Original Article
DOI: https://doi.org/10.70749/ijbr.v3i8.2284

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Influence of Leaf and Seed Powder Extracts of *Moringa oleifera* on Muscle Traits, Bone Morphometry and Retention of Tissue Minerals in Broilers

Shumaila Gul[1], Nida Khan[2], Rainaz Begum[3], Manahil Rafiq[4], Raheela Taj[5], Muneeb Islam[6], Ijaz Ahmad[7], Muhammad Sadeeq[8], Abrar Khan[4], Abdul Ghaffar Khan[9], Asad Ullah[10], Imad Khan[10]

[1]Department of Chemical and Life Sciences, Qurtuba University of Science and Information Technology Peshawar, KP, Pakistan.
[2]Department of Zoology, Women University Mardan, KP, Pakistan.
[3]Department of Chemistry, Abdul Wali Khan University Mardan, KP, Pakistan.
[4]Department of Zoology, Abdul Wali Khan University Mardan, KP, Pakistan.
[5]Institute of Chemical Sciences (ICS), University of Peshawar, KP, Pakistan.
[6]Department of Microbiology, Abdul Wali Khan University Mardan, KP, Pakistan.
[7]State Key Laboratory of Bioelectronics, School of Biological Sciences & Biomedical Engineering, Southeast University, Nanjing, China.
[8]University of Veterinary and Animal Sciences, Swat, KP, Pakistan.
[9]Livestock and Dairy Development Department (Extension), KP, Pakistan.
[10]College of Veterinary Sciences and Animal Husbandry (CVS and AH), Abdul Wali Khan University Mardan, KP, Pakistan.

ARTICLE INFO

Keywords: Birds, Medicinal Plants, Minerals, Selenium, Tibia.

Correspondence to: Imad Khan, College of Veterinary Science and Animal Husbandry (CVS & AH), Abdul Wali Khan University Mardan, KP, Pakistan.
Email: imadkhan@awkum.edu.pk
https://orcid.org/0000-0002-0252-0451

Declaration
Authors' Contribution
All authors equally contributed to the study and approved the final manuscript

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 27-05-2025 Revised: 09-08-2025
Accepted: 19-08-2025 Published: 30-08-2025

ABSTRACT

The current research was designed to determine the effect of leaf, and seed extract of *Moringa oleifera* on muscle characteristics, bone health and retention of minerals in chickens. Total 280 sample were divided into seven groups each having eight replicates (n=5). Chicks were fed corn-based basal diet (BD) supplemented with varying doses of MLE and MSE for 35 days. Group-A (control group) was given only BD, while Group-B was treated with BD+ 0.8% MLE, Group-C (0.8% MSE), Group-D (BD+1.2%MLE), Group-E (1.2% MSE), Group-F (BD+0.8%MLE+0.8% MSE) and Group-G was treated with BD+1.2% MLE+ 1.2% MSE. On 35th day, two chickens from each replica were slaughtered to collect the samples. The *pH* of the muscle was improved in group F (0.8%MLE+0.8% MSE) in comparison to group-A ($P<0.05$). The muscle fiber diameter and their cross-section area were also increased in all treated groups in comparison to the control group and group-G ($P<0.05$). The bone width, length and diaphysis diameter were increased in all treated groups in comparison to the control group and group-G ($P<0.05$). The selenium and calcium concentration improved in the serum and muscle, while in bone the calcium and phosphorus concentration were improved when birds diet supplement with MO leaf and seed extract in comparison to the control group ($P<0.05$). The conclusion came out from our results that the dietary combine used of MO leaf and seed extract (0.8% MLE, 0.8% MSE) improved the meat quality, bone health, and minerals levels in the tissues of the chickens.

INTRODUCTION

Moringa oleifera (MO) has been used by humans due to its edible nature since long time. This plant has been a tremendous source of digestible protein, vitamin C, calcium, iron, and carotenoid suitable for consumption in places where under nourishment is a major problem particularly in the emerging countries (Egbu *et al.,* 2022). Leaves of MO are rich source of protein, carotene, and ascorbic acid. These characteristics make it as a forage source for feeding animals, *Moringa oleifera* leaves considered to containing crude protein, 27.53%, crude fiber 19.26%, and unpolished fat 22.3%, Ash 7.14%, moistness content 76.56%, carbohydrate 44.11% and high in calories about 305.62% (Mahfuz and Piao, 2019). Leaves contain increased amount of entire phenol which is a good source of magnesium and potassium (Coppin, 2008). Fresh and dried leaves of *Moringa oleifera* reported to contain nutritional values like high lipid level and essential amino acid necessary for productivity of broiler chickens (Rehman *et al.,* 2018). Being a medicinal plant *Moringa oleifera* is used in the diet of broilers. Due to the presence of active substances it enhance digestion, improve metabolism and immunity of birds (Owolabi *et al.,* 2017). MO fruit extract have anti-fungal, anti-ulcerous, anti-hyperlipidemic, anti-inflammatory, and broad-spectrum antibacterial activity (Bukar *et al.,* 2010). It also has antioxidant activities helping in cell protection and boost immunity. The MO leaf has a high nutritional and

