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Research Article

Effect of *Moringa Oleifera* Leaf Powder Supplementation on Serum Parameters, Gut Micro-Flora, Muscle and Bone Health in Broilers under Dexamethasone-Induced Stress

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ABSTRACT

The research investigates the impact of *Moringa oleifera* leaf powder (MOLP) supplementation on broilers subjected to dexamethasone-induced stress. A total of 200-day old chicks were divided into five groups (A, B, C, D and E), each group having four replicates (ten chicks/replica). Negative control group (A) received only basal diet, positive control group (B) given dexamethasone (day 21st onward) in basal diet, group C, D, and E received 0.8%, 1.2%, and 1.6% MOLP respectively. Results indicate that significant ($P \leq 0.05$) improvements in liver health, evidenced by decreased levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphate (ALP) in MOLP-treated groups, urea and creatinine levels remained non-significant. Significant alterations in gut microbiota composition, with positive ($P \leq 0.05$) changes in Escherichia coli, Bifidobacteria, and Lactobacilli in MOLP-treated birds, indicated potential benefits for gut health. Mineral content analysis revealed higher ($P \leq 0.05$) phosphorus % in MOLP-administered groups, ash% and Ca% were non-significant. Bone morphological parameter demonstrated significant ($P \leq 0.05$) improvements in tibia length, body weight, tibiotarsal index and weight/length index in 1.2% and 1.6% MOLP groups, diaphysis diameter, medullary canal diameter, medial wall diameter, lateral wall diameter and robusticity index of tibial bones were non-significant. MOLP supplements significantly ($P \leq 0.05$) changed muscle fascicle diameter and muscle fascicle cross-sectional area, while muscle fiber diameter, muscle fiber cross-sectional area and muscle fiber density were remained non-significant. It was concluded that up to 1.6% MOLP in broiler feed improves broilers health.

Keywords: Broiler, Dexamethasone, Gut health, Muscle, Tibia.

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INTRODUCTION

Numerous environmental stressors, such as heat, resistance, transportation, cold, and oxidative stress, negatively affect the chicken performance, carcass quality, and the essence of the bird (Virden *et al.*, 2007).Stressors have a major side effect on broiler performance because they set off a hormonal chain reaction that raises glucocorticoid blood plasma concentrations.

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Samples of blood, colon, cecal, and feathers are utilized to assess elevated blood glucocorticoid levels as an indicator of ongoing stress (Weimer *et al.*, 2018). These hazardous substances change the structures of proteins, lipids, and deoxyribonucleic acid when they build up excessively, which in turn promotes the development of sickness in birds (Ademu *et al.*, 2021).

The primary balance that was thrown off by oxidative stress was that between the body's ability to combat reactive oxygen species (ROS) and their creation. Dexamethasone (Dex), a synthetic glucocorticoids analogue with established anti-inflammatory and cell-mediated immunosuppressive effects, has been used to simulate stress conditions in bone diseases and nutrient transport (Duff *et al.*, 2019). Dexamethasone also had an impact on ruminant metabolism, including food intake and glycometabolism (Hua *et al.*, 2018).

An immunosuppressive drug and an analog of stress hormones, Dex is widely used to investigate the impact of stressors in the genesis and progression of diseases in poultry. The glucocorticoid (Dex) is known to impair infection resistance and induce cell-mediated immunosuppression in several animal species, including birds (Sultana *et al.*, 2020). The Dex injection increases intramyocellular lipid storage in broiler chicks during their initial developmental stage by decreasing lipid oxidation, and it has been hypothesized that the adenosine-monophosphate-activated protein kinase pathway may be involved in the regulation of fatty acid consumption by glucocorticoids (Wang *et al.*, 2012). The Dex significantly reduced both bone growth and turnover. In addition, when the amount of bone matrix reduced, so did the biomechanical characteristics of bones (Liu *et al.*, 2011). Dex caused oxidative damage to the pectoral muscle of broilers, which affect their growth traits. The elimination of breast muscle drip, a rise in light and yellow values, and a decrease in redness value are all additional effects of Dex (Pan *et al.*, 2019). Dex negatively affected the weight of the body, visceral organs development, and its organs in addition to serum trace element concentrations. Nonetheless, the lipid profile showed a significant increase in cholesterol, low-density lipoprotein, and triglycerides. Dex induces oxidative stress has a comparable effect on the tissues of the kidney and liver, causing cellular vacuolation, lymphocyte infiltration, necrosis, and tissue degradation (Hammadi Jasim *et al.*, 2022).

