baghdad, in accordance with Ibraheem et al. (2023) using the high-performance liquid chromatography (HPLC) technique (Sykam S 3210, Germany). The active compounds detected in *M. oleifera* along with their concentrations were: flavonoids (quercetin

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1 088 ppm, kaempferol 281 ppm, rutin 147 ppm, apigenin 83.5 ppm, iso rhamnetin 67.9 ppm, luteolin 67.2 ppm, myricetin 57.6 ppm); alkaloids (moringine 139 ppm, moriginine 18.0 ppm, sparteine 5.20 ppm, nicotine 3.40 ppm); fat-soluble vitamins (Vit A 420 ppm, Vit E 150 ppm, Vit K 120 ppm, Vit D 4.30 ppm); water-soluble vitamins (Vit C 1 001 ppm, Vit B3 108 ppm, Vit B6 29.6 ppm, Vit B2 25.4 ppm, Vit B1 15.7 ppm, Vit B5 11.0 ppm, Vit B9 5.40 ppm).

Experimental design and birds' husbandry. The present experiment was conducted at the poultry project of the Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok, to evaluate the effect of *Moringa oleifera* seed powder on the performance and physiology of broiler chickens. *Moringa oleifera* (*Moringa*) seeds were obtained from the local market in Duhok province, Kurdistan Region, Iraq. *Moringa* seeds were shade-dried at room temperature to preserve their quality. The dried seeds were then ground and added as seed powder at different concentrations to the feeds of broilers. A total of 720-day-old Ross 308 chicks were randomly allocated into 12 treatment groups, each with 6 pen replicates of 10 birds each. Treatments were arranged in a 2 × 6 factorial arrangement, *Escherichia coli* (*E. coli*) challenge: yes (+) or no (–); and dietary treatments: control diet (no additive), control diet added with antibiotic oxytetracycline (OTC) at 0.5 g/kg, control diet added with *Moringa oleifera* at 1 g/kg (MO 0.1%), control diet added with *Moringa oleifera* at 2 g/kg (MO 0.2%), control diet added with *Moringa oleifera* at 4 g/kg (MO 0.4%), control diet added with *Moringa oleifera* at 8 g/kg (MO 0.8%). Birds were raised in floor pens (wire-mesh partitioned at 100 × 100 cm), bedded with wood shavings, in two separate, environmentally controlled halls under strict sanitation conditions to prevent non-challenged groups of birds from cross-contaminating each other with *E. coli.* At 7 days of age, all the birds were vaccinated against Newcastle disease. Birds were fed on starter, grower, and finisher diets from d 0 to d 10, d 11 to d 24, and d 25 to d 35, respectively. Three-phase basal diets were formulated using Concept5 feed formulation software (Creative Formulation Concepts, Pierz, Minnesota, USA; https://cfctech.com/contact.aspx) in accordance with the typical nutrient requirements of Ross 308 broiler chickens (Aviagen 2019; Table 1). Feed and water were avail-

Table 1. Ingredient and nutrient composition of the basal starter, grower, and finisher diets as a percentage

Ingredients	Starter	Grower	Finisher
Corn	57.9	59.1	64.0
Soybean meal	35.4	33.3	29.6
Soy oil	0.542	1.83	1.79
Limestone	1.10	0.890	0.753
Dicalcium phosphate	0.832	0.851	0.337
Salt	0.098	0.135	0.211
Sodium bicarbonate	0.373	0.318	0.217
Choline chloride	0.102	0.091	0.084
L-lysine hydrochloride	0.459	0.356	0.121
DL-methionine	0.437	0.364	0.330
L-threonine	0.232	0.167	0.105
Broiler chickens premix*	2.50	2.50	2.50
Nutrient composition (calculated)			
ME (MJ/kg)	12.3	12.8	13.0
Crude protein	23.0	22.0	20.0
Crude fat	3.62	4.92	5.00
Crude fibre	2.79	2.76	2.77
Arginine	1.57	1.51	1.39
Lysine	1.59	1.45	1.17
D. arginine	1.40	1.27	1.00
D. lysine	1.40	1.27	1.00
D. methionine	0.734	0.652	0.604
D. methionine cysteine	1.00	0.910	0.850
D. tryptophan	0.251	0.239	0.217
D. isoleucine	0.850	0.813	0.750
D. threonine	0.880	0.790	0.700
D. valine	0.984	0.944	0.880
Calcium	0.950	0.870	0.700
Phosphorus available	0.500	0.500	0.400
Sodium	0.180	0.180	0.180

*The broiler premix contained per kg: arginine, 2.2 g; valine, 1.4 g; threonine, 58.9 g; tryptophan, 0.4 g; methionine and cysteine, 113.9 g; methionine, 113.5 g; lysine, 113.2 g; choline, 10 411.2 mg; choline chloride, 12 000 mg; §d-pantothenate, 600 mg; calcium-§d-pantothenate, 600 mg; vitamin K3, 100 mg; folic acid, 40 mg; niacin, 1 600 mg; vitamin B12, 1 400 mcg; biotin, 4 mg; vitamin B6, 160 mg; vitamin B2, 280 mg; vitamin B1, 120 mg; vitamin E, 1 200 mg; vitamin D3, 160 000 IU; vitamin A, 400 000 IU; Se, 10 mg; I, 40 mg; Fe, 2.0 g; Zn, 2.4 g; Mn, 3.2 g; Cu, 0.4 g; chloride, 64.0 g; sodium, 50.0 g; available phosphorus, 121.3 g; calcium, 62.0 g; mycotoxin binder, 40 g; citric acid (E330), 0.2 g; propyl gallate (E310), 0.112 g; BHT (E321), 1.34 g; endo-1,4-beta-xylanase, 10 800 IU activity; endo-1,3(4)-beta-gluconate, 2 800 IU activity

D. = digestible; ME = metabolisable energy; MJ = megajoule

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able *ad libitum*. Temperature and light programmes in both facilities were adjusted according to the Ross 308 management guide (Aviagen 2018). From d 0 to d 10, d 11 to d 24, and d 25 to d 35 of age, birds and leftover feed were weighed on a pen base to measure feed intake (FI) and weight gain (WG). Also, mortal birds were recorded whenever they were found, and then the feed conversion ratio (FCR) corrected for mortality was calculated (consumed feed/weight gain).