medicinal value, It has antimicrobial activity, low anti-nutritional factors and having high crude protein (Ogbe and john, 2012). MO leaf supplementation in feed have shown antioxidant, anti-cancerous activities, immune modulatory and growth enhancing properties showed that it does not possess any serious life threating effects on livestock and humans (Khan *et al.,* 2017). The MO leaf supplementation have positive effect on intestinal integrity, digestive organ size, digestibility, bone breaking strength and bone residue content, as well as essence yield of broiler chickens throughout the production period. MO leaf meal enhances the growth traits without any harmful effects in bird and improve meat quality, carcass characteristics, minerals retention, bone and gut health. It also enhances the bird's genomic potential for finest output (Khan *et al.,* 2017; Rehman *et al.,* 2018). For healthy immune system animals need antibodies for which they require energy, wide range of proteins, minerals (selenium, coppers, and zinc etc.) and certain enzymes like vitamins A and E to communicate information in different parts of the animal body to fight against infections. Interestingly, MO is equipped with all the major proteins, carbohydrates, amino acids, minerals, and vitamins. Due to immune boosting effect and medicinal properties the demand of MO becomes high. It also has beneficial effect on organs immunity by enhancing the lymphocytes concentration (Balami *et al.,* 2018). The consumer acceptability depends on the texture, appearance, and sensory characteristics of broilers meat. The major factor for the consumer acceptance of the broiler's meat is the meat color which is important indicators for consumers. The *pH* of the white meat linked with biochemical status of the muscle at the slaughtering time, which is the cause of rigor mortis. Color and *pH* are strongly correlated with each other, the light color meat have low *pH* and dark or dark red meat indicating the high value of *pH*. The dietary MO have positive effects on meat *pH* and color, the dietary supplements having the capacity to hold the water contents in muscles fiber which maintained its *pH* and color (Mulaudzi *et al.,* 2022). Broiler bone's morphology were improved by using MO leaves supplementation (Sreelatha and Padma's, 2009). Adding powder of MO leaf to broiler feed improves the meat's *pH*, tenderness, breast muscle quality, and water holding capacity (Karthivashan *et al.,* 2015; Mahfuz and Piao, 2019). The *Moringa* leaf extract supplementation has positive effect on the morphology of muscle by increasing the fiber mass due to higher protein content and length of sarcomere of the pectoral muscle (Alabi *et al.,* 2017). The MO seed extract improved the morphology and histomorphometry of the tibial bone because MO seed extract are enriched of various vitamins, minerals, and antioxidants, which enhanced the processes of osteoblast genesis and suppress the process of osteoclast genesis and enhanced the osteoimmunological response (Egbu *et al.,* 2022; Nkukwana *et al.,* 2014).

The bioactive substances in MO leaves has a beneficial effect on digestive system i.e., enhance food and mineral absorption and reduce calcium excretion from the body, may be the cause of increased bone weight and ash percentage, which are indicators of healthy bone mineralization (Sirotkin and Harrath, 2014). This study aim to investigate the role of MO leaf and seed extract supplementation on growth traits, bone health and muscle characteristics of broilers. To the best of our knowledge, there is no/limited research conducted on both use of MO seed and leaf extract in broilers.

METHODOLOGY

Experimental Design

The study encompassed 280 chicks. This trial was set up in environmentally controlled experimental house. The shed was cleaned and fumigated before the arrival of chicks. On arrival the chicks were weighted and randomly divided into seven groups with eight replicates in each group (n=5).

The temperature and relative humidity (RH) on day 1 was maintained at 35 ± 1 °C and 65 ± 5%, respectively. Temperature was decreased by 3°C per week until it reaches 26 ±1°C on day 21, with RH 65 ± 5% and remains same up to the end of trial.

Preparation of *Moringa oleifera* Leaf and Seed Extract

The MO leaf and stem were collected from Muzaffar Guar, Punjab Pakistan and then identified from flora of Pakistan. The leaf and seed individually crushed to prepare a powder form. Then take 500 gm of powder and soaked for 48-hours in 5000ml (5liter) ethanol, then mixed properly and filtered with muslin cloth to obtain ethanolic extract, then weight and refrigerate the extract.