The maintenance of homeostasis depends on the liver, one of the body's most sensitive organs. This organ handles vital functions including metabolism and detoxification. Most of the toxic chemicals in the body are broken down in the liver. The hepatic vein transports these toxins to the liver after they are absorbed from the gastrointestinal tract, which is where they mostly enter the body (Asgari-Kafrani *et al.*, 2020). Nutritionists might be convinced to add dietary supplements to chicken diets since they have the potential to improve gut architecture and nutrient absorption, which would boost poultry production performance. Phytobiotics are plant-based organic compounds that are added to animal feed to improve the health and wellbeing of the animals (Soundararajan *et al.*, 2023). *Moringa oleifera* leaves, in the diet of birds have antimicrobial qualities. They nourish the birds and prevent Staphylococcus aureus from proliferating in animal intestines. With *Moringa oleifera* leaf powder (MOLP), the quantity of coliforms and intestinal microbiota were both significantly reduced. Several research claims that the essential oils like Artemisia camellia found in plants are helpful in regulating the bacteria in the stomach (Divya *et al.*, 2014). Caffeic acid from the Moringa plant enhances the density of the Tibia bone, inhibits the formation of osteoclasts, and naturally reduces the oxidation of meat lipids by natural antioxidants. Furthermore, prebiotic advantages of *Moringa oleifera* leaves are believed to exist (Rehman *et al.*, 2018).

One species in the Moringaceae family is *Moringa oleifera*. Vitamins, carotenoids, flavonoids, alkaloids, polyphenols, phenolic acids, and other macro- and micronutrients are rich in its leaves (Maizuwo *et al.*, 2017). Thus, the *Moringa oleifera* plant is used as an excellent food supplement to feed people and animals. The tree was referred to as "the miracle tree" due to its extensive use as a traditional medicinal source and for treating various illnesses (Nouman, 2019). Research has subsequently been conducted on the leaves' several therapeutic properties, which include their antifungal, hepatoprotective, anticancer, and anti-inflammatory properties (Bourais *et al.*, 2023). On the other hand, previous studies have demonstrated that the ethanolic extract of *Moringa oleifera* leaves is a useful means of shielding hepatic tissue from tissue damage caused by acetaminophen and antitubercular drugs (Fakurazi *et al.*, 2012). This tree has diuretic, anti-inflammatory, antibacterial, antioxidant, anticancer, hepatoprotective, antihypertensive, and anti-ulcer properties that make it useful for therapeutic purposes (Srinivas[1] *et al.*, 2013). Moringa's hepatoprotective, anti-cancer, anti-diabetic, and antioxidant properties are caused by quercetin, phenolic acid, tannins, and saponins. Its many pharmacological benefits are used by the traditional medical system as therapeutic treatments for a range of illnesses (Rode *et al.*, 2022).

Khan *et al.* (2021) found that MOLP has potent anti-inflammatory and analgesic properties, which can be attributed to its high content of bioactive compounds such as quercetin, kaempferol, and rutin also contain antidiuretic properties

and can significantly reduce blood glucose levels in diabetic animals. Moreover, MOLP has been found to modulate the gut microbiota, which is an important factor in maintaining overall gut health. MOLP supplementation increases the wealth of gainful microorganisms, like Lactobacillus and Bifidobacterium, in the gut of rats while decreasing the abundance of harmful bacteria, such as Enterobacteriaceae and Clostridium (Zhang *et al.*, 2023). MOLP may have a prebiotic effect that promotes the growth of beneficial gut bacteria while preventing the growth of detrimental ones. Powdered *Moringa oleifera* leaf contains bioactive chemicals that have been shown to enhance health. It is a potential natural supplement for both human and animal health due to its capacity to change the microbiota in your gut and improve a variety of health disorders (Abbas, 2013).

