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ORIGINAL ARTICLE

Dietary supplementation of *Moringa oleifera* leaf extract enhances productivity, carcass yield, gut microbiota and biomarker profiles in broiler chickens

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ABSTRACT. Plant feed additives produced as herbs, spices, and botanical extracts have presented themselves as possible substitutes for antimicrobial growth promoters (AGPs). These agents are valued for their growth-stimulating activities, antimicrobial effects, antioxidative properties, and immunomodulatory effects. This study aimed to determine the effects of *Moringa oleifera* leaf extract (MOLE) on the growth performance, carcass attributes, and physiological indicators of broiler chickens. A total of 240 one-day-old broiler chicks were assigned to four treatments: a control (no MOLE) and three levels of dietary MOLE (100, 200, and 400 mg MOLE/kg feed) for a feeding period of 42 days. Growth performance characteristics, such as weight gain, feed conversion ratio (FCR), and feed intake per day, were measured. Carcass characteristics and blood samples were examined at the end of the trial for liver and kidney function biomarkers, antioxidant enzymes, and lipid profiles. The supplementation of MOLE in diet significantly increased weight gain and FCR, especially at the level of 200 mg/kg. The inclusion of MOLE has been observed to improve carcass yield, particularly in the breast and thigh muscles of broilers. Furthermore, the MOLE-supplemented groups exhibited lower serum cholesterol levels, increased activity of some antioxidant enzymes (SOD and GPx), and improved liver and kidney function markers. In conclusion, MOLE has great potential as a feed additive to increase the productivity and improve the physiological health of broilers.

Keywords: *Moringa oleifera*, broiler chicken, growth performance, carcass traits, physiological biomarker, antioxidant activity.

INTRODUCTION

The global poultry industry plays a vital role in meeting the growing demand for high-quality animal protein. Broiler chickens have become essential to meat production systems globally because of their rapid growth, effective feed conversion, and brief production cycle (Maharjan *et al*., 2021). Intensive poultry farming frequently exposes birds to environmental, nutritional, and health stressors that can adversely affect their growth performance, carcass quality, and physiological health (Himu & Raihan, 2023). Traditionally, antibiotic growth promoters (AGPs) have been employed to address these concerns; however, their continued use has raised concerns regarding antibiotic resistance, meat drug residues, and adverse impacts on public health and the environment (Ahmad *et al*., 2022; Mesfin *et al*., 2024). Modern literature has also significantly highlighted the necessity of having some natural, sustainable options that can ensure the improvement of poultry well-being and productivity while helping maintain food safety and environmental sustenance (Abbasi *et al*., 2024). Plant feed additives produced as herbs, spices, and botanical extracts have presented themselves as possible substitutes for antimicrobial growth promoters (AGPs) (McGaw, 2025). These agents are valued for their growth-stimulating activities, antimicrobial effects, antioxidative properties, and immunomodulatory effects. Among the wide range of botanicals that have been studied, *Moringa oleifera*, also called the drumstick or miracle tree, is noteworthy (Mahfuz & Piao, 2019). Originating in the Indian subcontinent, *M. oleifera* has been widely cultivated in tropical and subtropical zones because it is a multipurpose plant with a long tradition of use owing to its nutritional and health benefits (Devkota & Bhusal, 2020). Its leaves are a source of bioactive compounds, including flavonoids (e.g., quercetin and kaempferol), phenolics, vitamins (A, C, and E), minerals (calcium, potassium, and iron), and essential amino acids (Jiménez-Aguilar & Grusak, 2017).

These phytochemicals have prominent antioxidant, anti-inflammatory, antimicrobial, and hypolipidemic effects, making *M. oleifera* a prospective functional ingredient for animal feed. The integration of *M. oleifera* leaf powder or extract as a dietary supplement in poultry has been studied at different levels (Mahfuz & Piao, 2019). The results

unanimously demonstrated that the intervention was always related to increased feed intake, weight gain, feed conversion efficiency, and improved immune responses. In addition, *M. oleifera* also adjusts the muscular composition and reduces fat storage, reducing the lipid metabolic rate, improving meat quality, and reducing oxidative stress (Alfifi *et al*., 2025). However, the type, time, and duration of *M. oleifera* supplementation had a significant effect on the reported results. Although the high inclusion level of tannins and other anti-nutritional components could worsen the nutrient utilization and palatability, their moderate or low inclusion has proved positive (Bhat *et al*., 2013; Alfifi *et al*., 2025). The microbiota residing in the gut of poultry is an essential asset to poultry health, as it affects nutrient absorption, immunological functions, and pathogen resistance. The growing demand for natural feed additives has led to the exploration of a plant-based supplement, *M. oleifera* leaf extract (MOLE), which is rich in bioactive compounds such as flavonoids, saponins, and phenolics. These compounds are believed to have antimicrobial and prebiotic effects on the intestinal microbial community, which may beneficially modulate the long-term intestinal results. The addition of MOLE to broiler diet can potentially inhibit the growth of pathogenic bacteria, such as *Escherichia coli*, and stimulate the growth of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, leading to overall improved behavioral and gut performance of the non-antibiotic raised poultry.