Dietary Plan

Chicks were fed starter and finisher commercial corn-based basal diet (BD) supplemented with different concentrations *of* MO leaf (MLE) and seed extract (MSE) for 35 days. Group-A was served as control group and fed only on BD, Group-B was supplemented with BD+ 0.8% MLE, Group-C (0.8% MSE), Group-D (BD+1.2%MLE), Group-E (1.2% MSE), Group-F (BD+0.8%MLE+0.8% MSE) and Group-G was given BD+1.2% MLE+ 1.2% MSE (Table 1).

Table 1

Grouping of birds.

Group	A	B	C	D	E	F	G
Feed Composition	BD*	B.D+0.8% MLE**	B.D+0.8% MSE	BD+1.2% MLE	B.D+1.2% MSE	B.D+0.8% MLE+0.8%MSE	B.D+1.2%MLE+1.2%MSE

***MLE =** Moringa oleifera Leaf extract, ***MSE=** Moringa oleifera Seed extract*

Parameters

The reported parameters include; Meat quality (*pH* and drip loss %), muscle morphology (number, diameter and cross-sectional area of muscles fiber, muscle fascicle diameter and their cross-sectional area), bone morphology (weight, length, and diameter, diameter of diaphysis and medullary canal, robusticity index, tibiotarsal index, Weight/length index), and minerals retention (calcium, phosphorus and selenium in serum, muscle, and bone).

Sampling

On day 35, two birds from each replicate (16 birds /group) were selected for sampling. After slaughtering blood was collected for hematological analysis. For serum analysis the blood was centrifuged at 1500rpm for 10 min and stored at -20˚C for further mineral analysis. For muscle sample about 1cm^3 pieces from left superficial pectoral muscle was selected and preserved in 10% buffered formalin solution. The collected muscle sample was labeled and stored in -4˚C for minerals analysis. The right and left tibial bones were collected by separating from drumsticks i.e., boiled for 10-12 minutes to detach the meat part and kept for overnight to dry at room temperature (25˚C). The whole bone used for morphometric study and grind some parts as powder for mineral analysis.

Meat Quality and Muscle Morphometry

For water holding capacity (WHC) sample of right pectoral muscle 35 to 40gm was collected and wrapped in labeled inflated plastic bags. The water holding capacity of meat samples were measured by using Honikels gravimetric method.

The formula used for drip loss WHC calculation is:

WHC = (W1-W2/W1) *100

pH of muscle sample was determined by conventional probe electrode which was inserted into muscle tissue at least 1cm deep into muscle.

Muscle Morphometry

On day 35th, tissue samples from pectoral muscle were collected and preserved in 10% buffered formalin. Tissue processing was performed by using paraffin embedding technique (khan *et al.,* 2017). The slides having tissue sections were then stained by using hematoxylin and eosin technique according to the protocol used by (Khan *et al.,* 2021). Muscle fiber and fascicle diameter were determined according to the protocol used by (Rehman *et al.,* 2018). The images were capture by using 4X and 10X objective lens. Muscle fascicle and fiber diameter (MFD) was determined by using histomorphometric program (Progress Capture-Pro 2.7.7 Labomed. USA).

To determine the cross-sectional area of fascicle, for that we analyze the diameter of three fascicle and then take there mean. The diameter (µm) of five muscle fibers in three fascicles was determined, by using formula the cross-sectional area of muscle fibers was determine from the muscle fiber diameter. To count muscle fibers, slides at 4X objective lens were examined and measurements were taken through histomorphometry program (Progress Capture-Pro 2.7.7 Labomed USA). The number of fibers was determined in three randomly selected fascicles. The fibers were counted in 0.5mm (Radius circle) and then determined in 1mm as documented by (Khan *et al.,* 2022).

Morphology of Tibial Bone

The tibia bone of each bird with flesh intact was separated as drumstick. The bone was labeled and immersed in boiling water at temperature of 100ºC for 10 minutes. Drumsticks were cooled for 42hr after removing from boiling water (Rehman *et al*.. 2018). The tibial bone width, length and weight were measured using a weight balance and digital vernier caliper. Diameter of each bone was measured outside and marked at mid-point. After breaking the bone at mid-point, diameter of medullary canal was measured with digital caliper. The bone weight / length index was determined by dividing the bone weight by its length and from bone length and cube root of bone weight, bone robusticity index will be find out (Khan *et al.,* 2022).

Analysis of Minerals in Different Tissue

Sample of dry tibia bones was collected in China crucible and burnt for 24 hours in a muffle furnace at 560ºC to measure the content of bone ash. In ash calcium, phosphorus and selenium was determined. For serum analysis the blood was centrifuged at 1500rpm for 10 min and stored at -20˚C for further mineral analysis. The muscle sample collected, labeled, and stored in -4˚C for minerals analysis. Acid digestion of 1 gm of muscle tissue, 1ml of serum and 1 gm of dried bone tissue were done according to the protocol of (Saeed *et al.,* 2017) and using distal water to adjust the final volume of the solution up to 50ml. The Phosphorus concentration was determined by using UV-spectrophotometer and calcium through flame photometry. The selenium concentration was analyzed using atomic absorption spectrophotometer.