In recent years, there has been a great deal of interest in MOLP due to its potential health benefits. Several studies have reported on the various pharmacological characteristics of MOLP, including its hepatoprotective, anti-inflammatory, antidiabetic, and antioxidant effects (Liu *et al.*, 2022). One of the primary areas of research is the potential use of MOLP as a nutritional supplement in animal feed. The effects of MOLP supplementation on the immune systems, gut microbiota, and growth performance of various species, including fish, pigs, and broiler chickens, have been the subject of several studies (Mohamed *et al.*, 2023). While producing more meat is a beneficial trait in broilers, it negatively impacts the growth of their bones. Fast development and increased muscle mass result in metabolic disorders that impair strength, and mobility (Szafraniec *et al.*, 2022). As reliable markers of the condition of the bones are bone length, weight, and ash content which depend on the minerals especially calcium that it contains. Chicken feed has always used antibiotics to boost meat production and bone health (Rehman *et al.*, 2018). Study by González-Burgos *et al.* (2021) reported that the growth performance and immunological response of broiler chickens were enhanced by MOLP supplementation. Moreover, MOLP has been shown to have a prebiotic effect, promoting the growth of beneficial stomach microbes like Bifidobacterium and Lactobacillus while preventing the growth of pathogenic bacteria like Clostridium perfringens and Escherichia coli. This inhibition raises the possibility that MOLP might be used in animal feed as a natural substitute for antibiotics (Khattak *et al.*, 2020; Soundararajan *et al.*, 2023). The specific objective of the study is to mitigate the adverse effect of oxidative stress over the bone, muscle, blood biochemistry and gut micro flora through a novel dose of MOLP mixed in per kg of basal diet.

MATERIALS AND METHODS

Birds Grouping and Feeding

A total of 200-day old broiler chicks were bought from a commercial hatchery. The trial was conducted at Abdul Wali Khan University Mardan, Pakistan in a controlled poultry shed. On the first day, temperature was kept at 35 °C and 65% relative humidity. After reaching 26 °C on day 21 with a relative humidity of 65%, the temperature was decreased by 2 to 3 °C per week and remained there until day 35th. Five treatment groups and five replicates for each group were used in the random design of the chicks. Group A was negative control group, only received basal diet, Group B, the positive control, received dexamethasone (Dex) in feed on day 21st onward and no MOLP. Group C received 0.8% MOLP + Dex (15 mg/kg); Group D received 1.2% MOLP + Dex (15 mg/kg); Group E received 1.6% MOLP + Dex (15 mg/kg).

Samples Collection

Serum Parameters

Blood samples were taken from each bird in order to examine serum parameters. Serum parameters were measured from blood samples taken from each bird's wing vein. These parameters included total protein, glucose, triglycerides, cholesterol, uric acid, and liver enzymes. The whole blood was drawn into an Ethylendiamine tetraacetic acid (EDTA) tube, and the serum was collected in a gel tube. Serum samples were collected, then brought in ice boxes to the lab where they were kept at the proper temperature until further examination. Before analysis, serum samples were allowed to thaw at room temperature and then centrifuged to eliminate any leftover cellular debris.

Gut Microflora

Fecal samples were taken from each bird, and the gut microbiome was examined. Fecal samples were taken straight out of the cloaca and kept in sterile tubes at -80°C for further examination. Fecal samples were collected, then brought in ice boxes to the lab where they were kept at the proper temperature until further examination. Using a selective medium, the number of bacteria in *Lactobacillus* species, *Bifidobacteria* species, and *Escherichia coli* was counted. Two birds' digesta were taken from each replication in order to count the diversity of certain microbes using a traditional cultivating method. Prior to adding 20 ml of the particular medium to sterile glass petri plates, the media

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was prepared for bacterial growth. For solidification, the medium was kept at room temperature.

