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**Research Paper**

# Effects of moringa oleifera leaf meal supplementation on growth performance, carcass characteristics, serum biochemistry, antioxidant status, and intestinal morphology of broiler chickens

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**ABSTRACT**

The dose dependent effect of Moringa oleifera leaf meal (MOLM) on growth performance, carcass yield, serum biochemical parameters, hepatic antioxidant enzyme activities and intestinal morphology was investigated in Cobb 500 broiler chickens. A basal control diet (T0) and diets fortified with 0.5% (T1), 1% (T2) and 1.5% MolM (T3) were fed to a total of 240 day-old chicks, equally distributed into four dietary treatments (60 chicks per treatment) with six replicates comprising 10 chicks each for 56 days. The final body weight, weight gain and breast meat yield significantly ($P<0.05$) increased with the increasing dose, and T3 exhibited the highest level of 410.4 g, 367.2 g weight gain and 32.3% breast meat yield, respectively. FCR was improved by the addition of MOLM in a gradual manner, starting from 1.86 in the control group to 1.53 in T3. In MOLM-fed birds, serum total protein, and albumin concentrations were raised, whereas serum cholesterol, triglycerides, and glucose concentrations were decreased. Activities of hepatic superoxide dismutase, catalase and glutathione peroxidase were elevated and concentration of malondialdehyde was found to be decreased by 36.8% in T3 as compared with control, which collectively suggested that there is a boost in the oxidative protection in T3. The mucosal integrity and absorptive capacity were enhanced in T3 as shown by the increased jejunal villus height (1,052 µm, compared to 843 µm in the control) and the villus height to crypt depth ratio (6.97, compared to 4.56). However, hepatotoxicity was not observed at these inclusion levels as indicated by the lack of effect of treatment on relative organ weights and serum AST/ALT activities. These results indicate the use of 1.5% MOLM in the diet would be ideal for broiler production and could provide a sustainable, cost-effective phytogenic feed additive with antioxidant, hypocholesterolaemic and growth promotion effects that can be used in broiler production systems without the use of antibiotics.

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## 1. INTRODUCTION

As the global poultry industry is increasingly under pressure to deliver lean and quality poultry products, it is also required to minimize or eliminate the use of synthetic growth promotants and antibiotic growth promoters (AGPs) [1]. In 2006, the EU adopted the ban on AGPs, and many other Asian and African countries are pursuing similar regulations that are now calling for safer, more effective and cost-effective natural alternatives [2]. The essential oils, botanical extracts and whole plant powders from plants are collectively known as phytogenic feed additives and have been a subject of great scientific interest because of their multifunctional properties, such as modulation of secretion of digestive enzymes, gut microbiota composition, antioxidant defence and immune responsiveness [3].

Moringa oleifera Lam. The “miracle tree” (family Moringaceae) is native to the sub-Himalayan region of South Asia but has been cultivated pantropically [4]. Crude protein (25-30%), essential amino acids, β-carotene, ascorbic acid, tocopherols, flavonoids (e.g., quercetin, kaempferol), glucosinolates and phenolic acids have all been found in the leaves at levels significantly higher than conventional forages [5]. Isothiocyanate and 4-(α-L-rhamnosyloxy)-benzyl isothiocyanate (moringin) are the compounds present in leaf meal which shows antimicrobial, anti-inflammatory and hepatoprotective properties in vivo [6]. The beneficial effect of these bioactive compounds makes Moringa oleifera leaf meal (MOLM) a candidate for being a multi-target feed additive for broiler nutrition. Despite this promise, however, results reported in the literature are quite variable, depending on the inclusion rate, agro-climatic zone, and duration of the trials, and the resulting doses-response relationships are reported in conflicting ways making it difficult to make practical recommendations for commercial producers [7], [8]. The mechanistic study of the action of MOLM on oxidative stress biomarkers and intestinal morphology, two important determinants of the efficiency of nutrient absorption, is still relatively limited, and very few studies have evaluated growth, feed efficiency, carcass merit, serum biochemistry, and the antioxidant-morphological axis in the same controlled experiment [9].

For this reason, the present study aimed to: (i) establish a dose-response relationship between graded MOLM inclusion (0, 0.5, 1.0 and 1.5%) and productive performance in Cobb 500 broilers, (ii) quantify the treatment effects on carcass characteristics and meat composition, (iii) assess serum biochemical alterations related to metabolic homeostasis, (iv) determine hepatic antioxidant enzyme activities and lipid peroxidation status, and (v) characterise small intestinal villus morphometry as an indicator of absorptive capacity and gut health. The rest of the written work is presented and organised as follows: Section 2 reviews related work on phytogenic and Moringa-based feed additives in poultry; Section 3 presents the methodology of experiments; Section 4 presents and discusses the results of the experiment; and Section 5 concludes the study with some practical suggestions.

## 2. RELATED WORK

There is a considerable amount of research on the use of plant based feed ingredients, which could be used as substitutes for AGPs in broiler nutrition. In the preliminary research on phytogenic additives, essential-oil mixtures and herbal extracts have been proven to increase digestibility of nutrients and, in some trials, to slightly increase growth rates despite some inconsistencies being found between trials due to variation in concentration of active ingredients in the essential-oils and in the extracts' bioavailability [10]. Later meta-analyses also found that the magnitude of growth response to phytogenics is highly dependent on the type of basal diet, the environmental stressor and the standardization of the additive [11]. Of this class, Moringa oleifera has been given special focus due to its unique high concentration of phytochemical and nutrients. A number of feeding trials has been conducted with broiler chickens that have shown that MOLM supplementation between 0.5% and 2.0% of the broiler diet improves FCR and BWG compared with the unsupplemented control, and the improvement was found to be greatest when the supplement level was below 2% and less at higher supplementation levels as a result of the presence of antinutritional tannin and saponin in MOLM leaf [12]. Such improvements in dressing percentage and breast meat yield have also been observed in MOLM fed broilers which is explained by the high EAA content of the leaf meal compared to other protein supplements like soybean meal [13]. In terms of serum biochemistry, adding Moringa leaf products to the diet has always been linked with a decrease in serum cholesterol, triglycerides, and glucose as well as an increase in total protein and albumin, which has been attributed to the hypocholesterolaemic effect and enhanced protein synthetic function in the liver [14]. Biochemical changes in laying hens and broilers have been correlated with the phytosterol and flavonoid component of Moringa leaves that disrupts cholesterol micellarisation in the intestine and regulates the activity of important enzymes involved in lipid metabolism [15].