Although the body of research evidence is growing significantly, relatively little research has been conducted to study the effect of MOLE on the growth performance of broilers, carcass yield, and physiological biomarkers within an environmental setting with conditions that more closely mimic or resemble conditions in commercial production settings. Most studies conducted so far have examined either the performance or the antioxidant effects. They have not examined physiological markers such as liver and kidney function, lipid profile, or oxidative stress indicators, which are important for determining the overall health and metabolic status of birds. Therefore, the present study aimed to evaluate the impact of graded levels of MOLE on growth performance, feed efficiency, carcass characteristics, and key physiological biomarkers in broiler chickens over a standard 42-day production cycle. By integrating growth and health parameters, this study sought to determine the efficacy and optimal dosage of MOLE as a natural alternative to synthetic growth enhancers. These findings are expected to contribute to the development of safer, eco-friendly, and more sustainable feeding strategies for poultry production systems.

MATERIALS AND METHODS

Experimental site and ethical approval

The research was conducted at the Poultry Research Unit (PRU) of the Department of Animal and Poultry Production, Faculty of Veterinary and Animal Science (FVAS), Gomal University DI Khan, in a traditional broiler raising unit. All animal-based experimental procedures were approved by the FVAS Institutional Animal Care and Use Committee (IACUC) according to the existing international ethical standards for animal experimentation.

Experimental design and feeding management of birds

Two-hundred-and-forty-day-old male and female Ross 308 broiler chicks were acquired from a commercial hatchery (Al Abbas Chicks Rawalpindi, Pakistan). The chicks were placed in four treatment groups in a completely randomized design (CRD) after a 3-day acclimation period, with each group randomly stocked with an exact number of chicks. There were three replicates of 20 birds in floor pens with the same set management regime: i) days 1 to 14: room temperature between 30 and 32 °C; ii) days 15 to 21: room temperature between 24 and 26 °C; and iii) days 22 to 42: room temperature between 22 and 34 °C. Four groups of MOLE-added (mg/kg) diets were established: T0 (control), no MOLE-based diet; T1: 100 mg/kg; T2: 200 mg/kg; and T3: 400 mg/kg. The birds were raised for 42 days under *ad libitum* supply of water and feed (Table 1). A nutritionally balanced starter diet (0-21 days) and finisher diet (22-42 days) were formulated to meet or exceed the requirements of the NRC (1994) recommendations, and the broilers were fed (Table 1). Both diets were isocaloric and isonitrogenous. Feeding was carried out twice a day, and birds were observed on random days to assess general health, behavior, and mortality. The temperature, humidity, and lighting conditions were controlled during the trial. Biosecurity measures and standard vaccination routines were followed during the study.

Table 1.
Composition and estimated nutrient values of the experimental diets.

Ingredient (%)	Starter (0–21 d)	Finisher (22–42 d)
Poultry Oil/Fat	1.840	2.630
46% Soybean meal	28.410	26.220
Corn	59.820	61.910
NaCl	0.350	0.350
APC	6	6
Limestone	1.450	1.321
Threonine	0.110	0.010
$NaHCO_3$	0.100	0.100
DL Methionine	0.320	0.220
Lysine Sulfate	0.390	0.2510
Phytase	0.010	0.010
Di Calcium Phosphate	0.870	0.640

Cont.

Ingredient (%)	Starter (0–21 d)	Finisher (22–42 d)
70% Choline Chloride	0.100	0.100
Mineral and Vitamin Premix	0.250	0.240
Calculated nutrients		
Ash	5.199	4.669
Ether extract	5.121	6.702
Dry matter	85.283	83.711
Crude Fiber	2.714	2.641
Protein	21.051	19.430
Cl	0.283	0.285
Na	0.180	0.180
Ca	0.900	0.760
K	0.849	0.796
Available Phosphorus	0.450	0.380
Tryptophan (D)	0.223	0.207
Methionine (D)	0.597	0.501
Lysine (D)	1.220	1.020
MetþCys (D)	0.311	0.800
AME (Kcal kg^{-1})	2975	3100

Supplementation with dietary MOLE (100, 200, and 400 mg/kg feed). Values are presented as percentages, unless otherwise stated. APC, animal protein; AME, apparent metabolizable energy concentrate.