To guarantee total medium sterility, all petriplates were then incubated for one night at 37°C in a temperature-controlled incubator. One gram of digesta was extracted, homogenized, and serially diluted (10 folds) in a standard saline solution. Using a spreader, diluted samples of 200 microliters or 0.2 milliliters were placed in petriplates containing certain bacterial growth medium. On eosin methylene blue (EMB) agar (Oxoid), Escherichia coli was cultured under aerobic conditions for 24 hours at 37°C. Due to their stringent anaerobic nature, lactobacilli spp. was cultivated on de Man, Rogossa, and Sharpe (MRS) agar at 37°C for 48 hours. After adding L-Cystine to MRS agar, bifidobacterium were cultivated and cultured for 24 hours at 37 degrees in an anaerobic jar. After 24h bacterial colonies were counted by conventional method using Colony Forming Unit formula.

Tissue Processing for Muscle Morphometric Parameters

The tissue processing technique employed was paraffin embedding (Suvarna *et al.*, 2018). The muscle slice was stained using hematoxylin and eosin (H&E) staining and the diameter of muscle fibers and fascicles was measured. To quantify the fascicle diameter, photographs were obtained at random spots on the slide using a 4X objective lens. The diameter of the muscle fibers (measured in millimeters) was determined by measuring the microscopical slides of the cross-section of muscles stained with H & E at 10X under a bright field microscope. The fiber diameter of five muscle fibers in three fascicles was measured in millimeters, and the results were averaged. The cross-sectional area was computed using the muscle fiber diameter. H & E-stained slides were examined at a 4X magnification, and histomorphometry calculations were performed using Labomed USA's Progress capture Pro 2.7.7. Muscle fibers were measured in circles with a 0.5 mm radius before being transformed to 1 mm (Suvarna *et al.*, 2018).

Tibia Bone Morphometric Characteristics

From each replica, two bird tibial bones with their flesh still intact were collected. After being tagged, the bone was immersed in boiling water (100°C) for ten minutes. Drumsticks were taken out of the boiling water and cool for 42 hours (Mutuş *et al.*, 2006). The weight, width, and length of the tibial bone were measured using a weighing scale and digital Vernier caliper. The exterior diameter of each bone was measured from medial to lateral and recorded at the midway. After the bone was split in the middle, the diameter of the medullary canal (MC) was measured with a digital calliper. The bone weight/length index was computed by dividing the weight of the bone by its length. The weight/length index was computed in this manner.

Weight (mg) / Length (mm) equals the weight/length index. The robustness index was calculated by dividing the bone's length by the weight of the cube root of the bone. Bone length (mm) / weight (mg) of the cube root of the bone the following formula was used to determine the tibiotarsal bone index based on the medullary canal and diaphysis diameters. Diaphysis diameter × 100 [Diaphysis diameter / Medullary canal diameter]. To determine the amount of bone ash present, samples of dry tibia bone fragments were gathered in a China crucible and burned for 24 hours at 560 degrees Celsius in a muffle furnace. The proportion of bone ash was computed in relation to the weight of the dry tibia.

Tissue Mineral Retention

The tibia bone was ground before being ground into a powder, which allowed for the determination of tissue mineral retention. A grinder led to the formation of a 1gm powder of tibia bone, which was then subjected to flame photometry (Biotech Engineering Management Co. Ltd. UK). Phosphorus and ash content using a UV spectrophotometer (V-1100, Thermo Fischer Scientific, USA) as followed by (Browning and Cowieson, 2013).

Data Analysis

Data were analyzed using analysis of variance (ANOVA) followed by Tukey's post hoc test to determine significant differences among treatments. A P-value of ($P \leq 0.05$) was considered statistically significant.