Regarding oxidative status, dietary supplementation with phenolic and flavonoid rich plant materials such as Moringa leaf meal, rosemary extract and grape pomace have been reported to have the ability to increase the activity of the antioxidant enzymes, namely superoxide dismutase, catalase and glutathione peroxidase in the liver, and decrease the malondialdehyde concentration in tissues of broilers, which is believed to occur via activation of the Nrf2/ARE antioxidant signalling pathway [16]. It has also been shown that there is an increase in villus height and villus height to crypt depth ratio after the inclusion of phytogenic feed additives such as additives based on Moringa in the diet, which is correlated to a higher efficiency of nutrient uptake and gut conditions in the poultry [17]. Despite this emerging body of evidence, there are still a number of areas of uncertainty. Few studies have examined the effects of MOLM over a sufficiently fine range of inclusion (0-1.5%) in one test to demonstrate a clear linear or quadratic dose-response curve. Second, the simultaneous evaluation of growth, carcass, serum biochemical, antioxidant as well as gut morphometric parameters in one experimental model, thus enabling the mechanistic correlation between systemic and local gut level response, has been rarely attempted. Third, most of the literature is based on a few limited agro-climatic conditions and broiler genotype, thus dose recommendations are not easily generalised to more popular commercial strains, such as Cobb 500. The aim of the present study was to overcome these limitations by testing a 4-level dose gradient (0, 0.5, 1.0 and 1.5% MOLM) in Cobb 500 broilers and examining performance, carcass, biochemical, antioxidant and histomorphometric endpoints in a common 56-day test.

## 3. METHODOLOGY

### 3.1. Moringa Leaf Meal Preparation and Proximate Analysis

Moringa oleifera leaves were collected from the mature trees (more than 2 years) in the University experimental farm from 07:00 – 09:00 h. Leaves were shade dried for 72 h at 38 ± 2 °C and ground to pass thru a 1.0 mm screen and stored in sealed high density polyethylene bags at 4 °C until use. The proximate composition was determined according to the method described by AOAC (2019) [18] including dry matter (DM), crude protein (CP, method 984.13), ether extract (EE, method 920.39), crude fibre (CF, method 973.18) and ash. Nitrogen free extract (NFE) was determined as a difference value. A phytochemical

analysis of the total phenolics, flavonoids and tannins was performed by following the spectrophotometric techniques [19].

### 3.2. Animals, Housing, and Experimental Design

The Institutional Animal Ethics Committee (IAEC/MPKV/2024/0047) approved all the experimental procedures. A total of 240 day-old Cobb 500 (mean body weight: 43.2 ± 0.4 g) broiler chicks were purchased from a commercial hatchery and randomly allocated into four dietary treatment groups as T0 (Cobb 500 basal diet), T1 (0.5% MOLM), T2 (1.0% MOLM), and T3 (1.5% MOLM) by complete randomised design (CRD) technique. The treatments consisted of 10 chicks per pen with 6 replicates in wire-floored battery brooders (1.5 m × 1.0 m). The lights were on continuously for the first 7 days and then a 16L:8D cycle was followed. All vaccinations were given as per the national programme for Marek disease, Newcastle disease, infectious bronchitis and Gumboro disease with the temperature remaining at 33 °C in the first week and then lowered by 3 °C per week to 24 °C.

### 3.3. Dietary Treatments and Feeding

To meet or exceed the NRC (1994) requirement for broilers [20], basal diets were formulated. The ingredient matrix in the starter (days 1-21) and finisher (days 22-56) phases were replaced by MOLM (at the same percentage as fed). The feed and water were offered freely during the experiment. The feed intake per replicate and individual bird weights were taken weekly to calculate the BWG and FCR. The experimental diets consisted of various ingredients and their calculated nutritive values are given in Table 1.

Table 1. Ingredient Composition and Calculated Nutritive Values of Experimental Diets (As-Fed Basis)

| Ingredient / Parameter | T0 (Control) | T1 (0.5%) | T2 (1.0%) | T3 (1.5%) |
|---|---|---|---|---|
| Maize (%) | 55.0 | 55.0 | 55.0 | 55.0 |
| Soybean meal (%) | 30.0 | 29.6 | 29.2 | 28.8 |
| Fish meal (%) | 5.0 | 5.0 | 5.0 | 5.0 |
| Wheat bran (%) | 5.5 | 5.4 | 5.3 | 5.2 |
| MOLM (%) | 0.0 | 0.5 | 1.0 | 1.5 |
| Limestone (%) | 1.5 | 1.5 | 1.5 | 1.5 |
| Dicalcium phosphate (%) | 1.5 | 1.5 | 1.5 | 1.5 |
| Premix (%) | 0.5 | 0.5 | 0.5 | 0.5 |
| Salt (%) | 0.3 | 0.3 | 0.3 | 0.3 |
| DL-Methionine (%) | 0.2 | 0.2 | 0.2 | 0.2 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 |
| ME (kcal/kg) | 3,100 | 3,102 | 3,105 | 3,108 |
| CP (%) | 22.0 | 22.1 | 22.1 | 22.2 |
| Calcium (%) | 1.00 | 1.00 | 1.00 | 1.00 |
| Available P (%) | 0.45 | 0.45 | 0.45 | 0.45 |
| Lysine (%) | 1.10 | 1.11 | 1.11 | 1.12 |
| Methionine + Cystine (%) | 0.80 | 0.80 | 0.81 | 0.81 |

Premix provided per kg diet: Vit A 8,000 IU, Vit D3 2,000 IU, Vit E 20 mg, Vit K3 2 mg, B1 1 mg, B2 4 mg, B6 2 mg, B12 12 µg, Niacin 40 mg, Pantothenic acid 8 mg, Folic acid 0.5 mg, Biotin 0.05 mg, Cu 8 mg, Fe 50 mg, Mn 80 mg, Zn 60 mg, I 0.6 mg, Se 0.2 mg.

