Effect of *Moringa oleifera* extracts on physiological and meat quality responses of broiler chickens

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Thesis accepted in fulfilment of the requirements for the degree *Doctor of Philosophy in Animal Science* at the North-West University

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DECLARATION

I, Chidozie Freedom Egbu (33102643), Department of Animal Science, North-West University (Mafikeng Campus), hereby present my thesis in fulfillment of the Doctor of Philosophy in Agriculture in Animal Sciences degree. I certify that the whole material included below is my original work and that I have not previously submitted it in its entirety or in part for any qualification. Other people's materials and information are completely recognized and acknowledged. This thesis was completed under the supervision of Dr. L.E. Motsei and Dr. A.O. Yusuf.

Signature and date

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C.F. Egbu

This thesis is submitted with my consent as a university supervisor, and I thus confirm that the requirements for the applicable Doctor's degree rules and regulations were satisfied.

Signature and Date	Signature and Date
……………………	………………….
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DEDICATION

I dedicate this thesis to GOD Almighty. Thank You for making this journey a success.

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Publication 1

Egbu, C.F.; Motsei, L.E.; Yusuf, A.O. and Mnisi, C.M. **Evaluating the Efficacy of *Moringa oleifera* Seed Extract on Nutrient Digestibility and Physiological Parameters of Broiler Chickens**. Agriculture 2022, 12, 1102. https://doi.org/10.3390/agriculture12081102

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CONFERENCES CONTRIBUTION

Conference Presentation 1

Egbu, C.F.; Motsei, L.E. and Yusuf, A.O. **Carcass, internal organs, and meat quality of broilers supplemented with *Moringa oleifera* leaf extracts**. Oral presentation at the 52nd Annual SASAS Congress, 10 – 12 August 2021, South Africa.

Conference Presentation 2

Egbu, C.F.; Motsei, L.E.;Yusuf, A.O and Mnisi C.M. **Effect of *Moringa oleifera* seed extract on growth performance and haematological parameters in Cobb 500 broiler chickens**. Oral presentation at the 53rd Annual SASAS Congress, 26 – 28 September 2022, Ascot Conference Venue, Pietermaritzburg, South Africa.

LIST OF ABBREVIATIONS

*a**	Meat colour for redness
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADWG	Average daily weight gain
ADWI	Average daily water intake
AGPs	Antimicrobial growth performance promoters
ALB/GLOB	Albumen/globulin ratios
ALKP	Alkaline phosphatase
ALT	Alanine transaminase
AOAC	Association of official analytical chemists
*b**	Meat colour for yellowness
BWG	Body weight gain
Ca	Calcium
Ca: P	Calcium-phosphorus ratio
CAT	Catalase
CCW	Cold carcass weight
CF	Crude fibre
CP	Crude protein
Cu	Copper
DFD	Dry, firm, and dark meat
DM	Dry matter
EE	Ether extract
FBW	Final body weight
FCR	Feed conversion ratio
Fe	Iron
FI	Feed intake
FPHT	Femoral side proximal head thickness
GC	Gas chromatography
GGT	Gamma-glutamyl transferase
GST	Glutathione-s-transferase
H&E	Haematoxylin and eosin
HB	Haemoglobin
HCW	Hot carcass weight
HDL	High-density lipoprotein cholesterol
HPLC	High-performance liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
K	Potassium
L^*	Meat colour lightness
LDL	Low-density lipoprotein cholesterol
LP	Lipid peroxide
MCD	Medullary canal diameter
MCH	Mean corpuscular haemoglobin
MCHC	Mean cell haemoglobin concentration
MCP	Monocalcium phosphate
MCV	Mean corpuscular volume
Mg	Magnesium
MHA	Methionine hydroxy analogue

mL	Millilitre
MLE	*M. Oleifera* leaf extract
MLSE	*M. Oleifera* leaf + seed extract
Mm	Millimetre
Mn	Manganese
MPHT	Metatarsal side proximal head thickness
MSE	*M. Oleifera* seed extract
Na	Sodium
NC	Negative control
NDF	Neutral detergent fibre
NIRs	Near-infrared reflectance spectroscopy
Nm	Nanometre
P	Phosphorus
PC	Positive control
PCV	Packed cell volume
pH_{24hr}	Ph at 24hr post-mortem
pH_{45min}	Ph at 45mins post-mortem
PSE	Pale, soft, and exudative meat
RBC	Red blood cell
RDW	Red cell distribution width
ROS	Reactive oxygen species
SDMA	Symmetric dimethylarginine
SLWT	Slaughtered weight
SOD	Superoxide dismutase
TBS	Tibia breaking strength
TC	Total cholesterol
TLC	Thin-layer chromatography
TLW	Thickness of the lateral wall
TMW	Thickness of the medial wall
TW/TL	Tibia weight/tibia length index
WBC	White blood cell
WHC	Water holding capacity
WI	Water intake
Zn	Zinc
μL	Microlitre
μm	Micrometre