RESULTS

Table 1 displays the results pertaining to the impact of *Moringa oleifera* leaf powder (MOLP) on blood parameters and liver enzyme levels. When compared to the control group, group B's (DEX-15 mg/kg) ALT, AST, and ALP levels rose, but group C's (MOLP 0.8%) and D's (MOLP 1.2%) supplements reduced ($P<0.05$). The results indicate that, as compared to the control group, uric acid levels were lower in group D (MOLP 1.2%) and higher in groups B (DEX-15 mg/kg), C (MOLP 0.8%), and E (MOLP 1.6%). However, uric acid levels rose ($P <0.05$) in all supplementation groups except for group D. When compared to the control group, the supplemented groups had lower glucose levels ($P <0.05$). A non-significant result was obtained for creatinine and urea.

It is predicted that changes in body metabolism, organ morphology, and eating behavior will occur, particularly when birds are fed alternative or supplemental diets (Hassan *et al.*, 2020). Serum biochemical parameters and the data

utilized for mitigation and improvement can be used to detect them. An animal's nutritional state can be determined from its serum glucose levels. High values partially signal replete status, while low levels suggest fasting. As a result, virtually all the chick groups fed the plant powders under investigation showed no appreciable variations in their glucose levels when compared to the control group. This indicates better nourishment and a replete state, which is consistent with animals' normal growth and well-being (Wang *et al.*, 2005).

Table 1. Effects of dietary *Moringa oleifera* leaf powder on serum parameters and liver enzymes.

Parameters	A	B	C	D	E	P-value
ALT	15±3^{b}	49±15^{a}	9±1^{b}	7±6^{b}	3±1.1^{b}	0.000
AST	194±8^{b}	229±17^{a}	141±5.2^{c}	122±5.7^{c}	94±2.9^{d}	0.000
ALP	2391±86^{b}	6867±558^{a}	2292±81^{b}	1335±80^{c}	2218±151^{b}	0.000
Uric Acid	5.05±0^{ab}	246±50^{a}	6.7±0.8^{a}	4.7±1.5^{b}	6.6±0.5^{a}	0.040
Glucose	162±16^{b}	142±0.3^{ab}	101±4^{c}	93±12^{c}	80±9^{d}	0.000
Urea	11±2.7	17±8.6	11±2.1	10±1.9	11±2.4	0.787
Creatinine	0.1±0.02	0.3±0.11	0.3±0.07	0.2±0.04	0.3±0.1	0.577

ALT= alanine aminotransferase, AST= aspartate aminotransferase, ALP= alkaline phosphate

The results of the study showed that adding *Moringa oleifera* to broiler diets at inclusion levels of 15% and 20% lowered the overall content of cholesterol. Similar to this, various dosages of M. oleifera supplementation increased blood concentrations of high-density lipoprotein cholesterol (HDL) while lowering those of low-density lipoprotein cholesterol (LDL) (Alnidawi *et al.*, 2016). It has been suggested that the increased natural fiber content of moringa leaves improves the host organism's lipid metabolism, which decreases cholesterol. Furthermore, compared to the control group, blood parameters such as hemoglobin percentage, total RBC count, and total PCV were shown to be higher at 20% supplementation levels (Mahfuz and Piao, 2019). Using leaf powder from *Moringa oleifera*, broilers were given nutritional supplements at concentrations of 0.6%, 0.9%, 1.2%, and 1.5% to improve their intestinal microarchitecture and growth performance (Khan *et al.*, 2017).

Table 2 shows the impact of MOLP supplementation on gut microflora, demonstrating a substantial improvement in gut microflora balance and a decrease in harmful bacterial counts in broilers. We saw improvements in the balance of gut microbiota in all the groups supplemented with MOLP; the group receiving 2.5% MOLP showed the greatest improvement.

Table 2. Effect of *Moringa oleifera* on gut microflora of broilers.