### 3.4. Slaughter and Carcass Evaluation

On day 56, two birds per replicate (12 birds/treatment) with the closest weight to the mean weight of the replicate were randomly selected, fasted for 12 hours, weighed and then humanely slaughtered (cervical dislocation). Hot carcass weight was measured right after evisceration. Dressing percentage was

determined as the percent of live weight. The breast, thigh, drumstick, wing, back and neck were dissected and weighed and expressed as percentage of the live weight. Liver, gizzard, spleen and bursa of Fabricius weights were calculated relative to body weight.

### 3.5. Serum Biochemistry

Blood samples (5 mL) were taken into vacutainers without anticoagulant, left to clot at ambient temperature and then centrifuged at 3,000 × g for 15 min. The level of total protein (biuret method), albumins (bromocresol green method), cholesterol (CHOD-PAP method), triglycerides (GPO-PAP method), glucose (GOD-POD method), uric acid, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using commercial diagnostic kits (Erba Diagnostics, Mannheim, Germany) in a semi-automated analyser [21].

### 3.6. Antioxidant Enzyme Assays

SOD (EC 1.15.1.1) activity was determined by the ability to inhibit the autoxidation of pyrogallol [22] in liver tissue homogenate (about 200 mg) prepared in ten volumes of buffer (0.1 M phosphate buffer, pH 7.4) and centrifuged at 10,000 × g for 20 min at 4 °C. Hydrogen peroxide decomposing activity of catalase (CAT, EC 1.11.1.6) was determined by measuring the decomposition of hydrogen peroxide at 240 nm [23]. The activity of glutathione peroxidase (GPx, EC 1.11.1.9) was determined by the coupled reaction with glutathione reductase [24]. Malondialdehyde (MDA) was used as a measure of lipid peroxidation, using the thiobarbituric acid reactive substances (TBARS) assay. The Bradford reagent was used to measure all enzyme activities and expressed as mg protein.

### 3.7. Intestinal Morphometry

A portion of the mid-jejunum (approx. 3 cm) was obtained, washed with buffered saline, fixed in 10% neutral buffered formalin, embedded in paraffin, cut to 4 µm and stained with haematoxylin and eosin (H&E). A well-oriented ten crypts and villi were measured in each section (three sections per bird, and two birds per replicate) using a calibrated digital microscope (Nikon Eclipse Ci-L, NIS-Elements v5.0). Villus height (VH), crypt depth (CD), villus width (VW) and VH:CD ratio were measured [25].

### 3.8. Statistical Analysis

One-way analysis of variance (ANOVA) was used to analyse data in IBM SPSS Statistics v.28 (IBM Corp., Armonk, NY, USA). The overall F-test was significant ($P < 0.05$) and means were compared using Tukey's Honestly Significant Difference (HSD) post-hoc test. Polynomial contrasts (linear and quadratic) were tested for dose-response modelling. All data are shown as means ± SEM. $P < 0.05$ was considered statistically significant.

## 4. RESULTS AND DISCUSSION

### 4.1. Proximate Composition of MOLM

The proximate and phytochemical composition of Moringa oleifera leaf meal fed in this trial are summarized in Table 2. The nutritional quality of the leaf meal was high, with 27.1% crude protein, and desirable amounts of total phenolics, flavonoids and condensed tannins, good concentrations of vitamin C and β-carotene, which indicated that the leaf meal was an excellent ingredient in feed for both nutritional and phytochemical value.

Table 2. Proximate and Phytochemical Composition of Moringa Oleifera Leaf Meal (% DM Basis)

| Parameter | Value (Mean ± SD) |
|---|---|
| Dry Matter (%) | 91.4 ± 0.38 |
| Crude Protein (%) | 27.1 ± 0.62 |
| Ether Extract (%) | 5.3 ± 0.21 |

| | |
|---|---|
| Crude Fibre (%) | 9.8 ± 0.44 |
| Ash (%) | 9.2 ± 0.37 |
| Nitrogen-Free Extract (%) | 48.6 ± 1.02 |
| Gross Energy (kcal/kg) | 3,842 ± 28.4 |
| Total Phenolics (mg GAE/g) | 38.6 ± 1.14 |
| Total Flavonoids (mg QE/g) | 22.4 ± 0.83 |
| Condensed Tannins (mg CE/g) | 4.12 ± 0.21 |
| Vitamin C (mg/100g) | 214 ± 8.2 |
| β-Carotene (mg/kg) | 112 ± 4.6 |

### 4.2. Growth Performance

The supplementation with MOLM was significantly (P< 0.05) improved in a linear dose-dependent manner the final body weight, total body weight gain and average daily gain as summarised in Table 1 and illustrated in Figure 1 and Figure 2. The final body weight of the T3 birds was largest (410.4 g more than the corresponding control birds throughout the trial) while the FCR of the T3 birds was gradually better than that of the control birds over the 56-day trial (1.86 in the control and 1.53 in the T3 birds). There was no significant difference between the average daily feed intake of the treatments indicating that MOLM did not increase feed intake, but instead improved feed utilisation efficiency.