Preparation and inoculation of E. coli inoculant. The *E. coli* O157:H7 bacterial strain was isolated from commercial broiler farms in Duhok Province, Kurdistan Region, Iraq, and was proliferated in the microbiology laboratory of the Animal Production Department at the University of Duhok. The isolated *E. coli* was first incubated for 24 h at 37 °C in the sterilised MacConkey broth (100 ml). Then, 0.1 ml of the previous broth was subsequently incubated overnight in eosin-methylene blue agar (EMB) for colony counting. 1 000 ml of MacConkey broth was used to inoculate a colony from EMB to get the challenge inoculant. Challenged groups of birds were then orally inoculated with 1.5 ml of the suspension of *E. coli* (3.8×10^8 CFU per ml) at 9 days of age using an automatic syringe. The non-challenged birds received 1.5 ml of 1% (w/v) of sterilised normal saline.

Sample collection. At 24 days of age, two birds per replicate were randomly selected, weighed, and blood samples (approximately 5 ml/bird) were taken from the jugular vein, placed into a non-heparinised tube, and allowed to clot. The birds were then euthanised by cervical dislocation. Clotted blood samples were centrifuged at 3 000 rpm for 15 min, and the serum was collected and stored at −20 °C until used for immune titre and serum biochemical measurements. Approximately 1 cm of the proximal jejunum tissue was collected from each killed chicken, gently flushed, and cleaned using phosphate-buffered saline (pH 7.4). The jejunal samples were then fixed in 10% buffered formalin for subsequent morphological analysis (Hussein et al. 2021).

Jejunum histomorphology. The fixed jejunum samples were cleaned, dried, and embedded in paraffin wax for histomorphometry analysis (Hussein et al. 2021). Consecutive 7 µm longitudinal sections of jejunum tissues were individually mounted on Superfrost® slides using a microtome (Thermo Scientific, Rockville, MD, USA) and stained with haematoxylin and eosin. Light microscopy (Olympus CX41 microscope, 10× objective, Tokyo, Japan) and Dino-eye software were used to analyse images of the sections captured with a colour video camera (DinoCapture 2.0; ANMO Electronics Corporation, Taiwan) and determine the morphometric indices of jejunum sections. On 10 well-oriented villi of each jejunum section, the villus height (VH), villus tip width (VTW), villus basal width (VBW), and crypt depth (CD), as well as the Cross-sectional jejunal muscle, were measured. For the calculation of the villus height : crypt depth (VH : CD), the average of 10 villus heights was divided by the average of 10 villus crypt depths. The apparent absorbent surface area of the villus was calculated using the formula: [(villus tip width + villus base width)/2] × villus height (Iji et al. 2001).

Serum biochemical indices. Serum samples were thawed and used for the determination of total protein (TP), albumin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) using an automatic analyser, Biolis 24i (Tokyo Boeki Medical System), with respective biochemical kits from the Cormay company. The estimated globulin value was obtained by subtracting the albumin value from the total protein value.

E. coli colony count. Caecal contents were collected and homogenised under sterile conditions. Six-fold serial dilutions (10^{-1} to 10^{-6}) were prepared using sterile phosphate-buffered saline (pH 7.4). For counting *E. coli*, aliquots of appropriate dilutions were plated onto MacConkey agar, a selective and differential medium for Gram-negative enteric bacteria. Plates were incubated aerobically at 37 °C for 24 hours. Following incubation, colonies of *E. coli* with characteristics of lactose-fermenting, pink colonies were counted. Colony-forming units (CFU) per gram of caecal content were calculated and expressed as $\log_{10}$ CFU.g^{-1}.

Newcastle disease antibody titres. Serum Newcastle disease virus (NDV) antibodies were measured by using the Biochek NDV ELISA kit. A Thermo Fisher ELISA plate reader with Optical density (OD) 450 nm was used to determine the antibodies according to Abdolmaleki et al. (2018). The antibody titres were automatically calculated by Biochek software, which converted the O.D. readings into $\log_{10}$ titres.

Statistical analysis. The SAS statistical package, v9.3 (Proc GLM; SAS Institute, Cary, NC,

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USA), was used to assess the homogeneity of variances and normality of data of each of the studied parameters within the respective dietary groups, as well as to determine the significance and interactions of the main effects of each experimental factor (SAS v9.3 2013; SAS Institute Inc., Cary, NC, USA). When interactions were detected ($P < 0.05$), Duncan's multiple range test was used to compare the individual treatment means.