Preparation of *Moringa oleifera* leaf extract (MOLE)

The leaves of *M. oleifera* are of trees grown in warm, sunny climates in well-drained soils. The harvesting of leaves starts 6-12 months after planting, and the crop can be harvested several times per year, with nutritional value being season-dependent. They are rich in protein, vitamins (A, C, E, and B-complex), minerals (calcium, potassium, and iron), and bioactive substances (flavonoids, glucosinolates, and phenolics) (Table 2). Fresh *M. oleifera* leaves were brought to the laboratory and washed as soon as they were obtained to remove debris on their surface. Thereafter, they were dried in the shade at room temperature (25-28 C°) within a period of 7 days. The dried leaves were then ground in a mechanical grinder to obtain a fine powder. To prepare an extract, 500 g of the powder was repleted in 70 % ethanol (w/v 1:5) and left in agitation for 3 days. The content was filtered through Whatman No. 1 filter paper, and the filtrate was evaporated under vacuum at 40 °C in a rotary evaporator to yield a crude ethanolic extract. The extract was preserved in airtight and amber-colored bottles and was kept at 4 °C before being added to the feed as per the given dosage of the treatments (Khan *et al*., 2022).

Table 2.
Chemical characterization of *Moringa oleifera* leaf extract (MOLE)

Parameter	Method of analysis	Concentration (mean ± SD)	Unit
Total phenolic content	Folin–Ciocalteu method	85.4 ± 3.2	mg GAE/g extract
Total flavonoid content	Aluminum chloride colorimetric method	42.7 ± 2.1	mg QE/g extract
Vitamin A (β-carotene)	HPLC	18.6 ± 1.4	mg/100 g extract
Vitamin C (ascorbic acid)	HPLC / Titrimetric	112.3 ± 5.8	mg/100 g extract
Vitamin E (α-tocopherol)	HPLC	27.9 ± 2.0	mg/100 g extract
Crude protein	Kjeldahl method	24.8 ± 1.3	% (dry basis)
Moisture content	AOAC method	6.2 ± 0.4	%
Ash content	AOAC method	9.6 ± 0.5	%

Performance assessment

Feed intake and weight were predicted weekly. The difference between the initial and final weights was measured as weight gain. The feed conversion ratio (FCR) was calculated using the following formula: FCR = Feed Intake (g) / Body Weight Gain (g). Mortality was recorded daily and used to calculate the adjusted feed intake and performance.

Carcass assessment

During the 42-day trial, the animals were housed at an average of five birds per replicate, with 15 replicates per treatment. At the end of the experiment, 10 birds were randomly selected from each treatment and placed in temperature-controlled cages, where they were provided with 12 h of *ad libitum* access to water. The birds were slaughtered, weighed, and exsanguinated following standard halal procedures. The carcass traits measured were live weight, dressed weight, dressing percentage, breast weight, thigh weight, and liver weight. All values are presented as percentages of live body weight.

Biochemical analysis and blood sampling

At the end of the experiment, 10 birds were randomly selected from each treatment group before slaughter, and 5 mL of whole blood was collected through the jugular vein using non-heparinized syringes. The samples were allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 min. The outcome of the reaction in serum was collected as a supernatant and stored at -20 °C for later biochemical analysis (Khan *et al*., 2019). Serum biochemical indicators were determined using an automated UV-

Vis spectrophotometer (IRMECO Model U2020, Mountain View, CA, USA) and a commercial kit (Randox Laboratories Ltd. United Kingdom, BT29 4 QY). Liver function tests included alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Kidney tests included creatinine, urea, lipid profile included total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides. Biomarkers of antioxidants: superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) (determined by enzyme-linked immunosorbent assay kits and spectrophotometric analysis) (Shah *et al*., 2019).

Cultivation and counting of bacteria from intestinal microflora

Six samples from the cecum and ileum were collected from each group on day 42 of the study to analyze the microbiome. The contents were combined with nine milliliters of sterile saline and homogenized. The suspensions were then serially diluted with sterile saline (101 to 106). To duplicate plates, 100 µl of the suspension was added to each plate, and the plates were incubated anaerobically at 37°C. For anaerobic assays, *Lactobacillus* was cultured on Lactobacilli MRS agar (Fisher Scientific, Ottawa, ON, Canada) after 48 h of incubation at 37°C. Wilkins-Chalgren agar (Oxoid, Nepean, ON, Canada) with glacial acetic acid (1 ml/L) and mupirocin (100 mg/L), extracted from antibacterial discs (Oxoid, London, UK), was used to enumerate Bifidobacteria. The plates were incubated at 37°C for 20 h. *Escherichia coli* was cultured on MacConkey agar (Oxoid, UK) plates. The mean number of colony-forming units in triplicate plates was used to determine the logarithm of colony-forming units per gram of gut content of each bird sample (Shah, Wu *et al*., 2020).