Table 3. Growth Performance Parameters of Broiler Chickens Fed MOLM-Supplemented Diets (Means ± SEM)

| Parameter | T0 (Control) | T1 (0.5%) | T2 (1.0%) | T3 (1.5%) | P-value |
|---|---|---|---|---|---|
| Initial BW (g) | 43.2 ± 0.4$^{a}$ | 43.1 ± 0.3$^{a}$ | 43.3 ± 0.5$^{a}$ | 43.2 ± 0.4$^{a}$ | 0.987 |
| Final BW (g) | 342.1 ± 4.8$^{d}$ | 368.4 ± 5.2$^{c}$ | 389.2 ± 5.9$^{b}$ | 410.4 ± 6.1$^{a}$ | < 0.001 |
| Total BWG (g) | 298.9 ± 4.2$^{d}$ | 325.3 ± 5.0$^{c}$ | 345.9 ± 5.4$^{b}$ | 367.2 ± 5.8$^{a}$ | < 0.001 |
| ADFI (g/bird/d) | 82.4 ± 1.2$^{a}$ | 83.1 ± 1.4$^{a}$ | 83.8 ± 1.3$^{a}$ | 84.2 ± 1.5$^{a}$ | 0.742 |
| ADG (g/bird/d) | 5.34 ± 0.08$^{d}$ | 5.81 ± 0.09$^{c}$ | 6.18 ± 0.10$^{b}$ | 6.56 ± 0.11$^{a}$ | < 0.001 |
| FCR (overall) | 1.86 ± 0.04$^{a}$ | 1.74 ± 0.03$^{b}$ | 1.63 ± 0.03$^{c}$ | 1.53 ± 0.02$^{d}$ | < 0.001 |
| Mortality (%) | 3.3 ± 0.8 | 1.7 ± 0.6 | 1.7 ± 0.6 | 1.7 ± 0.6 | 0.431 |

BW = Body weight; BWG = Body weight gain; ADFI = Average daily feed intake; ADG = Average daily gain; FCR = Feed conversion ratio. a-d: within a row, means with different superscripts differ significantly (P < 0.05).