Strain	A	B	C	D	R	P-value
E. coli	6.85 ± 0.40^{ab}	7.20±0.2 0^{a}	6.10±0.10^{b}	6.40±0.40^{bc}	6.60±0.30^{bc}	0.05
Bifidobacteria	6.75 ± 0.30^{b}	6.60±0.2 0^{b}	8.00±0.40^{a}	7.80±0.50^{a}	7.80±0.40^{b}	0.03
Lactobacilli	7.00 ± 0.50^{c}	6.90±0.5 0^{c}	7.45±0.33^{b}	7.90±0.20^{a}	7.60±0.40^{ab}	0.03

The leaves of *Moringa oleifera* extract have antioxidant and antibacterial qualities, and it is widely used as a feed additive in poultry production (Kumar *et al.*, 2012). Previous research has linked antimicrobial-mediated improvements in broiler chicken development, which are made possible by a decrease in harmful bacteria in the gut that lessens nutritional competition. Moreover, a decrease in the microbial population also results in a decrease in microbial metabolites, which hinder the healthy growth of chickens (Mandal *et al.*, 2014; Yadav and Jha, 2019). Antibiotics thereby ensure a healthy microbiota and, through a series of different processes, boost the energy return from the eaten feed, which improves growth performance (Liao and Nyachoti, 2017). It has been shown that the Moringa species contains antibacterial properties. Perhaps these plants' antimicrobial properties explain the gut microbiota equilibrium seen in chickens fed Moringa species. Studies have indicated that extended usage and high dosages of synthetic antibiotics might have negative health consequences (Taufek *et al.*, 2022). Furthermore, even though they enhance growth performance, their body's buildup as a result of ineffective metabolism and elimination has been linked to antibiotic resistance in 146 cases (Edens, 2003). According to the results, the development and health of the experimental chicks were benefited by the greatly improved beneficial gut microbiota resulting from the use of powdered leaves and extracts of *Moringa oleifera* and *Moringa stenopetala* (Nathaniel, 2021). These results were in line with past research that showed broiler chickens' gut microbiome and growth performance were

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considerably enhanced when leaves of Moringa spp. were added (Abu Hafsa *et al.*, 2020).

Effect of *Moringa oleifera* over the minerals content of broilers bone is shown in table 3. It is observed that the effect of *Moringa oleifera* leaf powder improve the phosphorous content in bone which higher (P≤ 0.05) in MOLP supplemented group C, D and group E as compared to the negative control group B. The Ash % and Ca % remained unaffected among different supplemented groups when compared to positive and negative control.

Table 3. Effect of *Moringa oleifera Moringa oleifera* on mineral content of broilers chickens.

Parameters	A	B	C	D	E	P-value
Ash %	52.0±3.08	43.75±2.13	51.0±3.02	56.75±4.99	49.0±5.61	0.175
Ca %	24.25±2.59	17.25±0.62	20.75±2.65	24.5±1.8	25.25±2.65	0.108
P %	10.0±1.29^{a}	8.25±0.47^{b}	10.25±1.7^{a}	13.75±1.31^{a}	13.50±1.19^{a}	0.030

The differences in bone ash Ca and P between treatments, according to Williams *et al.* (2000), have nothing to do with how these elements are found in the diets (Nkukwana *et al.*, 2014). Most of the components found in poultry diets come from plants; between 60% and 80% of P comes from phytic acid, which is physiologically inert and so cannot be used. Rather, it is released into the surroundings (Kornegay, 1999). While the availability of calcium is generally rather high from most feedstuffs, the availability of phosphorus varies greatly depending on the source (Waldenstedt, 2006). Therefore, if Ca was similarly limiting in P shortage, it is doubtful that birds would respond to phytase supplementation (Yan *et al.*, 2001). However, a dietary ratio of 2Ca:1P is thought to optimize dietary Ca and P absorption and homeostasis (Reis *et al.*, 2023).