Statistical analysis

The treatment mean differences were compared using one-way ANOVA in SPSS software (version 22), and significant differences were found using the Tukey post-hoc test. Means and standard errors (SEM) are provided, and significance was set at $P < 0.05$. Correlation analysis was performed using the Pearson correlation coefficient to evaluate the strength and direction of the linear relationships among the measured variables.

RESULTS

The effects of *M. oleifera* leaf extract supplementation on broiler growth performance are summarized in Table 3. No significant differences ($P > 0.05$) were observed in the initial body weights among the treatment groups, confirming homogeneous allocation. However, the final body weight, total weight gain, and feed conversion ratio (FCR) were significantly influenced by the dietary MOLE inclusion.

The carcass evaluation results are presented in Table 4. A significant improvement ($P < 0.05$) in dressing percentage was observed in the MOLE-supplemented groups compared to the control, with the highest value recorded in the T2 group (74.5 ± 1.3%). Breast muscle yield, a key indicator of meat quality and market value, was also significantly enhanced ($P < 0.05$) in birds receiving 200 mg/kg (26.1 ± 0.7%) and 400 mg/kg (25.8 ± 0.8%) MOLE compared to the control group (23.5 ± 0.8%). The thigh yield was numerically higher in the treated groups but not

Table 3.
Effects of MOLE on the growth performance of broilers (Day 1–42).

Parameter	T0 (Control)	T1 (100 mg/kg)	T2 (200 mg/kg)	T3 (400 mg/kg)	SEM	*P*-value
Initial weight (g)	42.1 ± 1.2	42.3 ± 1.3	42.4 ± 1.1	42.5 ± 1.4	0.15	ns
Final weight (g)	2150 ± 45^{a}	2220 ± 50^{ab}	2310 ± 48^{b}	2265 ± 52^{ab}	35.7	0.04
Weight gain (g)	2107 ± 44^{a}	2177 ± 48^{ab}	2267 ± 46^{b}	2223 ± 50^{ab}	33.4	0.03
Feed intake (g/bird)	3700 ± 65	3735 ± 70	3760 ± 72	3850 ± 75	54.2	ns
FCR	1.76 ± 0.03^{b}	1.72 ± 0.02^{b}	1.66± 0.02^{a}	1.73 ± 0.03^{b}	0.02	0.01

Values with different superscripts ($^{a, b}$) in a row are significantly different ($P < 0.05$).

Table 4.
Carcass traits of broiler chickens on day 42.

Trait (%)	T0 (Control)	T1 (100 mg/kg)	T2 (200 mg/kg)	T3 (400 mg/kg)	SEM	*P*-value
Dressing percentage	69.1 ± 1.2^{a}	72.2 ± 1.1^{ab}	73.5 ± 1.3^{b}	72.8 ± 1.2^{b}	0.9	0.02
Breast yield	23.5 ± 0.8^{a}	24.2 ± 0.9^{ab}	26.1 ± 0.7^{b}	25.8 ± 0.8^{b}	0.6	0.03
Thigh yield	15.4 ± 0.6	15.8 ± 0.7	16.1 ± 0.5	16.2 ± 0.6	0.4	ns
Liver weight	2.5 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	2.6 ± 0.3	0.1	ns

Values with different superscripts ($^{a, b}$) in a row differ significantly ($P < 0.05$).

statistically significant ($P > 0.05$). Relative liver weights showed no significant differences among the treatments, suggesting that MOLE had no adverse effects on internal organ development.

Table 5 and Figure 1 show the effects of MOLE supplementation on serum biochemical parameters. Broilers supplemented with MOLE exhibited significantly reduced ($P < 0.05$) serum total cholesterol and LDL levels, particularly in the T2 group, which recorded the lowest values (150.5 ± 6.0 mg/dL and 85.0 ± 3.7 mg/dL, respectively). HDL concentrations were significantly higher in all MOLE-treated groups, with T2 showing the most notable increase (48.9 ± 2.5 mg/dL) (Figure 1). Triglyceride levels were also reduced in the T2 and T3 groups, although the reduction was not statistically significant. The liver function enzyme levels (ALT and AST) in the MOLE-treated groups were significantly lower than those in the control group, indicating better hepatic function. The ALT level decreased in the control by 32.4 U/L, with a standard deviation of 2.2 to 27.2 U/L in T2 ($P < 0.05$), while the AST values declined among treatments, with the lowest value observed in T2 (40.2 ± 2.3 U/L) (Figure 2). Kidney function markers (urea and creatinine) remained within normal physiological ranges and showed no significant differences ($P > 0.05$) among the groups, suggesting that MOLE supplementation did not impair renal function.