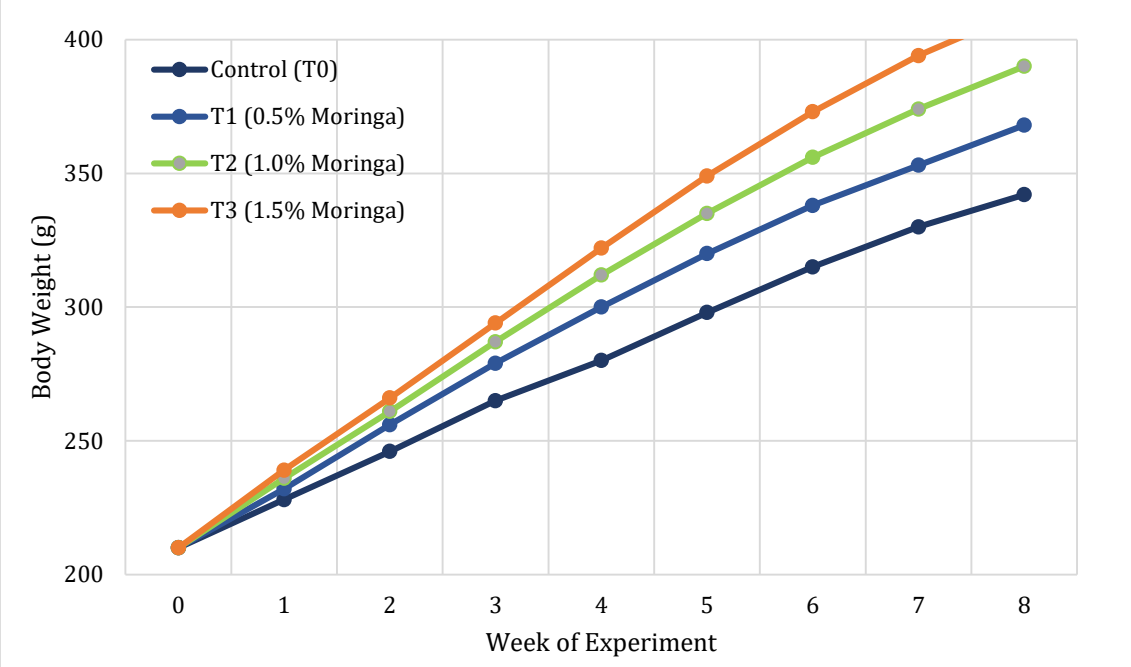


Figure 1. Weekly Body Weight of Cobb 500 Broilers Fed MOLM Diets

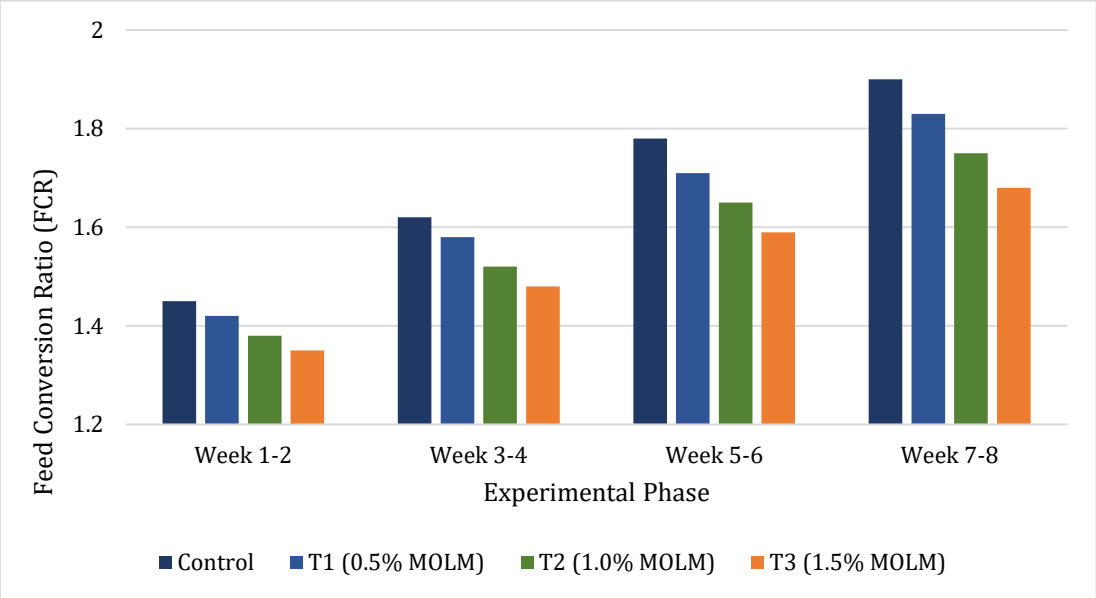


Figure 2. Feed Conversion Ratio (FCR) of Broiler Chickens Fed MOLM Diets

The results of improved body weight gains and FCR obtained for all MOLM treatments is in line with previous work with broilers that used phytogenic feed additives [11] and could be explained by three synergistic effects; the first is the direct effect of MOLM protein and essential amino acids, the second is through the action of digestive enzymes, and the third is due to the better villus morphology observed, leading to a larger surface area of nutrients that can be absorbed. The amino acid composition of MOLM, especially its high Met and Lys (g/100g Liveweight) as compared to the basal diet likely added to the amino acid matrix of the basal diet, which may explain the better protein deposition, as evidenced by higher breast meat yield reported in Section 4.3 [13]. MOIM contains polyphenolic compounds such as quercetin and kaempferol which has been reported to stimulate secretion of pancreatic lipase, amylase and protease through stimulation of enteroendocrine I-cells and release of cholecystokinin, leading to improved digestibility of the nutrients [26].

### 4.3. Carcass Characteristics

As MOLM was increased in the feed, the dressing percentage and breast meat yield both significantly ($P < 0.05$) improved Table 4 and Figure 3. T3 birds had a 4.7 percentage-point higher dressing yield and 3.9 percentage-point higher breast meat proportion compared to controls. Relative weight of the organs (liver, gizzard, and spleen) was not affected by the treatment ($P > 0.05$) and was within the physiologic range, suggesting nonvisceral hypertrophy and nonorgan level stress response upon MOLM inclusion.

Table 4. Carcass Characteristics (% of Live Weight) of Broiler Chickens Fed MOLM-Supplemented Diets

| Trait | T0 | T1 | T2 | T3 | P-value |
|---|---|---|---|---|---|
| Dressing Yield (%) | $72.1 \pm 0.6^{c}$ | $73.5 \pm 0.7^{c}$ | $75.2 \pm 0.8^{b}$ | $76.8 \pm 0.9^{a}$ | < 0.001 |
| Breast Meat (%) | $28.4 \pm 0.5^{c}$ | $29.6 \pm 0.6^{bc}$ | $31.0 \pm 0.7^{b}$ | $32.3 \pm 0.8^{a}$ | < 0.001 |
| Thigh (%) | $15.2 \pm 0.3^{b}$ | $15.8 \pm 0.4^{ab}$ | $16.5 \pm 0.4^{a}$ | $17.1 \pm 0.5^{a}$ | 0.008 |
| Drumstick (%) | $12.8 \pm 0.3^{a}$ | $13.1 \pm 0.3^{a}$ | $13.5 \pm 0.4^{a}$ | $13.9 \pm 0.4^{a}$ | 0.093 |
| Wings (%) | $8.6 \pm 0.2^{a}$ | $8.7 \pm 0.2^{a}$ | $8.8 \pm 0.3^{a}$ | $8.9 \pm 0.3^{a}$ | 0.780 |
| Liver (% LBW) | $2.35 \pm 0.12^{a}$ | $2.28 \pm 0.10^{a}$ | $2.20 \pm 0.09^{a}$ | $2.15 \pm 0.11^{a}$ | 0.412 |
| Gizzard (% LBW) | $2.12 \pm 0.09^{a}$ | $2.10 \pm 0.08^{a}$ | $2.08 \pm 0.09^{a}$ | $2.06 \pm 0.08^{a}$ | 0.921 |

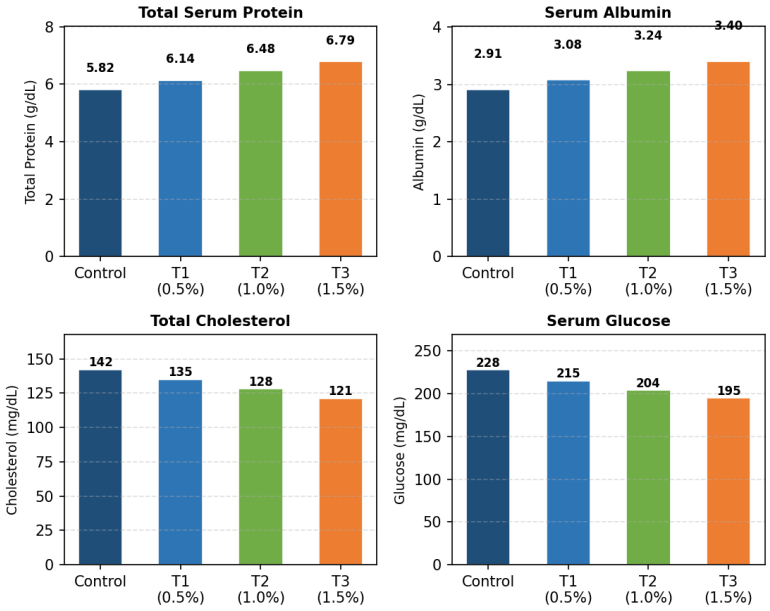


Figure 3. Serum Biochemical Parameters of Broiler Chickens Fed MOLM Diets

### 4.4. Serum Biochemical Profile

Table 5 and Figure 4 show that dietary MOLM significantly increased the levels of total protein and albumin, while decreasing the levels of total cholesterol, triglycerides and glucose ($P < 0.05$). However, the activities of AST and ALT were slightly lower in the MOLM groups than in the controls, but the difference was not significant ($P > 0.05$) and indicates that no hepatotoxicity was observed at the levels used in this study.