RESULTS

Broiler performance. At day 10, the interaction of the studied factors was not significant on FI and WG (Table 2). However, the interaction of the studied factors was significant for FCR ($P = 0.031$) and mortality rate ($P < 0.001$) revealing a lower FCR in the unchallenged control group, followed by unchallenged birds that were on OTC, and challenged

Table 2. Effect of dietary addition of different doses of *Moringa oleifera* on weight gain (WG), feed intake (FI), feed conversion ratio (FCR), and mortality rate up to 10 days of age in broilers challenged to *E. coli* infection ($n = 6$ pens)

Treatments	*E. coli* challenge	FI (g)	WG (g)	FCR	Mortality (%)
Control	no	277	255	1.09^{c}	0^{b}
OTC	no	279	248	1.12^{bc}	0^{b}
MO 0.1%	no	274	241	1.14^{bc}	0^{b}
MO 0.2%	no	278	235	1.18^{abc}	0^{b}
MO 0.4%	no	281	242	1.17^{abc}	0^{b}
MO 0.8%	no	262	233	1.13^{bc}	0^{b}
Control	yes	295	239	1.24^{ab}	42^{a}
OTC	yes	306	242	1.27^{a}	42^{a}
MO 0.1%	yes	304	246	1.25^{ab}	52^{a}
MO 0.2%	yes	290	248	1.18^{abc}	48^{a}
MO 0.4%	yes	288	237	1.22^{ab}	62^{a}
MO 0.8%	yes	290	260	1.12^{bc}	47^{a}
Pooled SEM		3.07	2.54	0.010	3.40
		Main effect			
Challenge					
Yes		296^{a}	245	1.21^{a}	49^{a}
No		275^{b}	242	1.14^{b}	0^{b}
Additive					
Control		286	247	1.16	21
OTC		293	245	1.2	21
MO 0.1%		289	243	1.19	26
MO 0.2%		284	242	1.18	24
MO 0.4%		284	239	1.19	31
MO 0.8%		276	246	1.13	23
P-value					
Challenge × additive		0.136	0.643	0.031	<0.001
Challenge		0.001	0.552	0.002	<0.001
Additive		0.741	0.959	0.544	0.965

$^{a–c}$Means within the same column with different superscripts differ significantly ($P < 0.05$)

Control = basal diet; MO 0.1% = basal diet supplemented with *Moringa oleifera* at 1 g/kg; MO 0.2% = basal diet supplemented with *Moringa oleifera* at 2 g/kg; MO 0.4% = basal diet supplemented with *Moringa oleifera* at 4 g/kg; MO 0.8% = basal diet supplemented with *Moringa oleifera* at 8 g/kg; OTC = basal diet supplemented with oxytetracycline; SEM = standard error of the mean

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birds that were on MO 0.8%. Whereas the higher FCR was recorded in the OTC received challenged birds. The higher mortality rate was in challenged birds that were on MO 0.4%. Regardless of the additive, compared to non-challenged birds, the challenged ones fed higher (P = 0.001) amounts of feed, had deteriorated FCR (P = 0.002), and a higher (P < 0.001) rate of mortal birds. During the period from 0 to 24 days of age, statistical outcomes indicated that the interaction of studied factors had no significant effect on the amount of feed consumed by birds (Table 3).

However, challenge × additive interactions were observed for WG, FCR, and mortality rate. In general, the lower WG (P < 0.001) was recorded in the challenged control group, followed by the challenged group fed an OTC-supplemented diet. Also, the lower FCR (P < 0.004) was recorded in the unchallenged group that was on MO 0.8%, followed by the unchallenged control, and those birds that

Table 3. Effect of dietary addition of different doses of *Moringa oleifera* on weight gain (WG), feed intake (FI), feed conversion ratio (FCR), and mortality rate up to 24 days of age in broilers challenged to *E. coli* infection (n = 6 pens)

Treatments	*E. coli* challenge	FI (g)	WG (g)	FCR	Mortality (%)
Control	no	1 313	1 101^{a}	1.19^{cd}	0^{b}
OTC	no	1 325	1 104^{a}	1.20^{cd}	0^{b}
MO 0.1%	no	1 342	1 059^{ab}	1.27^{abc}	2^{b}
MO 0.2%	no	1 306	1 096^{a}	1.19^{cd}	0^{b}
MO 0.4%	no	1 291	1 079^{ab}	1.20^{cd}	0^{b}
MO 0.8%	no	1 305	1 105^{a}	1.18^{d}	0^{b}
Control	yes	1 188	907^{c}	1.31^{ab}	45^{a}
OTC	yes	1 338	1 008^{b}	1.33^{a}	42^{a}
MO 0.1%	yes	1 316	1 058^{ab}	1.25^{bcd}	53^{a}
MO 0.2%	yes	1 302	1 042^{ab}	1.25^{bcd}	48^{a}
MO 0.4%	yes	1 316	1 061^{ab}	1.24^{bcd}	62^{a}
MO 0.8%	yes	1 262	1 034^{ab}	1.22^{cd}	48^{a}
Pooled SEM		9.68	9.50	0.010	3.47
			Main effect		
Challenge					
Yes		1 287	1 018^{b}	1.27^{a}	50^{a}
No		1 314	1 091^{a}	1.21^{b}	0.28^{b}
Additive					
Control		1 251	1 004	1.25	23
OTC		1 331	1 056	1.27	21
MO 0.1%		1 329	1 058	1.26	28
MO 0.2%		1 304	1 069	1.22	24
MO 0.4%		1 303	1 070	1.22	31
MO 0.8%		1 284	1 070	1.20	24
P-value					
Challenge × additive		0.107	<0.001	<0.004	<0.001
Challenge		0.168	<0.001	<0.002	<0.001
Additive		0.148	0.307	0.176	0.973

$^{a–d}$Means within the same column with different superscripts differ significantly (P < 0.05)