MOLE supplementation significantly enhanced the serum antioxidant status (Table 6). The birds in the T2 and T3 groups exhibited higher superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities than those in the control group ($P < 0.05$). SOD activity in T2 reached 124.4 ± 6.5 U/mL, whereas it was 96.6 ± 5.2 U/mL in the control group. Similarly, GPx activity was highest in T2 (37.5 ± 2.1 U/mL), indicating an improved oxidative stress resistance. Malondialdehyde (MDA) levels, a marker of lipid peroxidation, were significantly lower in MOLE-supplemented birds. The lowest MDA concentration was recorded in T2 (1.39 ± 0.08 nmol/mL), suggesting reduced oxidative damage to the cell membranes.

Table 5.
Serum biochemical parameters of broiler chickens

Biomarker	T0 (Control)	T1 (100 mg/kg)	T2 (200 mg/kg)	T3 (400 mg/kg)	*P*-value
Total cholesterol (mg/dL)	180.3 ± 6.5	165.2 ± 5.8	150.5 ± 6.0	153.7 ± 5.7	0.01
Urea (mg/dL)	22.5 ± 1.5	21.3 ± 1.4	20.1 ± 1.2	20.4 ± 1.3	ns
Creatinine (mg/dL)	0.84 ± 0.05	0.80 ± 0.04	0.78 ± 0.03	0.79 ± 0.04	ns

Values with different superscripts ([a, b]) in a row differ significantly ($P < 0.05$).

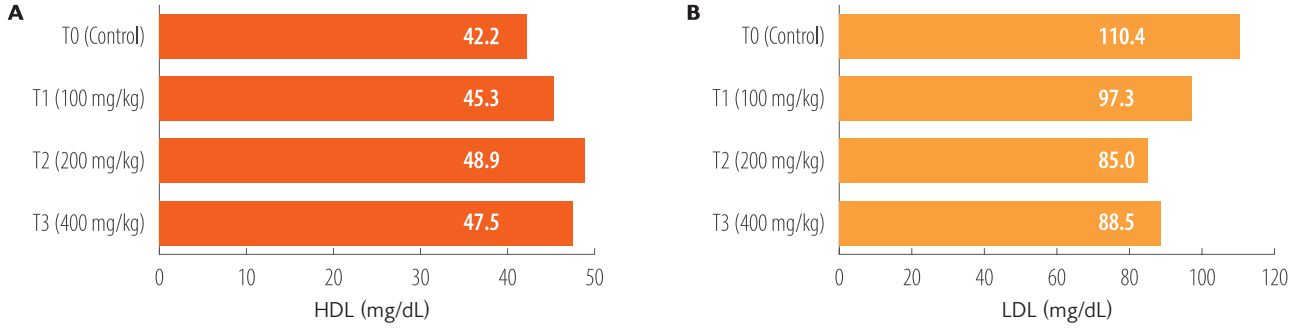

Figure 1.
Serum biochemical high-density lipoprotein (HDL) (A) and low-density lipoprotein (LDL) (B) parameters of broiler chickens

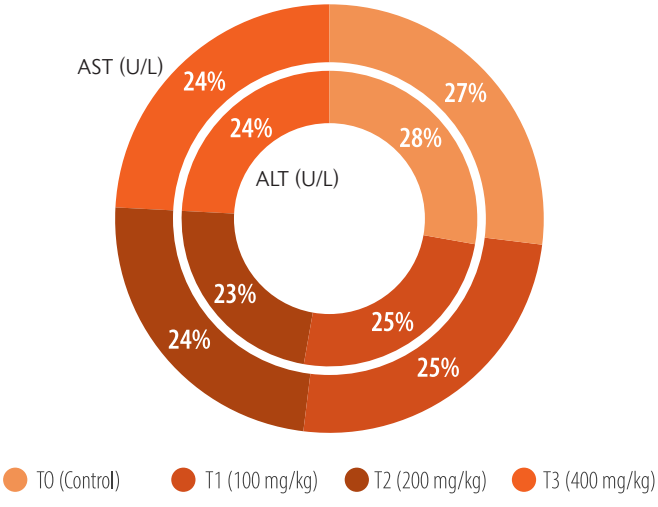

Figure 2.
Liver functions enzyme alanine aminotransferase (ALT) and aspartate aminotransferase (AST) parameters of broiler chickens

Table 6.
Antioxidant status of broiler chickens supplemented with MOLE

Parameter	T0 (Control)	T1 (100 mg/kg)	T2 (200 mg/kg)	T3 (400 mg/kg)	SEM	P-value
Superoxide dismutase (SOD, U/mL)	96.6 ± 5.2[a]	111.3 ± 6.1[ab]	124.4 ± 6.5[b]	118.5 ± 5.9[b]	4.35	0.01
Glutathione peroxidase (GPx, U/mL)	29.6 ± 1.9[a]	33.1 ± 2.0[ab]	37.5 ± 2.1[b]	36.4 ± 2.3[b]	1.52	0.02
Malondialdehyde (MDA, nmol/mL)	2.17 ± 0.10[a]	1.70 ± 0.09[b]	1.39 ± 0.08[c]	1.48 ± 0.07[c]	0.06	< 0.001

Values are expressed as mean ± SEM (n = 10 birds/treatment). Different superscripts within a row indicate significant differences (P < 0.05).