Statistical Investigation

The collected data were statistical examine with Statistical Packages for Social Sciences (SPSS Inc.) Version 13.3 (Chicago IL, USA). Data were presented as mean ± SEM. The Kolmogorov Smirnov test was employed to test the normal distribution of the data. The data was analyzed using one-way analysis of variance. The group differences were measured when the significant difference was at P <0.05.

RESULTS

Muscle Characteristics

Drip loss percentage, muscle fascicle diameter and their cross-sectional area remain unaffected among different treated and control groups (P<0.05) (Table 2). Initial *pH* of the muscle was higher in group-F (B. D+0.8% MLE+0.8%MSE) than the (control) A-group (P<0.05). Furthermore, Initial *pH* of the muscle was also higher in B (B. D+0.8% MLE), D (BD+1.2% MLE) groups than the A-group (P<0.05). After 24hr, the *pH* of the muscle was higher in B (B. D+0.8% MLE), D (BD+1.2% MLE) and F (B. D+0.8% MLE+0.8%MSE) groups than the A-group (P <0.05). Muscle fiber density, muscle fascicle diameter and their cross-sectional area was higher in B (B. D+0.8% MLE), D (BD+1.2% MLE) and F (B. D+0.8% MLE+0.8%MSE) groups than the A-group (P <0.05), but muscle fiber density was also higher in C (B. D+0.8% MSE) and E (B. D+1.2%MSE) groups than the A-group (P <0.05).

Table 2

Effect of leaf and seed powder extracts of Moringa oleifera on pectoral muscle of broiler chicken of broiler chickens

Parameters	A	B	C	D	E	F	G	SEM	P-Value
Bone-length(mm)	73.80^{b}	86.00^{a}	80.30^{ab}	83.80^{a}	82.10^{ab}	88.45^{a}	74.20^{b}	1.09	**0.009**
Bone-weight(gm)	5.01^{b}	5.75^{ab}	5.58^{ab}	5.48^{ab}	5.90^{a}	6.30^{a}	5.20^{b}	0.00	**0.048**

Dia*PH*ysis-Diameter (mm)	8.40^{b}	8.90^{a}	8.60^{ab}	8.70^{ab}	8.90^{a}	8.95^{a}	8.68^{ab}	0.03	**0.030**
Medullary Canal Diameter (mm)	4.66	5.34	5.05	5.21	5.18	5.46	4.78	0.02	0.156
Robusticity Index	4.31	4.80	4.47	4.75	4.54	4.79	4.04	0.05	0.290
Tibiotarsal index	44.50	40	41.30	40.15	41.80	39.00	44.93	0.6	0.770
Weight/length index	67.88	66.86	69.50	65.40	71.86	71.23	70.10	0.90	0.560

$^{a-c}$ Means with different superscripts within the same row are significantly different (P <0.05) from each other.
MFD= Muscle Fascicle Diameter, MFCSA: muscle fiber diameter cross sectional area, MFSD: muscle fascicle diameter; MFSCSA: muscle fascicle cross sectional area, MFDs= Muscle fiber density (total number of muscle fibers/mm^2 of muscle area)

Morphometry of bone

Tibial bone robusticity Index, medullary canal diameter, tibiotarsal and Weight/length did not affect among different treated and control groups (P <0.05) (Table 2). The bone length and diaphysis diameter were higher in B (B. D+0.8% MLE), D (BD+1.2% MLE) and F (B. D+0.8% MLE+0.8%MSE) groups than the A-group (P <0.05). The bone weight was higher (P <0.05) in E (B. D+1.2%MSE) and F (B. D+0.8% MLE+0.8%MSE) groups than the control group.

Table 4.2

Effect of leaf and seed powder extracts of Moringa oleifera on morphometric parameters of Tibia bone of broiler chickens.

Parameters	A	B	C	D	E	F	G	SEM	P-Value
pH (at 0 hour)	6.11^{c}	6.50^{ab}	6.40^{b}	6.51^{ab}	6.19^{bc}	6.55^{a}	6.15^{c}	0.02	0.009
pH(at 24 hour)	5.46^{b}	5.92^{a}	5.81^{ab}	5.90^{a}	5.85^{ab}	6.03^{a}	5.52^{b}	0.02	0.018
Drip loss (%)	3.68	2.93	3.27	2.97	3.13	3.30	3.68	0.02	0.79
MFD (µm)	40.21^{b}	43.59^{a}	41.79^{ab}	42.79^{a}	42.08^{ab}	44.41^{a}	40.32^{b}	0.19	0.019
MFCSA(mm^2)	1.27^{b}	1.50^{a}	1.37^{ab}	1.44^{ab}	1.39^{ab}	1.55^{a}	1.28^{b}	0.01	0.018
MFSD (mm)	0.76	0.80	0.77	0.82	0.79	0.83	0.78	0.01	0.169
MFSCSA(mm^2)	0.45	0.50	0.47	0.53	0.49	0.54	0.48	0.00	0.179
MFDs	569.92^{c}	519.87^{a}	544.80^{b}	505.70^{a}	541.20^{b}	511.75^{a}	566.20^{c}	3.9	0.040

$^{a-b}$ Means with different superscripts within the same row are significantly different (P <0.05) from each other.