Table 4 shows the impact of *Moringa oleifera* on the morphometric characteristics of the tibia bone. It has been established that when *Moringa oleifera* was supplemented in the diet, group E saw a greater (P≤0.05) effect on bone length than did group B, the positive control. Comparing the C and D groups to the positive control, however, the bone length in those groups was greater (P≤ 0.05). All supplemented groups showed increased bone weights (P≤0.05) than the positive control group (group B).

Table 4. Impact of *Moringa oleifera* on morphological parameter of tibia bone in broilers chickens.

Parameters	A	B	C	D	E	P-value
BL (mm)	82.73±1.07^{b}	72.25±1.29^{c}	83.87±0.39^{b}	83.25±0.92^{b}	92.75±1.56^{a}	0.00
BW (mg)	7500.0±327.32^{a}	4250.0±590.09^{b}	7125.0±398.09^{a}	7375.0±263.05^{a}	8500.0±462.91^{a}	0.00
DD (mm)	0.85±0.4	0.98±0.7	0.945±0.66	0.887±0.39	0.7963±0.21	0.138
MCD (mm)	0.45±0.025^{a}	0.35±0.025^{b}	0.425±0.18^{b}	0.43±0.02^{ab}	0.47±0.016^{ab}	0.009
MWD	0.22±0.013	0.18±0.18	0.20±0.011	0.18±0.012	0.21±0.01	0.179
LWD	0.1±00	0.1±00	0.1±00	0.1±00	0.1±00	1.000
RI	4.23±0.06	4.56±0.17	4.38±0.08	4.28±0.03	4.56±0.05	0.66
TI	45.8±3.7^{bc}	61.99±4.18^{a}	53.97±2.76^{ab}	50.37±3.99^{abc}	40.09±2.47^{b}	0.001
W/LI	90.6±3.7^{a}	58.76±7.94^{b}	84.94±4.7^{a}	88.49±2.64^{a}	91.37±4.05^{a}	0.001

BL= Bone length, BW= Bone weight, DD= Diaphysis diameter, MCD= medullary canal diameter, MWD= Medial wall diameter, LWD= Lateral wall diameter, RI= Robusticity index, TI= Tibiotarsal index, W/LI= Weight/length index.

Compared to the positive control (group B), the medullary canal width was shown to be significantly larger in the *Moringa oleifera* supplemented groups E, D, and A (negative control). Group E's tibial tarsal index was greater (P≤0.05) than that of the positive control group. In comparison to the positive control group (group B), the impact of *Moringa oleifera* on the weight/length index was seen to be larger (P≤ 0.05) in all supplemented groups. In contrast to control groups (positive and negative), diaphysis diameter, medial wall diameter, lateral wall diameter, and robustness index did not change across the various supplemented groups.

According to (Nkukwana *et al.*, 2014), *Moringa oleifera* leaf meal improved weight gain and feed conversion ratio (FCR) but had no influence on tibia bone characteristics. They ascribed this to the amount and method of moringa inclusion in broiler diets. On the other hand, it is generally observed in some research trials which is more important that dietary antioxidants can change the color of meat, lessen rancidity, and decrease lipid peroxidation, all of which contribute to the preservation of meat quality (Mir *et al.*, 2017). The oxidative condition of meat muscle affects cooking loss, drip loss, meat color, and pH negatively and is strongly correlated with meat quality (Nawaz *et al.*,

2022). Therefore, adding antioxidant-rich moringa extracts to feed may be a practical way to improve the quality of meat produced by broiler chickens. Moreover, it has been demonstrated that phytosterols increase the concentration of glutathione (GSH) and decrease malondialdehyde (MDA) in the breast muscle of experimental broiler chickens (Egbu, 2023). Broiler meat's fat content was increased by moringa leaf meal, but its moisture, ash, or protein content remained unchanged (Nduku *et al.*, 2020). The high fat content was thought to be associated with the amount of saturated fatty acids present in moringa leaves, according to the scientists.