Table 5. Serum Biochemical Parameters of Broiler Chickens Fed MOLM-Supplemented Diets (Means ± SEM)

| Serum Parameter | T0 | T1 | T2 | T3 | P-Value |
|---|---|---|---|---|---|
| Total Protein (g/dL) | $5.82 \pm 0.18^{c}$ | $6.14 \pm 0.20^{bc}$ | $6.48 \pm 0.22^{b}$ | $6.79 \pm 0.24^{a}$ | < 0.001 |
| Albumin (g/dL) | $2.91 \pm 0.09^{b}$ | $3.08 \pm 0.11^{ab}$ | $3.24 \pm 0.12^{a}$ | $3.40 \pm 0.13^{a}$ | 0.003 |

| | | | | | |
|---|---|---|---|---|---|
| Globulin (g/dL) | 2.91 ± 0.10 | 3.06 ± 0.11 | 3.24 ± 0.13 | 3.39 ± 0.14 | 0.072 |
| Total Cholesterol (mg/dL) | 142 ± 3.8$^{a}$ | 135 ± 3.5$^{ab}$ | 128 ± 3.2$^{b}$ | 121 ± 2.9$^{c}$ | < 0.001 |
| Triglycerides (mg/dL) | 86.4 ± 2.9$^{a}$ | 82.1 ± 2.6$^{ab}$ | 77.8 ± 2.4$^{b}$ | 73.2 ± 2.1$^{c}$ | 0.002 |
| Glucose (mg/dL) | 228 ± 5.4$^{a}$ | 215 ± 4.8$^{ab}$ | 204 ± 4.2$^{b}$ | 195 ± 3.9$^{c}$ | < 0.001 |
| Uric Acid (mg/dL) | 4.82 ± 0.22$^{a}$ | 4.64 ± 0.20$^{a}$ | 4.48 ± 0.18$^{a}$ | 4.32 ± 0.17$^{a}$ | 0.387 |
| AST (U/L) | 42.4 ± 2.1$^{a}$ | 40.8 ± 1.9$^{a}$ | 39.2 ± 1.8$^{a}$ | 38.1 ± 1.7$^{a}$ | 0.542 |
| ALT (U/L) | 18.6 ± 1.2$^{a}$ | 17.9 ± 1.1$^{a}$ | 17.1 ± 1.0$^{a}$ | 16.5 ± 0.9$^{a}$ | 0.621 |

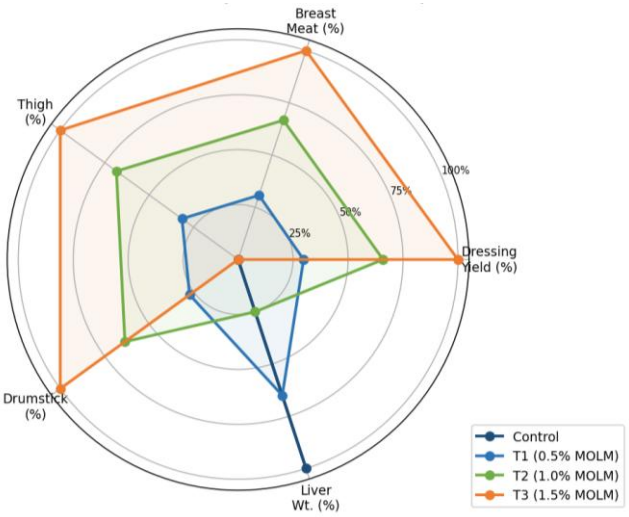


Figure 4. Relative Carcass Performance Indices of Broiler Chickens Fed MOLM Diets

Earlier, some phytochemicals of M. oleifera have been reported to exhibit hypocholesterolaemic activity [15] and in this study, the significant reduction in the serum cholesterol and triglycerides in MOLM-supplemented birds could be attributed to the presence of β-Sitosterol which competes with dietary cholesterol for incorporation in the intestinal lumen which consequently reduces cholesterol absorption [27]. In addition, MOLM flavonoids show inhibitory activities on HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis, which is similar to statins [28] but acts in a natural manner. Concomitant increase in serum protein and albumin Table 5 indicates improved hepatocellular protein synthesis which may be mediated by the cytoprotective flavonoids which reduce the damage caused by the oxidative process in the hepatocytes [29].

### 4.5. Antioxidant Enzyme Activity and Lipid Peroxidation

The activity of hepatic SOD, CAT and GPx significantly ($P < 0.05$) increased with the increasing dose of MOLM as shown in Table 6 and Figure 5. In contrast, the concentration of MDA which reflects lipid peroxidation decreased linearly with the increase of MOLM concentration ($R^2 = 0.94$), suggesting dose-dependent antioxidant activity of MOLM. The antioxidant responses have been in agreement with high levels of flavonoids and phenols in MOLM as revealed in Table 2.