Table 7.
Mortality rate and general health observations in broiler chickens

Treatment group	No. of Birds	Mortality (%)	Observed health issues	Behavioral abnormalities
T0 (Control)	60	2 (3.3%)	Mild lethargy, occasional pasting	None
T1 (100 mg/kg)	60	1 (1.7%)	None	None
T2 (200 mg/kg)	60	0 (0%)	None	None
T3 (300 mg/kg)	60	0 (0%)	None	None

All deaths occurred within the first two weeks and were not related to treatment. No signs of toxicity, diarrhea, or respiratory distress were observed in any of the groups throughout the experiment.

Throughout the 42-day trial, overall mortality remained low and did not significantly differ among the treatment groups (0–2.5 %). No signs of toxicity or abnormal behavior were observed in the MOLE-supplemented birds, indicating the safety and tolerance of the extract at the tested doses (Table 7).

An increased antibacterial effect of *M. oleifera* leaf extract was observed in relation to cecal content E. coli and *Bifidobacterium* spp. counts in a group of broilers on supplemented diets than in control group. At the same time, the population of beneficial microbes, especially *Lactobacillus* spp., showed a significant increase, which both reaffirms and signifies a change in the composition of the intestinal microbiota in the direction of a healthier domain (Figure 3). Such modifications were greatest at the moderate inclusion level of MOLE (e.g. 200-400 mg/kg), indicating a dose-reactivity to microbial modulation.

The heatmap labelled correlation between gut microbiota and biomarker profiles in broiler chickens visualizes the interactions between the most important populations of the gut microbial community (*Lactobacillus* and *E. coli*) and several physiological biomarkers, such as total antioxidant capacity (TAC), serum cholesterol, and triglycerides. There was a positive correlation between *Lactobacillus* and total antioxidant capacity (r = +0.95), suggesting a positive relationship between high levels of beneficial gut bacteria and high levels of antioxidant capacity in broilers. The correlation of *Lactobacillus* with serum cholesterol and triglyceride levels also showed that *Lactobacillus* was inversely related to serum cholesterol (r =-0.94) and triglyceride (r =-0.92) levels, indicating that the higher the levels of *Lactobacillus*, the better the lipid profile (Figure 4). In turn, E. coli demonstrated a negative correlation with the total antioxidant capacity (r = -0.91) and a positive correlation with both serum cholesterol (r = +0.93) and triglycerides (r = +0.89), indicating that an increased level of pathogenic bacteria is associated with oxidative stress and worse lipid metabolism. These associations substantiate the theory that the manipulation of the gut microbiome using *M. oleifera* leaf extract has a remarkable impact on the systemic indicators of health by inspiring antioxidant defense as well as lessening the condition of metabolic syndromes in broilers.

DISCUSSION

The current study evaluated the effects of dietary *M. oleifera* leaf extract (MOLE) on growth patterns, carcass composition, and physiological responses in broiler chickens. The results showed that supplementation, particularly at a dose of 200 mg/kg, significantly increased growth, feed conversion ratio (FCR), and carcass characteristics, as well as increased the activity of antioxidants in the serum and lipid levels. Therefore, these findings prove that MOLE may serve as an effective natural dietary supplement for poultry growth. One of the most striking results is the high-performance effect that was evidenced in the treatment group administered 200 mg/kg MOLE, which exhibited extremely rapid weight gain and high FCR (Rossi *et al*., 2020; Singh *et al*., 2024). The suggested bioactive elements of *M. oleifera* leaves are flavonoids and phenolic