Table 5 displays the impact of *Moringa oleifera* on muscular health. Group E and group A, the negative control, had greater muscle fascicle diameters (P≤0.05) than the positive control. Compared to other supplemented groups, the muscle fascicle diameter cross sectional area was observed to be greater (P≤ 0.05) in group A, the negative control. The cross-section area and diameter of the muscle fibers were not significantly affected by *Moringa oleifera* leaf powder supplementation throughout the experimental trial. Furthermore, there was no difference in fiber density between the supplemented groups and the control groups (positive and negative).

Table 5. Effect of *Moringa oleifera* on muscle health of broiler chickens.

Parameters	A	B	C	D	E	P-value
MFD (mm)	0.14±007	0.14±0.01	0.17±0.5	0.14±0.02	0.13±0.27	0.822
MFCSA(mm)	0.017±0.001	0.15±0.003	0.02±0.014	0.015±0.004	0.01±0.00	0.761
MFSD(mm)	$2.82±0.2^{a}$	$3.5±0.01^{b}$	$3.6±0.19^{b}$	$3.22±0.31^{b}$	$4.3±0.59^{ab}$	0.016
MFSCSA (mm^2)	$8.79±1.54^{a}$	$3.58±0.08^{b}$	$5.39±0.78^{b}$	$9.96±1.1^{b}$	$13.79±3.07^{b}$	0.010
MFDen	95±21.0	83±3.0	109±41.0	106±6.0	97±3.0	0.903

MFD= Muscle fiber diameter, MFSCA= Muscle fiber cross-sectional area, MFSD= Muscle fascicle diameter, MFSCSA= Muscle fascicle cross-sectional area, MFDen= Muscle fiber density

The animal's protein composition affects the levels of blood urea and creatinine. Moreover, high levels of creatinine indicate higher muscle metabolism since it is a measure of muscular activity (Suprataman *et al.*, 2020). Studies show that because of their lower turnover rates, older broiler chicken had lower levels of urea and creatinine (Tesseraud *et al.*, 1996). The creatinine levels of broiler chickens in this investigation were not significantly impacted by the addition of *Moringa oleifera* and *Moringa stenopetala* to their diets. This implies, in part, that these meals helped prevent waste by maintaining muscle turnover in one way or another. The development and overall well-being of these experimental chickens served as proof of this.

Enhancing the quality of meat in chickens can be achieved by nutritional intervention (Egbu *et al.,* 2022). Because broiler chicken meat is low in fat, high in vitamins and minerals, and an excellent source of protein, there is a significant demand from consumers (Mir *et al.*, 2017). Meat's pH, softness, color (lightness, redness, and yellowness), and ability to hold water are highly valued by consumers The effects of supplementing broilers with *M. oleifera* leaf powder on the quality of their meat and bone were examined by (Rehman *et al.*, 2018). The authors proposed that adding 12 g/kg of leaf powder to the breast muscle of experimental broilers improved its pH, water-holding capacity, and muscle fiber diameter. Moreover, broilers fed moringa leaf diet exhibited higher tibia bone density, weight, and ash % (Rehman *et al.*, 2018). Egbu (2023) proposed that the experimental groups may be linked to myofibril stability by avoiding free radicals and activating antioxidant properties. The increased breast muscle weight in the moringa-supplemented groups was due to an increased rate of protein deposition, whereas the tibia bone weight and ash % were boosted by phytoestrogen flavonoids detected in powdered Moringa leaves.

CONCLUSION

The current research work concluded that both the phosphorus content deposition and the shape of the tibia bone are observed to improve with MOLP supplementation in broilers. Furthermore, supplementing with MOLP has enhanced the meat quality, muscle histomorphometry and gut microflora of broilers chickens under dexamethasone induced stress. However, the effectiveness of MOLP supplementation promoting bone health and tissue minerals retention.

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COMPETING OF INTEREST

The author(s) declared no potential conflicts of interest with respect to research, authorship, and/or publication with the work submitted.

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