Control = basal diet; MO 0.1% = basal diet supplemented with *Moringa oleifera* at 1 g/kg; MO 0.2% = basal diet supplemented with *Moringa oleifera* at 2 g/kg; MO 0.4% = basal diet supplemented with *Moringa oleifera* at 4 g/kg; MO 0.8% = basal diet supplemented with *Moringa oleifera* at 8 g/kg; OTC = basal diet supplemented with oxytetracycline; SEM = standard error of the mean

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were on MO 0.2%, whereas the higher FCR was recorded in OTC received challenged birds. The higher mortality rate ($P < 0.001$) was in challenged birds that were on MO 0.4%. Regardless of feed additives, unchallenged birds gained ($P < 0.001$) more weight, had better ($P < 0.002$) FCR, and had a lower ($P < 0.001$) rate of mortal birds. Although compared to the control group, broilers in the OTC and *Moringa oleifera* supplemented groups consumed more feed, gained more weight, and had a higher rate of mortality (except for the OTC group), the differences were not significant.

From 0 to 35 days of age**,** challenge × additive interactions were noted for WG, FCR, and mortality % (Table 4).

In general, the lower WG ($P < 0.001$) was recorded in the challenged control group, followed by the non-challenged control and OTC challenged groups. Birds that received MO gained more weight than the other experimental groups. The higher

Table 4. Effect of dietary addition of different doses of *Moringa oleifera* on weight gain (WG), feed intake (FI), feed conversion ratio (FCR), and mortality rate up to 35 days of age in broilers challenged to *E. coli* infection ($n = 6$ pens)

Treatments	*E. coli* challenge	FI (g)	WG (g)	FCR	Mortality (%)
Control	no	2 816	1 877^{bc}	1.50^{ab}	2^{b}
OTC	no	2 884	1 999^{a}	1.44^{cd}	2^{b}
MO 0.1%	no	2 912	2 019^{a}	1.44^{cd}	2^{b}
MO 0.2%	no	2 873	2 016^{a}	1.43^{d}	0^{b}
MO 0.4%	no	2 945	2 036^{a}	1.45^{cd}	0^{b}
MO 0.8%	no	2 852	1 952^{ab}	1.46^{bcd}	0^{b}
Control	yes	2 713	1 779^{c}	1.53^{a}	50^{a}
OTC	yes	2 806	1 889^{b}	1.49^{abc}	46^{a}
MO 0.1%	yes	2 878	1 963^{ab}	1.47^{bcd}	61^{a}
MO 0.2%	yes	2 865	1 972^{ab}	1.45^{bcd}	54^{a}
MO 0.4%	yes	2 971	2 047^{a}	1.45^{bcd}	69^{a}
MO 0.8%	yes	2 964	2 032^{a}	1.46^{bcd}	58^{a}
Pooled SEM		18.22	13.01	0.010	3.91
			Main effect		
Challenge					
Yes		2 866	1 947	1.47	56^{a}
No		2 880	1 983	1.45	0.920^{b}
Additive					
Control		2 764^{b}	1 828^{c}	1.51^{a}	26
OTC		2 845^{ab}	1 944^{b}	1.46^{b}	24
MO 0.1%		2 895^{a}	1 991^{ab}	1.45^{b}	32
MO 0.2%		2 869^{ab}	1 994^{ab}	1.44^{b}	27
MO 0.4%		2 958^{a}	2 042^{a}	1.45^{b}	34
MO 0.8%		2 908^{a}	1 992^{ab}	1.46^{b}	29
P-value					
Challenge × additive		0.184	<0.001	0.002	<0.001
Challenge		0.703	0.167	0.050	<0.001
Additive		0.049	<0.001	<0.003	0.980

$^{a–d}$Means within the same column with different superscripts differ significantly ($P < 0.05$)

Control = basal diet; MO 0.1% = basal diet supplemented with *Moringa oleifera* at 1 g/kg; MO 0.2% = basal diet supplemented with *Moringa oleifera* at 2 g/kg; MO 0.4% = basal diet supplemented with *Moringa oleifera* at 4 g/kg; MO 0.8% = basal diet supplemented with *Moringa oleifera* at 8 g/kg; OTC = basal diet supplemented with oxytetracycline; SEM = standard error of the mean

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WG was recorded in the challenged birds that received MO 0.4%. The higher FCR (P = 0.002) was found in both challenged and non-challenged control groups. The higher mortality rate (P < 0.001) was in challenged birds that were on MO 0.4%. The interaction of studied factors has not considerably changed the FI of broilers. Irrespective of the challenge, broilers on diets supplemented with all concentrations of Moringa, except those on 0.2% Moringa diet, consumed more feed (P = 0.049) compared to those on the control diet. Also, compared to the control group of birds, the OTC and Moringa-supplemented broilers gained more weight (P < 0.001) with respect to (0.4% Moringa),

Table 5. Effect of dietary addition of different doses of *Moringa oleifera* on villus height (VH), crypt depth (CD), VH : CD, villus tip width (VTW), villus base width (VBW), villus surface area (VSA), jejunum muscle thickness, and *E. coli* colony count in 24-day-old broilers challenged to *E. coli* infection (n = 6 pens)