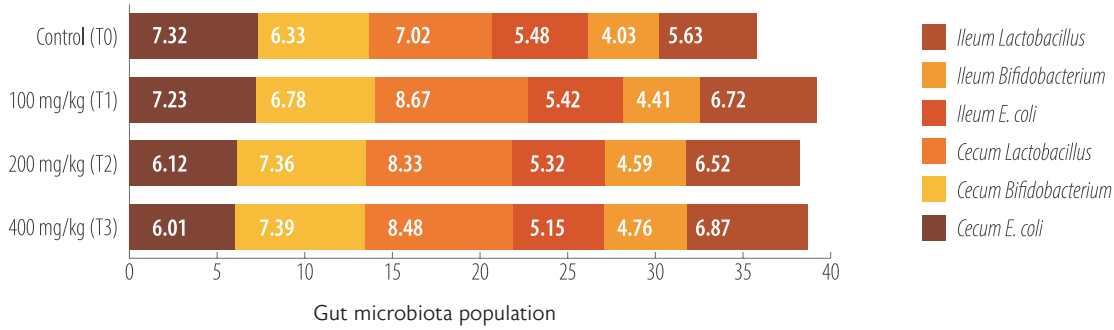

Figure 3.
Gut microbiota population in broiler chickens.

Figure 4.
Correlation between gut microbiota and biomarker profile in broiler chickens.

compounds, including quercetin and kaempferol, which are hypothesized to ease nutrient absorption and stimulate digestive enzymes. Furthermore, essential amino acid and micronutrient supplementation could enhance protein deposition and energy metabolism, leading to better growth performance. Similar findings have been reported by Melesse *et al*. (2018) and Ayssiwede *et al*. (2011). The chemical characterization of *M. oleifera* leaf extract provides a crucial foundation for future diet formulation and useful applications in broiler nutrition. The measured concentrations of bioactive substances, such as proteins, phenolics, flavonoids, and vitamins, aid in determining the minimum amounts necessary to improve the physiological biomarkers, growth performance, carcass yield, and gut microbiota modulation (Abu Hafsa *et al*., 2020). To ensure that results are consistent, effective, and repeatable across various production systems, it is essential to establish baseline concentrations. Higher inclusion levels may present risks due to the excessive intake of specific phytochemicals; therefore, safety concerns must be prioritized (Rossi *et al*., 2020; Singh *et al*., 2024). Higher concentrations should not be exceeded without specific toxicological evaluation because the upper inclusion level (400 mg/kg diet) in the current study did not provide any additional benefits and may approach a threshold beyond which adverse or suboptimal responses may occur. To ensure repeatability and reliable biological reactions, natural extracts must be standardized according to their active compound profiles (Melesse *et al*., 2018). In antibiotic-free broiler production systems, determining effective mini-

mum doses also makes it possible to evaluate the viability of adding MOLE to commercial feed formulations by balancing biological efficacy with practicality and safety (Abu Hafsa *et al*., 2020; Rossi *et al*., 2020; Singh *et al*., 2024).

This study ranked the effects of dietary addition of *M. oleifera* leaf extract (MOLE) to broiler chicken diets on body development, carcass output, and physiological measurements. In the designed experiment, it was shown that the addition of MOLE at the level of 200 mg/kg promoted the improvement of body weight, feed conversion ratio (FCR), carcass characteristics, serum antioxidant status, and plasma lipid content. Therefore, the data showed that MOLE can be useful as a natural additive to enhance the development and growth of poultry. In addition, the essential amino acids and micronutrients present in MOLE could increase the levels of protein synthesis and improve the use of energy, leading to better animal growth. Likewise, the findings of Melesse *et al*. (2018) and Abu Hafsa *et al*. (2020) on the performance of broilers included with moderate level of *M. oleifera* diet also showed comparable results in improvements in performance. Therefore, strict control of dosing is necessary to maximize the positive effects of MOLE and decrease negative responses. The improvements seen in performance, carcass yield, and health show the many benefits of MOLE as a feed additive. Unlike synthetic growth promoters that focus on specific pathways, plant extracts such as MOLE provide a wider range of benefits. These include nutrient support, antioxidant protection and metabolic control (Singh *et al*., 2024). The leaves of *M. oleifera* are sources of flavonoids (e.g., quercetin and kaempferol), phenolic acids (e.g., chlorogenic acid), vitamins, and isothiocyanates that are complementary to one another and operate in myriad ways (Menichetti *et al*., 2025). Antioxidant compounds neutralize the effects of reactive oxygen species and improve endogenous antioxidant enzymes, thus alleviating oxidative stress (Singh *et al*., 2024). NF-KB signaling is prevented by anti-inflammatory isothiocyanates and flavonoids, which reduce pro-inflammatory cytokines (Coello *et al*., 2020). Antimicrobial benzyl isothiocyanates destabilize microbial membranes and interfere with protein production (Singh *et al*., 2024). Antidiabetic phenolics enhance insulin sensitivity and prevent the action of carbohydrate-digesting enzymes, such as alpha-amylase and alpha-glucosidase. Flavonoids also promote cardioprotective properties by suppressing LDL oxidation and enhancing lipid metabolism (Coello *et al*., 2020).