Minerals retention in different tissue

Phosphorus in serum and muscles, and selenium in bone tissue did not affect among different treated and control groups (P<0.05) (Table 3). The calcium level in muscle was higher (P<0.05) in all treated groups than the A-group. Selenium concentration in muscle tissue was higher in D (BD+1.2% MLE) and F (B. D+0.8% MLE+0.8%MSE) groups than the A (control) group (P<0.05). In serum, the calcium concentration was increased in all treated groups than the control group (P<0.05). The selenium level in serum was increased in D (BD+1.2% MLE), E (B. D+1.2%MSE) and F (B. D+0.8% MLE+0.8%MSE) groups than the A-group (P<0.05). In bone, the calcium concentration was high in all treated groups than the A and G (B. D+1.2%MLE+1.2%MSE) group (P<0.05). Phosphorus concentration in tibial bone was higher in B (B. D+0.8% MLE), D (BD+1.2% MLE) and F (B. D+0.8% MLE+0.8%MSE) groups than the control group (P<0.05).

Table 4.3

Effect of leaf and seed powder extracts of Moringa oleifera on mineral content of broiler chickens

	A	B	C	D	E	F	G	SEM	P-Value
Muscle									
Ca(mg/g)	0.24^{b}	0.34^{a}	0.32^{a}	0.34^{a}	0.33^{a}	0.35^{a}	0.30^{a}	0.01	0.045
P (mg/g)	6.86	7.06	6.98	7.21	7.10	7.02	6.88	0.03	0.100
Se(µg/g)	0.094^{c}	0.175^{b}	0.139^{bc}	0.223^{ab}	0.174^{b}	0.289^{a}	0.102^{c}	0.00	0.003
SERUM									
Ca(mg/dl)	10.10^{c}	10.85^{ab}	10.60^{b}	10.90^{ab}	10.85^{ab}	11.35^{a}	10.45^{bc}	0.3	0.024
P (mg/dl)	6.49	6.90	6.88	6.62	6.85	6.95	6.53	0.03	0.180
Se(mg/l)	0.22^{b}	0.28^{ab}	0.27^{ab}	0.42^{a}	0.41^{a}	0.46^{a}	0.24^{b}	0.02	0.028
TIBIAL BONE									
Ca (gm/kg)	152.83^{b}	176.20^{a}	185.10^{a}	191.65^{a}	189.50^{a}	183.83^{a}	156.75^{b}	1.90	0.025
P(gm/kg)	102.10^{b}	111.75^{a}	106.00^{ab}	115.50^{a}	110.80^{ab}	113.85^{a}	104.60^{b}	0.90	0.028
Se(mg/kg)	0.30	0.32	0.44	0.31	0.44	0.47	0.31	0.02	0.060

$^{a-c}$ Means with different superscripts within the same row are significantly different (P <0.05) from each other.

DISCUSSION

The study conducted by (Cardinali *et al.,* 2015) align our research in certain areas i.e., they revealed that phytogenic additions can improve effectiveness, meat quality, and lipid oxidation protection. To achieve optimal development and feed efficiency for better growth in animals, herbal supplements (oregano) was the most promising natural product, whereas rosemary and vitamin E produced results like those of the untreated group (Cardinali *et al.,* 2015).

We concluded that initial *pH* of the muscle was higher in group-F than the A-group (P<0.05). Furthermore, Initial *pH* of the muscle was also higher in B and D groups than the A-group. After 24hr, the *pH* of the muscle was increased. Muscle fiber density, muscle fascicle diameter and their cross-sectional area was found higher in B, D and F groups than the A-group, but muscle fiber density was