Treatments	*E. coli* challenge	VH (μm)	CD (μm)	VH : CD	VTW (μm)	VBW (μm)	VSA (mm^2)	Muscle thickness (μm)	*E. coli* colony count log_{10} $CFU.g^{-1}$
Control	no	1 247^{d}	245^{d}	5.20^{c}	181^{bc}	222	0.286^{a}	257^{abc}	0.000^{d}
OTC	no	1 339^{c}	272^{abc}	5.07^{c}	196^{abc}	234	0.287^{a}	265^{ab}	0.000^{d}
MO 0.1%	no	1 393^{b}	257^{bcd}	5.62^{abc}	196^{abc}	225	0.293^{a}	255^{abc}	0.333^{d}
MO 0.2%	no	1 399^{ab}	241^{d}	5.92^{ab}	206^{ab}	230	0.305^{a}	218^{e}	0.667^{d}
MO 0.4%	no	1 455^{a}	249^{cd}	5.97^{ab}	199^{abc}	247	0.324^{a}	236^{cde}	0.167^{d}
MO 0.8%	no	1 404^{ab}	240^{d}	5.98^{ab}	204^{ab}	235	0.306^{a}	225de	0.000^{d}
Control	yes	1 161^{e}	289^{a}	4.18^{d}	175^{c}	235	0.237^{b}	268^{ab}	17.0^{a}
OTC	yes	1 391^{b}	253^{cd}	5.61^{abc}	212^{a}	230	0.307^{a}	253^{abc}	4.83^{c}
MO 0.1%	yes	1 406^{ab}	266^{abcd}	5.51^{bc}	193^{abc}	225	0.295^{a}	275^{a}	7.50^{b}
MO 0.2%	yes	1 399^{ab}	278^{ab}	5.30^{c}	210^{a}	241	0.315^{a}	274^{a}	6.50^{bc}
MO 0.4%	yes	1 411^{ab}	244^{d}	6.11^{a}	204^{ab}	235	0.308^{a}	244^{bcde}	4.50^{c}
MO 0.8%	yes	1 422^{ab}	249^{cd}	5.99^{ab}	193^{abc}	227	0.298^{a}	249^{abcd}	4.00^{c}
Pooled SEM		6.27	2.39	0.060	2.25	2.31	0.003	2.51	0.610
					Main effect				
Challenge									
Yes		1 366	263^{a}	5.46	199	233	0.294	260^{a}	7.39^{a}
No		1 369	251^{b}	5.60	197	232	0.292	244^{b}	0.194^{b}
Additive									
Control		1 203^{c}	267^{a}	4.68^{c}	178^{b}	228	0.242^{c}	263^{a}	8.50^{a}
OTC		1 365^{b}	263^{ab}	5.33^{b}	204^{a}	232	0.297^{ab}	259^{a}	2.42^{b}
MO 0.1%		1 399^{ab}	261^{abc}	5.56^{b}	194^{a}	225	0.294^{b}	265^{a}	3.92^{b}
MO 0.2%		1 399^{ab}	264^{a}	5.54^{b}	209^{a}	237	0.311^{ab}	252^{ab}	3.58^{b}
MO 0.4%		1 429^{a}	246^{bc}	6.05^{a}	202^{a}	240	0.314^{a}	241^{b}	2.33^{b}
MO 0.8%		1 414^{a}	245^{c}	5.99^{a}	198^{a}	231	0.302^{ab}	238^{b}	2.00^{b}
P-value									
Challenge × additive		<0.001	<0.001	<0.001	0.016	0.660	<0.001	<0.001	<0.001
Challenge		0.778	0.014	0.227	0.675	0.879	0.699	0.002	<0.001
Additive		<0.001	0.016	<0.001	0.002	0.469	<0.001	0.005	0.018

$^{a–e}$Means within the same column with different superscripts differ significantly (P < 0.05)

Control = basal diet; MO 0.1% = basal diet supplemented with *Moringa oleifera* at 1 g/kg; MO 0.2% = basal diet supplemented with *Moringa oleifera* at 2 g/kg; MO 0.4% = basal diet supplemented with *Moringa oleifera* at 4 g/kg; MO 0.8% = basal diet supplemented with *Moringa oleifera* at 8 g/kg; OTC = basal diet supplemented with oxytetracycline; SEM = standard error of the mean

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and had better FCR ($P < 0.003$). Furthermore, no significant effect of additives was recorded on the mortality rate among the broiler groups. Regardless of the type of additives used in the present study, the *E. coli* challenge had no significant effect on the FI and WG of birds; however, unchallenged broilers tended to have better FCR ($P = 0.050$) compared to the challenged ones. In addition, the challenged birds had a higher mortality rate ($P < 0.001$) compared to the unchallenged broilers.

Jejunum histomorphology. At 24 days of age, the interaction between challenge and additives was observed for all studied parameters, except for VBW (Table 5). At both challenged and non-challenged conditions, birds on diets supplemented

Table 6. Effect of dietary addition of different doses of *Moringa oleifera* on serum biochemistry, NDV titre in 24-day-old broilers challenged to *E. coli* infection ($n = 6$ pens)