An increased carcass yield was also noted in MOLE-fed broilers, especially in the T2 group (ideal dose), which had the highest dressing percentage and breast muscle weight. Such gains may be ascribed to the high levels of phytochemicals in *M. oleifera*, with a special prevalence of flavonoids, vitamins (A, C, and E), and high-quality proteins that, combined, improve nutrient absorption, muscle accretion, and overall growth performance (Nantapo *et al*., 2024). The antioxidant capacity of MOLE also contributes to the reduction of oxidative stress in muscular tissues, which could result in enhanced protein production and muscle hypertrophy (Soni *et al*., 2022; Singh *et al*., 2024).

There was no noticeable increase in the size of the liver, gizzard, and heart recorded among the treatments, implying that there were no hypertrophic effects of MOLE on internal organs compared to some of the synthetic growth promoters, which have been reported to alter the size of the liver (hepatomegaly) (Attah *et al*., 2022; Kashyap *et al*., 2022). However, the absolute weights of the internal organs were still within the physiological range, indicating the tolerance and safety of the extract. Additionally, the higher percent yield of breast meat and weights of thigh and drumstick in MOLE-supplemented birds is in agreement with the previous findings of Zanu *et al*. (2012), who observed higher lean tissue deposition in broilers fed Moringa-based diets. This enhancement in edible portions is financially desirable and resonates with the increasing inclination of consumers to use natural additives in preference to artificial antibiotics or hormones.

Supplementation with MOLE also affected the major physiological and biochemical indicators that reflected a systemic improvement in the immunity and metabolism of broilers. The T2 group reduced serum cholesterol and triglyceride levels significantly as compared to the control and fed birds, hence the birds in the T2 group had hypolipidemic effects. The decrease in lipid levels could be attributed to the actions of saponins and polyphenols found in MOLE, which have been reported to reduce the production of lipids and encourage their breakdown through biliary secretion and emulsification of lipids (Elahwl *et al*., 2025). In addition, there was a significant increase in serum total protein and albumin levels in the MOLE-treated groups. This enhancement implies better digestion and absorption of proteins, which could be facilitated by the amino acid content of *M. oleifera* and its effects on proliferating digestive enzymes (Abdel-Raheem & Hassan, 2021). Improved nutritional conditions and liver functions are also indicated by high albumin levels, which means that the nutritional status of the broilers and their antioxidant status improved significantly with MOLE supplementation, as shown by increased superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels and decreased malondialdehyde (MDA) levels in the serum. Such enzymes are of utmost importance in the neutralization of free radicals, thus safeguarding tissues from free radicals (Shah *et al*., 2016). The marker of oxidative stress was MDA, which was extremely low in the T2 group, indicating reduced oxidative stress. The results of this study are similar to those of Mohamed Kamil *et al*. (2024), who showed the antioxidative properties of Moringa supplements in poultry and other livestock. The reduced mortality and overall health of the treated populations can also be supported by increased levels of antioxidant enzymes (Awan *et al*., 2024). Oxidative stress is a major cause of immune suppression, metabolic diseases, and organ malfunction in fast-growing

broilers. MOLE reduces the risk of diseases by improving physiological survival and immune performance through the reduction of oxidative stress (Shah, Liu *et al*., 2020; Elahwl *et al*., 2025).

The effects of *M. oleifera* leaf extract on the composition of intestinal bacteria revealed that the extract has beneficial effects on the regulation of intestinal microbiota and, hence, the health of the host. The decrease in pathogenic bacteria could be explained by the antimicrobial effect of phenolic substances and saponins in MOLE, which increase the permeability of microbial cell membranes and prevent colonization. Simultaneously, the increase in probiotic bacteria, including *Lactobacillus*, implies a prebiotic effect that can bolster the strength of the gut barrier and augment nutrient uptake. Such alterations in the microbiota have been associated with better immune response and feed efficiency, which underscores MOLE as a potential natural supplement for the enhancement of gut health and antibiotic-free broiler production systems.

CONCLUSION

In conclusion, the supplementation of broiler chickens with *M. oleifera* leaf extract (MOLE) had a significant effect on growth performance, gut microbiota, carcass yield, and health biomarkers of broiler chickens. The extract increased the antioxidant status and population of beneficial microbes, especially *Lactobacillus* spp., decreased lipid levels, and facilitated immune functions, particularly at moderate inclusion levels. These results indicate that MOLE may be a viable natural substitute for synthetic growth promoters, enhancing poultry health and productivity.

Ethical approval statement

All procedures involving animals in this study were conducted in strict accordance with ethical standards and guidelines for the care and use of animals in research. The experimental protocol was reviewed and approved by the Faculty of Veterinary and Animal Sciences, Gomal University DI Khan.

Declarations of competing interest

The authors declare no conflicts of interest.

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