found higher in C and E groups than the A-group (P <0.05). Rehman *et al.,* 2018 agreed with our finding stated that *Moringa* leaf supplementation effects on carcass quality and morphometric characteristic of tibial bone in chickens. The *pH* and drip loss percentage are the main indicators of chicken's meat which affect its qualities (meat tenderness, juiciness, cooking loss and taste) which is main demands of the consumers. The higher water holding capacity of muscle resulted in higher *pH*. *Moringa oleifera* is rich source of mineral and trace elements, these elements stabilized the muscles membrane by scavenging the free radicals and activation the antioxidant system. Higher *pH* and WHC prevent the lactic acid production and protein denaturation which resulted in stabilizing the myofibrils volume (Haidy *et al.,* 2022). The weight of muscle is due to total number of fibers present in fascicles along with cross section area. The finding showed that MLE supplementation increased the diameter of muscle fiber. The increased in fiber size due to higher content of protein and length of sarcomere of the pectoral muscle (Alabi *et al.,* 2017). Tenderness of the meat is a function of heat stability, content of collagens, and structure of myofibrillar of the muscle. Tenderness is improved with the aging of muscle due to breakdown of proteins in myofibrillar. The MO leaf supplementation improve meat tenderness (Karthivashan *et al.,* 2015). Similar results was documented by Talukder they revealed that the presence of dietary fiber has a variable effect on the sensory qualities of processed beef products such as texture, juiciness, and color. When oxygen is deprived during slaughter, oxidative phosphorylation, and cellular respiration cease, leading to glycogen depletion. Glycogen reserves provide energy needed by muscles (Talukder *et al.,* 2020). Other similar study showed that the impact of medicinal plant leaf extract supplemented in diets of broilers determine the meat quality and economic effectiveness (Khan *et al.,* 2023). The findings of this study suggest that a leaf extract diet can enhance live, carcass, breast, and thigh weight in broiler chickens while encouraging decreases in collagen and lipid contents in the muscles of the breast and thigh. Furthermore, compared to control group, supplemented chicks indicate improved outcomes for enhancing meat quality (Sakr *et al.,* 2022 and Kirkpinar *et al.,* 2014).

In our findings Tibial bone robusticity Index, medullary canal diameter, tibio-tarsal and Weight/length did not affect among different treated and control groups (P <0.05) (Table 2). The bone length and diaphysis diameter were higher in B, D and F groups than the control group (P <0.05). The bone weight was higher in E and F groups than the A-group (P <0.05). Egbu *et al;* revealed that the structural properties and morphology of tibial bone are crucial parameters in determining the ability of bones to achieve the basic functions, which give structural support and help in locomotion (Egbu *et al.,* 2022). The MO seed extract increased the bone length, weight, and medullary canal diameter of tibial bone (Khan *et al.,* 2023). The MLE were enriched of vitamins, minerals, and antioxidants, which enhanced the processes of osteoblast genesis and suppress the process of osteoclast genesis and enhanced the osteoimmunological response. The bone weight, weight/length index and ash percentage were increased with dietary ML in broilers. This improvement is main indicator of good mineralization of bone due to dietary supplementation of *MO*, having bioactive elements which is easily absorbed by intestinal tract (Nkukwana *et al.,* 2014). The tibial bone weight, robusticity index, and percentage of ash significantly increased with dietary supplements of herbal additives (MO powder) at the rate of 1.2 % in the diet of birds. Improvement in the percentage of ash and weight of the bone shows bone mineralization, it indicate that *MO* have bioactive ingredients that have positive effect on gut foremost to enhanced minerals and nutrients absorption through mucosa (Khan *et al.,* 2023).

We analyze that Phosphorus in serum and muscles, and selenium in bone tissue did not affect among different treated and control groups (Table 3). The Ca level in muscle was higher in all treated groups than control-group. Selenium concentration in muscle tissue was higher in D and F groups than the control group (P<0.05). In serum, the Ca concentration was increased in all treated groups than the A group (P<0.05). The selenium level in serum was increased in D, E and F groups than A-group (P<0.05). In **bone,** the Ca concentration was high in all treated groups than the A and G groups (P<0.05). Phosphorus concentration in tibial bone was higher in B, D and F groups than the A-group (P<0.05). The similar finding of Karthivashan *et al*; reported that the concentration of Ca and selenium was higher with the inclusion of the dietary MO leaf. The retention of these minerals showed higher bioavailability and absorption of these minerals for vital body function. Higher selenium and calcium retention in pectoral muscle is due to optimum dos MO. MO showed strong antioxidant capacity and scavenging the free radical (Karthivashan *et al.,* 2015). The tibia bone ash content and minerals contents (Ca, Mg, P, Zn) positively effects with the MO seed extract supplementation. Ca and P are most abundant in tibial bones, and the distribution of minerals effects the mineralization, development, and growth of the bone (Egbu *et al.,* 2022). Other study also reported that *Moringa oleifera* meal improved macro-minerals (Ca, P) in tibial bone. This showed that *MO* directly has relationship with bone quality (Faustin-Evaris *et al.,* 2022).

CONCLUSION

The MO seed and leaf extract supplemented in feed of broilers have positive influence on retention of minerals in different tissue, meat quality, morphology of bone, and muscle. The selenium and calcium concentration improved in the serum and muscle, while in bone the calcium and phosphorus concentration were improved when birds diet supplement with MO leaf and seed extract in comparison to the control group.