Treatments	*E. coli* challenge	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	AST (IU/l)	ALT (IU/l)	NDV titre
Control	no	32.1	20.5^{abc}	11.7	360	2.33^{abc}	1 264^{a}
OTC	no	30.4	22.6^{a}	7.60	402	2.20^{abc}	1 149^{ab}
MO 0.1%	no	30.6	20.2^{abc}	10.4	342	0.30^{c}	1 368^{a}
MO 0.2%	no	30.2	21.2^{ab}	9.20	349	2.00^{abc}	1 318^{a}
MO 0.4%	no	32.5	22.0^{ab}	10.5	477	1.33^{bc}	1 318^{a}
MO 0.8%	no	33.5	23.0^{a}	10.5	334	0.50^{bc}	1 358^{a}
Control	yes	28.2	18.5^{bc}	11.7	375	4.33^{ab}	953^{bc}
OTC	yes	30.0	16.8^{c}	12.6	301	5.40^{a}	750^{dc}
MO 0.1%	yes	29.6	18.4^{bc}	11.4	389	4.40^{ab}	629^{d}
MO 0.2%	yes	29.8	20.8^{ab}	9.20	360	1.50^{bc}	668^{d}
MO 0.4%	yes	30.8	21.8^{ab}	8.8	290	1.00^{bc}	826^{dc}
MO 0.8%	yes	30.0	22.0^{ab}	7.8	288	0.60^{bc}	849^{dc}
Pooled SEM		0.400	0.390	0.440	15.2	0.372	40.1
				Main effect			
Challenge							
Yes		29.7^{b}	19.7^{b}	10.3	336	2.88^{a}	779^{b}
No		31.7^{a}	21.6^{a}	10.0	378	1.41^{b}	1 296^{a}
Additive							
Control		30.3	19.5	11.7	367	3.33	1 108
OTC		30.2	19.7	10.1	352	3.80	950
MO 0.1%		30.1	19.3	10.9	366	2.20	998
MO 0.2%		30.0	21.0	9.20	355	1.75	993
MO 0.4%		30.3	21.9	9.70	392	1.18	1 072
MO 0.8%		31.9	22.5	9.30	313	0.550	1 104
P-value							
Challenge × additive		0.266	0.019	0.408	0.439	0.047	<0.001
Challenge		0.012	0.015	0.805	0.169	0.048	<0.001
Additive		0.554	0.061	0.517	0.802	0.092	0.823

$^{a–d}$Means within the same column with different superscripts differ significantly ($P < 0.05$)

Control = basal diet; MO 0.1% = basal diet supplemented with *Moringa oleifera* at 1 g/kg; MO 0.2% = basal diet supplemented with *Moringa oleifera* at 2 g/kg; MO 0.4% = basal diet supplemented with *Moringa oleifera* at 4 g/kg; MO 0.8% = basal diet supplemented with *Moringa oleifera* at 8 g/kg; NDV = Newcastle disease virus; OTC = basal diet supplemented with oxytetracycline; SEM = standard error of the mean

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with Moringa and OTC had taller jejunal villi ($P < 0.001$), with respect to unchallenged MO 0.4% and challenged MO 0.8% groups, compared to those of challenged and unchallenged controls. In addition, at both challenged and unchallenged conditions, the villus height of broilers increased as the in-feed concentration of Moringa was increased. Also, *E. coli*-infected control birds had deeper crypts ($P < 0.001$), lower VH : CD ($P < 0.001$), narrower VTW ($P = 0.016$), and lower villi surface area ($P < 0.001$). Furthermore, unchallenged birds on 0.2% Moringa-supplemented diets possessed thinner jejunum muscle ($P < 0.001$). In addition, there was no effect of the studied factors on the VBW. Moreover, the caeca content of challenged control birds had a higher number of *E. coli* ($P < 0.001$) colony counts compared to all other challenged and non-challenged groups. Also, in infected birds, by increasing the concentration of Moringa, the number of *E. coli* colony counts was decreased. Regardless of the additives, the *E. coli*-challenged broilers had deeper crypts ($P = 0.014$) and thicker jejunum muscle ($P = 0.002$). In addition, the caeca content of challenged birds had higher ($P < 0.001$) *E. coli* colony counts. Irrespective of the challenge, broilers on OTC and all Moringa concentrations had taller jejunum villi ($P < 0.001$) compared to those in Controls, and villi of 0.4% and 0.8% fed birds were significantly taller than those of OTC fed broilers. Also, crypt depth of broilers on 0.4% and 0.8% of MO, with respect to MO 0.8%, was less ($P = 0.016$) than those on control and 0.2% MO diets. In addition, compared to the control group, the VH : CD ratio was higher ($P < 0.001$) in birds that received diets supplemented with OTC, and all concentrations of *Moringa oleifera* outperformed in 0.4% and 0.8% Moringa-fed birds. On the other hand, the jejunum villi tip was wider ($P = 0.002$) in broilers of OTC and all Moringa supplemented groups compared to those in the control group; however, dietary groups were not different from each other in terms of villi base width ($P = 0.469$). In addition, the villi surface area of the jejunum of broilers on OTC, and all *Moringa oleifera* levels, was larger ($P < 0.001$) than that of broilers in the control group. Moreover, the thickness of the jejunum muscle of broilers fed control, OTC, and MO 0.1% supplemented diets was significantly larger ($P = 0.005$) than that of broilers fed diets supplemented with Moringa at 0.4 and 0.8 percentages. Also, compared to control diet-fed broilers, OTC and all *Moringa oleifera*-fed diet broilers had significantly lower ($P = 0.018$) caeca content of *E. coli* colony counts, and the lowest colony count was recorded in the MO 0.8% group.

Serum biochemical indices. At 24 days of age, the interaction of studied factors had no significant effect on the serum content of total protein, globulin, and AST activity (Table 6). However, the challenge OTC fed birds had lower ($P = 0.019$) serum albumen content and higher ($P = 0.047$) serum ALT activity compared to challenged birds fed Moringa at 2–8 g/kg and unchallenged birds in MO 0.1%, MO 0.4%, and MO 0.8% groups. Furthermore, serum level of antibodies against NDV was higher ($P < 0.001$) in non-challenged control and all the unchallenged MO-fed groups of broilers compared to all other challenged and non-challenged groups of birds.