Table 6. Hepatic Antioxidant Enzyme Activities and MDA Levels in Broiler Chickens Fed MOLM-Supplemented Diets

| Enzyme / Marker | T0 | T1 | T2 | T3 | P-Value |
|---|---|---|---|---|---|
| SOD (U/mg protein) | 12.4 ± 0.6$^{c}$ | 13.8 ± 0.7$^{bc}$ | 15.2 ± 0.8$^{b}$ | 16.9 ± 0.9$^{a}$ | < 0.001 |
| CAT (U/mg protein) | 8.7 ± 0.4$^{c}$ | 9.5 ± 0.5$^{bc}$ | 10.4 ± 0.6$^{b}$ | 11.6 ± 0.7$^{a}$ | < 0.001 |
| GPx (nmol/min/mg) | 5.2 ± 0.3$^{c}$ | 5.9 ± 0.3$^{b}$ | 6.7 ± 0.4$^{b}$ | 7.5 ± 0.5$^{a}$ | < 0.001 |
| MDA (nmol/mg protein) | 3.8 ± 0.2$^{a}$ | 3.4 ± 0.2$^{ab}$ | 2.9 ± 0.2$^{b}$ | 2.4 ± 0.1$^{c}$ | < 0.001 |
| GSH (µmol/g tissue) | 18.6 ± 0.8$^{c}$ | 20.4 ± 0.9$^{bc}$ | 22.8 ± 1.0$^{b}$ | 25.1 ± 1.2$^{a}$ | < 0.001 |

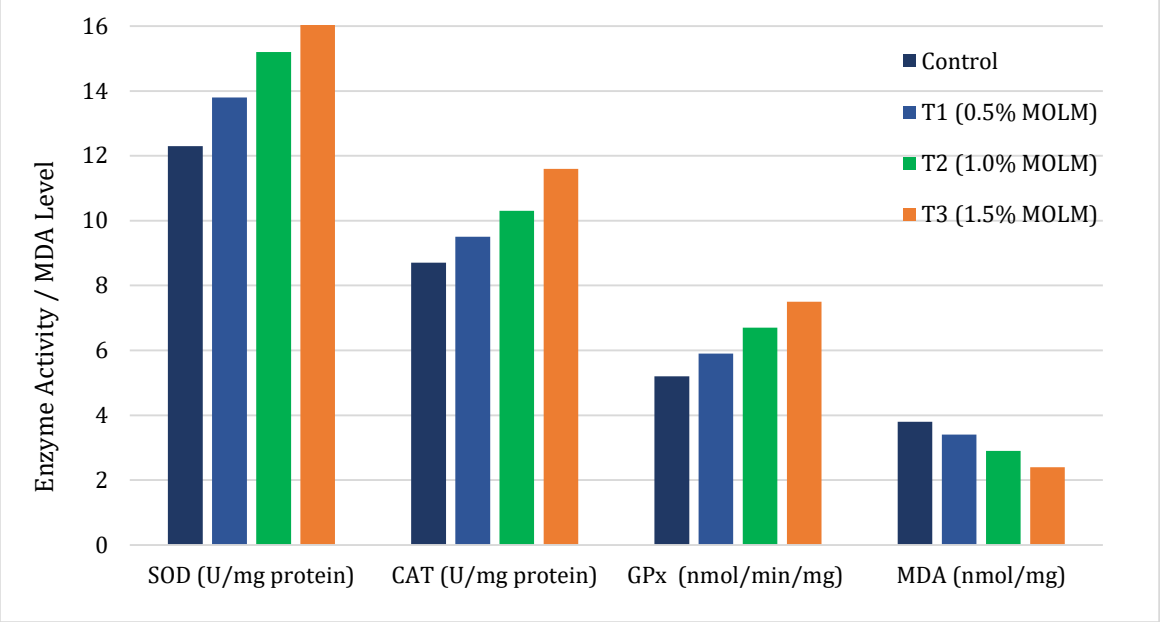


Figure 5. Hepatic Antioxidant Status of Broiler Chickens Fed MOLM Diets

The observed enhancement of SOD, CAT, and GPx activities and the reduction of MDA in the hepatic tissue in a dose-dependent manner as presented in Table 6 is a strong proof of systemic augmentation of antioxidant activities. These enzymes are involved in the dismutation of superoxide radical, the decomposition of hydrogen peroxide and the reduction of lipid hydroperoxides, respectively, and their coordinated upregulation suggests that they provide a broad anti-ROS protection along the entire ROS-cascade [30] The MOLM in this study contained both quercetin and kaempferol at significant levels Table 2, which are both strong activators of Nrf2 transcription factor, which translocates into the nucleus and leads to the induction of Phase II detoxifying enzymes such as SOD, CAT and GPx, which are also antioxidants [16]. The phenolic-rich plant additives caused a significant decrease of MDA in T3 (- 36.8%), as observed in broilers fed these additives before [26].

### 4.6. Intestinal Morphology

Table 7 and Figure 6 showed that there was significantly higher jejunal villus height and VH:CD ratio in MOLM-supplemented birds ($P < 0.05$), and lower crypt depth in T3 than control birds. The overall trend of increased villus height and decreased crypt depth suggests overall improved mucosal integrity and decreased cell turnover, which is consistent with improved absorptive surface area with MOLM feeding.