The leaf extract of MO group D (MLE-1.2% in diet of the broilers) and combined used of MLE and MSE, group F (B. D+0.8% MLE+0.8%MSE) is higher for enhancing the broilers minerals retention in different tissue. In chickens Phyto-biotics combination enhance the *morphometric* characteristics of muscle and meat quality.

REFERENCES

Egbu, C. F., Motsei, L. E., Yusuf, A. O., & Mnisi, C. M. (2022). Effect of moringa oleifera seed extract administered through drinking water on physiological responses, carcass and meat quality traits, and bone parameters in broiler chickens. *Applied Sciences*, *12*(20), 10330. https://doi.org/10.3390/app122010330

Mahfuz, S., & Piao, X. (2019). Application of moringa (Moringa oleifera) as natural feed supplement in poultry diets. *Animals*, *9*(7), 431. https://doi.org/10.3390/ani9070431

Coppin, J. (2008). *A study of the nutritional and medicinal values of Moringa oleifera leaves from sub-Saharan Africa: Ghana, Rwanda, Senegal and Zambia* (Doctoral dissertation, Rutgers University-Graduate School-New Brunswick). https://rucore.libraries.rutgers.edu/rutgers-lib/24288/PDF/1/play/

Rehman, H., Zaneb, H., Masood, S., Yousaf, M., Ashraf, S., Khan, I., Shah, M., Khilji, M., & Rehman, H. (2018). Effect of moringa Oleifera leaf powder supplementation on pectoral muscle quality and Morphometric characteristics of tibia bone in broiler chickens. *Brazilian Journal of Poultry Science*, *20*(4), 817-824. https://doi.org/10.1590/1806-9061-2017-0609

Owolabi, S. J., Alabi, O. J., Malik, A. D., Jiya, E. Z., & Adama, T. Z. (2020). Effects of aqueous moringa oleifera leaf extracts on gut morphology and pH of Hubbard broiler chickens. *Nigerian Journal of Animal Production*, *44*(1), 97-105. https://doi.org/10.51791/njap.v44i1.493

Bukar, A., Uba, A., & Oyeyi, T. (2010). Antimicrobial profile of moringa oleifera lam. Extracts against some food – borne microorganisms. *Bayero Journal of Pure and Applied Sciences*, *3*(1). https://doi.org/10.4314/bajopas.v3i1.58706

Ogbe, A. O., & Affiku, J. P. (2011). Proximate study, mineral and anti-nutrient composition of Moringa oleifera leaves harvested from Lafia, Nigeria: potential benefits in poultry nutrition and health. *Journal of Microbiology Biotechnology and Food Sciences*, *1*(3), 296–308. https://office2.jmbfs.org/index.php/JMBFS/article/view/4464

Khan, I., Zaneb, H., Masood, S., Yousaf, M. S., Rehman, H. F., & Rehman, H. (2017). Effect of *Moringa oleifera* leaf powder supplementation on growth performance and intestinal morphology in broiler chickens. *Journal of Animal Physiology and Animal Nutrition*, *101*(S1), 114-121. https://doi.org/10.1111/jpn.12634

Awad, W., Ghareeb, K., & Böhm, J. (2008). Intestinal structure and function of broiler chickens on diets supplemented with a Synbiotic containing Enterococcus faecium and Oligosaccharides. *International Journal of Molecular Sciences*, *9*(11), 2205-2216. https://doi.org/10.3390/ijms9112205

Mulaudzi, A., Mnisi, C. M., & Mlambo, V. (2022). Simultaneous pre-treatment of dietary Moringa oleifera leaf meal with polyethylene glycol and fibrolytic enzymes: effect on growth performance, physiological indices, and meat quality parameters in jumbo quail. *Frontiers in Animal Science*, *3*, 960233. https://doi.org/10.3389/fanim.2022.960233

Sreelatha, S., & Padma, P. R. (2009). Antioxidant activity and total phenolic content of moringa oleifera leaves in two stages of maturity. *Plant Foods for Human Nutrition*, *64*(4), 303-311. https://doi.org/10.1007/s11130-009-0141-0

Karthivashan, G., Arulselvan, P., Alimon, A. R., Safinar Ismail, I., & Fakurazi, S. (2015). Competing role of Bioactive constituents in*Moringa oleifera*Extract and conventional nutrition feed on the performance of Cobb 500 broilers. *BioMed Research International*, *2015*, 1-13. https://doi.org/10.1155/2015/970398

Alabi, O., Malik, A., Ng'ambi, J., Obaje, P., & Ojo, B. (2017). Effect of aqueous moringa Oleifera (Lam) leaf extracts on growth performance and carcass characteristics of Hubbard broiler chicken. *Revista Brasileira de Ciência Avícola*, *19*(2), 273-280. https://doi.org/10.1590/1806-9061-2016-0373