Regardless of additives, compared to unchallenged groups of broilers, serum of the challenged ones had lower ($P = 0.012$) content of total protein, lower ($P = 0.015$) content of albumen, higher ($P = 0.048$) ALT activity, and lower ($P < 0.001$) content of NDV antibodies. Irrespective of the challenge condition, additives had no significant effect on the broilers' serum content of total protein, albumen, globulin, NDV titers, and ASTand ALT activities.

DISCUSSION

In the present study, the early and long-term impact of *E. coli* on the birds through clear clinical signs and poor performance confirms the success of the challenge. Challenged birds experienced laboured respiration and gasping, weakness, ruffled feathers, reduced feed intake, dropped weight gain, and deteriorated FCR. In addition, the high mortality percentage among challenged birds and the high number of *E. coli* colonies in the caeca content of infected birds were two other strong pieces of evidence for the success of the *E. coli* challenge.

The challenge concentration of *E. coli* O157 (3.8×10^8 CFU per bird) was selected based on the reported *E. coli* populations in the digestive tract of chickens, where total coliform and *E. coli* counts can naturally reach 10^6–10^9 CFU/g of intestine content, particularly in the caeca under cases of stress, immune function suppression, or enteric discomfort. While commensal *E. coli* is a component of the standard and healthy flora found within the

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intestine, pathogenic strains such as O157 can increase speedily and control the gut environment during infection, leading to superior bacterial loading and acute clinical symptoms (Shang et al. 2018). Therefore, the used dose exemplifies the biologically realistic upper range of exposure and was purposely applied to set a vigorous challenge model, guaranteeing consistent colonisation and allowing the subsequent evaluation criteria of the host response and treatment efficiency under high pathogenic stress.

The results of the present study demonstrated the potential of *Moringa oleifera* as an alternative to antibiotics in mitigating the adverse impacts of *E. coli* in broiler chickens and improving their performance. The body weight gain of birds at the starter phase was not affected by additives. Similar results were also reported by Khan et al. (2017), who noticed that MO did not improve the BWG of broilers in the starter period. This might be due to the underdeveloped gut of the birds at an early age (Gul et al. 2024). However, at 24 and 35 days of age, compared to control birds, the *Moringa oleifera*-fed birds had significantly increased FI and WG, and improved FCR, and this improvement was somewhat superior to the positive effect of the antibiotics. Similar findings have been reported by Akib et al. (2024), who demonstrated that dietary *Moringa oleifera* leaf powder at 100 mg/kg significantly improved FI, WG, and FCR of broilers at day 35. The improvement in FI of MO-fed broilers and their subsequent growth performance could be due to the bioactive compounds content of MO, like flavanol glycosides and moriginine, which potentially stimulate appetite, enhance the flavour and palatability of feed, reduce the gut pathogenic counts, and increase digestive enzyme production (Mahfuz et al. 2021).

Findings of the present study were also aligned with results of Gul et al. (2024), who indicated that giving broiler chickens Moringa leaf and seeds extracts at 0.8 and 1.2 percentages individually or combined significantly improved BWG and FCR. The enhancement of WG and FCR in the MO-fed broilers of the present study could be attributed to antimicrobial and antioxidant properties, immune stimulation ability, high polyphenols, flavonoids, highly digestible protein, and essential amino acids, significant amounts of vitamins (A, D, E), and minerals (iron, calcium, phosphorus) as bioactive compounds and natural growth promoters, as well as the low tannin content of Moringa (Akib et al. 2024; Gul et al. 2024). These bioactive compounds suppress the formation of free radicals, activate antioxidant enzymes, prohibit oxidases, and have protective impacts on the cytoplasmic membrane. This will subsequently enhance digestive physiology and metabolism; improve gut function; increase nutrients availableness, absorption, and utilisation by birds (Ullah et al. 2022).

Contrarily, Nantapo et al. (2024) reported that adding Moringa leaf powder to broilers' diet at (1, 3, and 5 percentages) did not affect the FI and WG but significantly improved the FCR at 3 and 5 percentages. Authors have revealed the low FI and WG to the form and way of feed offering, high tannin, and high fibre content of Moringa (up to 3.5%), which adversely affect the palatability of the feed and its digestibility, resulting in reduced growth of broilers.

In the present study, the improved performance of the challenged birds fed different levels of the MO compared to the challenged unsupplemented group can be clearly seen as a result of reduced *E. coli* colony counts and subsequent enhancement of gut health. This includes increased villus height and a higher villus height to crypt depth ratio; Moringa's high content of flavonoids like quercetin, apigenin, and kaempferol inhibits the growth and multiplication of harmful microorganisms such as *E. coli*, impacts oxidase activity, and stimulates antioxidant enzymes (Abd Rani et al. 2018). These effects lead to better nutrient digestion and greater nutrient absorption (Khan et al. 2017). The poor performance observed in the *E. coli*-challenged controls confirms its strong pathogenic impact, consistent with reports that identify *E. coli* as one of the most harmful infections in broilers. *E. coli* causes damage to the intestinal mucosa and disrupts the gut lining, resulting in malabsorption of protein and other nutrients (Mohebodini et al. 2019).

Mortality was the most striking observation at this stage. Nearly half of the challenged chicks died, while none of the non-challenged birds were lost. This highlights how severe *E. coli* infection can be during the starter phase. At day 10, neither antibiotics nor Moringa offered strong protection, which indicates that the beneficial effects of these additives may require more time to become fully effective.