Table 7. Jejunal Morphometric Parameters of Broiler Chickens Fed MOLM-Supplemented Diets (µm; Means ± SEM)

| Parameter | T0 | T1 | T2 | T3 | P-value |
|---|---|---|---|---|---|
| Villus Height (µm) | $843 \pm 14.2^{c}$ | $912 \pm 16.8^{b}$ | $978 \pm 18.4^{ab}$ | $1052 \pm 20.1^{a}$ | < 0.001 |
| Villus Width (µm) | $118 \pm 4.2^{b}$ | $125 \pm 4.8^{ab}$ | $133 \pm 5.1^{a}$ | $141 \pm 5.6^{a}$ | 0.012 |
| Crypt Depth (µm) | $185 \pm 5.8^{a}$ | $172 \pm 5.2^{ab}$ | $162 \pm 4.9^{b}$ | $151 \pm 4.4^{c}$ | < 0.001 |
| VH:CD Ratio | $4.56 \pm 0.12^{c}$ | $5.30 \pm 0.14^{b}$ | $6.04 \pm 0.16^{ab}$ | $6.97 \pm 0.19^{a}$ | < 0.001 |
| Goblet Cell Count/Villus | $18.4 \pm 0.9^{b}$ | $20.1 \pm 1.0^{ab}$ | $22.6 \pm 1.1^{a}$ | $24.8 \pm 1.3^{a}$ | 0.003 |

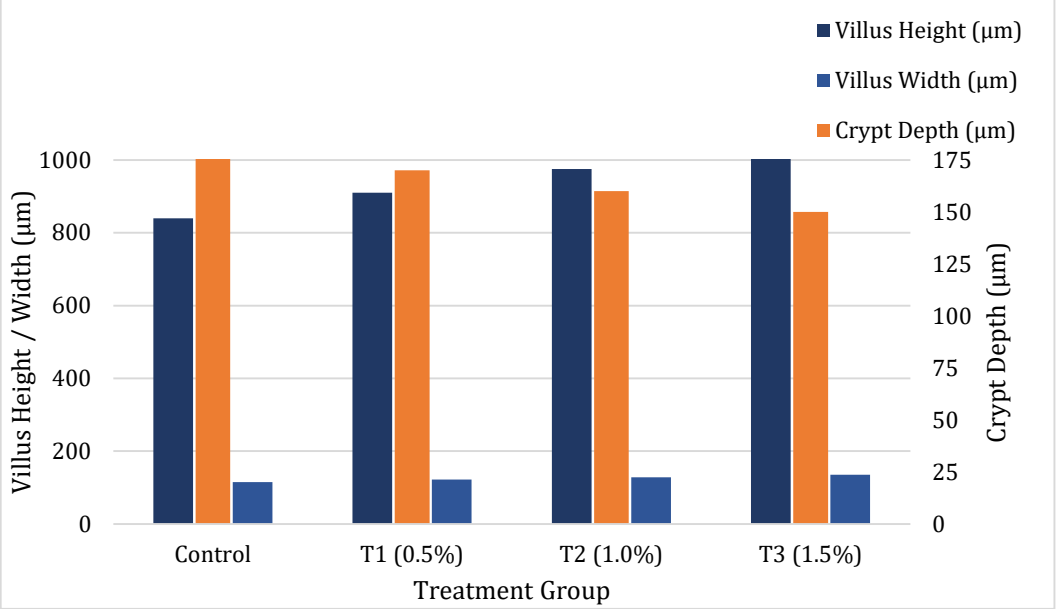


Figure 6. Intestinal Morphometry of Broiler Chickens Fed MOLM Diets

In poultry, the most accepted structure which correlates best with absorptive capacity and mucosal health is intestinal villus height [17]. The 24.8% increase in jejunal villus height from T0 (843 µm) to T3 (1,052 µm), as reported in Table 7, is a significant improvement in the absorptive surface area which for cylindrical approximations is proportional to the square of villus height. Crypt depth, an indicator of the rate of enterocyte proliferation needed to replace the villus epithelium, was significantly lower in T3 birds, indicating a decreased turnover due to inflammation. The VH:CD ratio was increased from 4.56 to 6.97, which is similar to the VH:CD ratio usually obtained with antibiotic growth promoters in diets, making MOLM a promising substitute for antibiotic use in this area [31]. The result in Table 7 shows an increase in the density of goblet cells which leads to increased mucin secretion and an enhanced barrier to pathogen invasion as well as to facilitating a lubricated micro-environment for commensal microbiota [32].

### 4.7. Practical Implications and Safety Considerations

Practically, the additional cost of feeding 1.5% M. oleifera in commercial broiler diets in tropical and sub-tropical agriculture systems where M. oleifera is abundantly available and is considered largely under utilised agro-industrial biomass, is relatively low [33]. As seen no mortality difference among treatments in Table 3 and no significant liver enzymes AST and ALT elevations (that would have indicated hepatotoxicity) in Table 5, MOLM can be considered safe in use at the inclusion rates tested. The multi-level evidence from the combination of better growth performance, superior carcass traits, favourable serum lipid and protein parameters, hepatoprotection by antioxidant activity and improved intestinal architecture from Table 3, Table 7 allows for the conclusion that MOLM is a promising phytogenic feed additive in commercial broiler production.

## 5. CONCLUSION

Significant and dose-dependent improvements in final BW, FCE, dressing percentage, BM%, serum protein and lipid metabolism, antioxidant enzyme activity in the liver, and morphometric parameters of the jejunal villi were observed in Cobb 500 broiler chickens fed with various levels of Moringa oleifera leaf meal (0.5, 1.0, and 1.5%). Based on the performance, biochemical and morphological evidence presented here, the optimal dose is recommended to be 1.5% MOLM. The results indicate that the use of MOLM could be an effective, low-cost sustainable feed ingredient in antibiotic-free broiler production systems and especially in tropical and sub-tropical environments where raw material is readily available. This research

should be supplemented with further investigation of MOLM in combination with other phytogenic additives; investigation of effects on meat quality attributes and shelf-life; investigation of effects on the composition of the gut microbiome using molecular methods; and calculation of economic returns over multiple commercial production cycles.


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## Author Contributions Statement

| Name of Author | C | M | So | Va | Fo | I | R | D | O | E | Vi | Su | P | Fu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ahadov Akobir | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | ✓ | ✓ | ✓ | | | |

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

## Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## Informed Consent

All participants were informed about the purpose of the study, and their voluntary consent was obtained prior to data collection.

## Ethical Approval

Not Applicable.

## Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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