Nkukwana, T., Muchenje, V., Masika, P., Hoffman, L., Dzama, K., & Descalzo, A. (2014). Fatty acid composition and oxidative stability of breast meat from broiler chickens supplemented with moringa oleifera leaf meal over a period of refrigeration. *Food Chemistry*, *142*, 255-261. https://doi.org/10.1016/j.foodchem.2013.07.059

Sirotkin, A. V., & Harrath, A. H. (2014). Phytoestrogens and their effects. *European Journal of Pharmacology*, *741*, 230-236. https://doi.org/10.1016/j.ejphar.2014.07.057

Khan, I., Zaneb, H., Masood, S., Ashraf, S., Rehman, H. F., Rehman, H. U., ... & Rahman, S. U. (2022). Supplemental selenium nanoparticles-loaded to chitosan improves meat quality, pectoral muscle histology, tibia bone morphometry and tissue mineral retention in broilers. *Pakistan Veterinary Journal*, *42*(2). https://doi.org/10.29261/pakvetj/2022.007

Khan, I., Zaneb, H., Masood, S., Ashraf, S., Rehman, H. F., Tahir, S. K., Rehman, H. U., Khan, A., Taj, R., Rahman, S. U., & Shah, M. (2021). Supplementation of selenium nanoparticles-loaded Chitosan improves production performance, intestinal morphology, and gut microflora in broiler chickens. *The Journal of Poultry Science*, *59*(3), 272-281. https://doi.org/10.2141/jpsa.0210026

Khan, I., Zaneb, H., Masood, S., Ashraf, S., Rehman, H., Ullah, A., Ataya, F., Batiha, G. E., Taj, R., Elgazzar, A., & Din, S. (2023). Effects of selenium nanoparticles coated with chitosan supplementation on morphometry of immune organs, redox status, and immune response in broiler chicken. *Journal of Applied Poultry Research*, *32*(4), 100377. https://doi.org/10.1016/j.japr.2023.100377

Cardinali, R., Cullere, M., Dal Bosco, A., Mugnai, C., Ruggeri, S., Mattioli, S., Castellini, C., Trabalza Marinucci, M., & Dalle Zotte, A. (2015). Oregano, Rosemary and vitamin E dietary supplementation in growing rabbits: Effect on growth performance, carcass traits, bone development and meat chemical composition. *Livestock Science*, *175*, 83-89. https://doi.org/10.1016/j.livsci.2015.02.010

Mohamed, H. E., Ibrahim, W. A., & Gaafar, R. E. (2022). Impact of moringa olifera leaves or saccharomyces supplementation on carcass quality, mRNA of heat shock proteins and antioxidants in broilers exposed to heat stress. *SVU-International Journal of Veterinary Sciences*, *5*(4), 193-227. https://doi.org/10.21608/svu.2022.167661.1232

Talukdar, A., Choudhury, D., Kalita, K. P., & Saikia, R. (2020). Effect of using Moringa oleifera (drumstick) leaf meal on performance of Japanese quail. *JEZS*, *8*(2), 1485-1490.

Sakr, S. A., EL-Emam, H. A., Naiel, M. A., Wahed, N. M., Zaher, H. A., Mohamed Soliman, M., Shukry, M., Shehata, A. M., Alkhedaide, A., & Elghareeb, M. M. (2022). The effects of paulownia (*Paulownia tomentosa*) leaf extract enriched diets on meat quality, sensory attributes, and the potential economic impact of broilers. *Italian Journal of Animal Science*, *21*(1), 1430-1441. https://doi.org/10.1080/1828051x.2022.2121665

Kirkpinar, F., Ünlü, H., Serdaroğlu, M., & Turp, G. (2014). Effects of dietary oregano and garlic essential oils on carcass characteristics, meat composition, colour, pH and sensory

quality of broiler meat. *British Poultry Science*, *55*(2), 157-166. https://doi.org/10.1080/00071668.2013.879980

Evaris, E. F., Franco, L. S., Castro, C. S., Correa, J. S., & Maldonado, J. C. (2022). Male layer chicken's response to dietary moringa oleifera meal in a tropical climate. *Animals*, *12*(14), 1843. https://doi.org/10.3390/ani12141843

Saeed, A. A., Sandhu, M. A., Khilji, M. S., Yousaf, M. S., Rehman, H. U., Tanvir, Z. I., & Ahmad, T. (2017). Effects of dietary chromium supplementation on muscle and bone mineral interaction in broiler chicken. *Journal of Trace Elements in Medicine and Biology*, *42*, 25-29. https://doi.org/10.1016/j.jtemb.2017.03.007