The present study demonstrated that the *E. coli* challenge caused significant changes in jejunal

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histomorphometry and caecal colony counts. After inoculation, *E. coli*-challenged birds showed a marked increase in crypt depth, jejunal muscle thickness, and cecum *E. coli* colonies, along with a numerical decrease in the VH : CD ratio. This aligns with the findings of Ali et al. (2025), who also observed that *E. coli* O78 significantly impaired the ileum's integrity by increasing CD, reducing the VH : CD ratio, and increasing colony numbers. The outcomes of this study were also similar to those of Ali et al. (2025), who found that challenged birds fed Moringa had increased VH, VH : CD, VTW, and VSA, along with reduced CD and cecum *E. coli* colony counts compared to challenged birds fed a basal diet. This also agrees with Khan et al. (2017), who showed that Moringa leaf powder increased VH in the duodenum, jejunum, and ileum; VSA in the duodenum; and VH : CD in the ileum of broiler chickens. Additionally, the results align with those of Akib et al. (2024), who found that Moringa leaf powder significantly reduced caecal *E. coli* populations.

Furthermore, the antimicrobial efficacy of Moringa in the present study is confirmed by Nantapo et al. (2024), who reported that Moringa inhibited the growth of harmful microorganisms, such as *E. coli* and *C. perfringens*, and enhanced the proliferation of *Lactobacillus* spp.; Rifat et al. (2024), who observed that Moringa significantly decreased the *E. coli* and total aerobic bacteria counts in the caeca of broiler chickens. The ameliorative role of Moringa in improving the gut health and integrity of broilers could be attributed to its content of quercetin, kaempferol, and moringine, which possess strong anti-inflammatory, antimicrobial, and antioxidant properties (Prajapati et al. 2022). The increased height of villi and VH : CD ratio in the present study might be a result of controlled damage to enterocytes, which subsequently reduces the demand for their turnover and stimulation of enzyme production from the villus tip (Khan et al. 2017). Reduction in the *E. coli* population in the moringa seeds-fed broilers in the present study might be due to their high content of antibacterial bioactive phytochemical 4-(α-L-rhamnosyloxy)-benzyl isothiocyanate (moringin), which inhibits the growth of *E. coli* by disrupting the formation of the cell membrane or crucial enzymes (Rifat et al. 2024).

In the present study, *E. coli* challenge significantly altered the serum levels of biochemicals and NDV immune titres. Infected birds showed a notable decrease in serum total protein, albumen, and NDV titres, along with an increase in serum ALT levels. These findings are consistent with Nair (2018), who also found that *E. coli* challenge reduced serum total protein and albumen levels and elevated serum ALT; they are also in line with the results of a study by Awad et al. (2019), who reported that *E. coli* suppressed the immunity of broiler chickens through hampering the production of titres against NDV. The *E. coli*-induced hypoproteinaemia, hypoalbuminemia, and high serum ALT are possibly due to liver injury and dysfunction, inflammation, septicaemia, hepatitis, and potential degenerative alterations in hepatocytes, either directly or by endotoxins, resulting in impaired liver protein synthesis, including albumen and immediate elevation of serum (Tan et al. 2024). The decreased NDV titres in the serum of *E. coli*-infected birds may result from their suppressed immune function, either directly caused by endotoxins or indirectly due to stress or systemic disease.

Thereby, the bird's overall body health and maintenance will be impaired due to the preoccupation of the immune system to fight bacterial infections, so the body's capacity to produce sufficient antibody titres against NDV will be weakened (El Tayeb and Hanson 2002). Furthermore, *E. coli* sometimes causes lymphoid depletion in the bursa of Fabricius, thymus, and spleen, which impairs humoral immune responses and antibody production (Nakamura et al. 1986).

The findings of the present study also showed that high Moringa level-fed, challenged, and non-challenged birds had significantly increased serum albumin levels, decreased ALT levels as a liver health marker, and improved NDV titres. This was also confirmed in a study by Egbu et al. (2022), who found that Moringa seed extract at 60 and 90 ml/l of drinking water significantly increased serum albumin content; aligned with Elkloub et al. (2015), who observed that Moringa at different levels (0.2–0.6%) significantly reduced ALT plasma levels in Japanese quails. The increased serum albumin concentration might be due to the large number of antioxidants present in Moringa, which inhibit the corticosterone release, decreasing protein catabolism under challenge conditions and resulting in higher serum albumin content (Khan et al. 2021). The reduced serum levels of ALT, as a liver disfunction marker, in the Moringa-fed

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broilers could be attributed to its high content of flavonoids (e.g. quercetin, kaempferol, rutin, etc.), alkaloids (e.g. moringine, moriginine, etc.), vitamins (e.g. Vit A, Vit E, Vit K, Vit D, Vit C, Vit B3, etc.), which possess antioxidant and hepatoprotective properties, scavenging free radicals and reducing oxidative stress in hepatocytes, and stabilising hepatocellular membranes (Arshad et al. 2025). The lower serum ALT content might be due to Moringa's antimicrobial and anti-inflammatory activities, reducing systemic inflammation, and protecting the liver from infection or endotoxin-caused damage (Villegas-Vazquez et al. 2025).

CONCLUSION

In conclusion, the present study successfully established an *E. coli* infection model. Evaluation of WG, FCR, mortality rate, gut morphology, caeca *E. coli* counts, serum AST and ALT activities, and immune function in challenged broilers receiving either OTC or Moringa demonstrated that Moringa seed powder was generally comparable to, and in some studied parameters superior to, OTC in mitigating the negative effects of *E. coli* infection on broiler performance, health, and physiological status.

Moreover, since dietary inclusion of Moringa at 0.4% and 0.8% showed similar protective potential against *E. coli*, the use of 0.4% Moringa is recommended as a cost-effective dietary preventive strategy in broiler production.

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Conflict of interest

The authors declare no conflict of interest.

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