CHAPTER 1: INTRODUCTION

1.1 Background

The vulnerability of broiler chickens to stressors such as transportation, pathological, nutritional, and environmental factors (like heat stress) has hindered the improvement of broiler chickens' production (Stęczny and Kokoszyński, 2020). These stressors stimulate the synthesis of reactive oxygen species (ROS), which disrupt the equilibrium between the chicken's oxidative and antioxidant defense systems, resulting in oxidative stress (Farahat et al., 2017). Also, increased ROS, such as free radicals, cause damage to cellular components (lipid, protein, and DNA). As a result, ROS negatively impacts on physiological performance and meat quality traits (Waheed et al., 2017; Akuru et al., 2021). Conventional antioxidants such as vitamins C and E are normally used as feed additives in broiler diets to mitigate the impact of ROS but are limited in bioavailability when high levels of polyunsaturated fatty acids are included in the feed and there are concerns about synthetic sources of antioxidants (Hajati et al., 2015; Sigolo et al., 2019). Furthermore, antibiotics were formerly utilized to promote feed utilization efficiency in broiler chickens, but a restriction enforced in the European Union has created a vacuum in safeguarding chickens against stresses such as infections (Kleczek et al., 2012; Stęczny and Kokoszyński, 2020). As a result, prebiotics, probiotics, and organic phytogenic additives such as herbs are being adopted as alternatives.

The leaves and seeds of plants that are rich in polyphenols are gaining popularity as growth enhancers in broiler diets. *Moringa oleifera* leaves and seeds contain a variety of nutrients (calcium, phosphorus, protein, carbohydrates, and vitamins A, C, and E) and bioactive compounds (alkaloids, flavonoids, phenols, saponins, and tannins) with antimicrobial and antioxidant capabilities (Stevens et al., 2015; Mune et al., 2016; Ashour et al., 2020). They are thought to elicit prebiotic, antibacterial, antioxidant, and immune stimulants, resulting in higher broiler chicken performance (Elbushra et al., 2019). However, earlier studies on the effects of including *M. oleifera* leaves/seeds powder/meal in broiler diets on physiological performance and meat quality traits are few and conflicting. Mulaudzi et al. (2019) noted that *M. oleifera* leaf meal supplemented at 5% improved growth performance but not haemo-biochemical indices of female Japanese quail. When *M. oleifera* seed powder was included in diets at levels more than 0.5% a significant reduction in feed intake (FI) but no effect in feed conversion ratio (FCR) or body weight gain (BWG) of broilers was reported (Ochi et al., 2015). Likewise, when *M. oleifera* seed meal was supplemented in broiler diets at a level greater than 2% a better FCR and poor weight gain were observed (Sugiharto and Toana, 2021). This might be because the leaf/seed meal/powder was not processed into a leaf/seed extract, leaving the birds to rely exclusively on their digestive systems to extract the moringa leaf/seed's bioactive compounds. These earlier authors suggested that the decreased physiological performance at increased inclusion levels might be attributed to the high amount of fibre and condensed tannins of the leaf/seed powder/meal.

Furthermore, studies in which the leaves/seeds of polyphenol-rich plants that were extracted before being supplemented in broiler feeds reported a negative effect on broiler physiological performance when administered at high doses (Hajati et al., 2018; Ao and Kim, 2020). Polyphenol-rich plant leaves/seeds are typically extracted using an organic solvent or water. The extraction solvent influences the phytochemical profile of the resultant extract; water-based extracts should include monomeric flavonoids, dimer, and trimer procyanidins, however, organic solvents extract larger molecular weight procyanidins (Farahat et al., 2017). Previous studies on the effect of leaf/seed extracts of polyphenol-rich plants administered through feeds are littered with contradictory data on broiler chicken physiological performance and meat quality responses (Chamorro et al., 2013; Hajati et al., 2018, Rezvani et al., 2018; Panggabean et al., 2019). It was against this backdrop that this current study was conducted to determine the effect of oral administration of *M. oleifera* leaf and seed extracts on physiological performance and meat quality responses of broiler chickens.

1.2 Problem Statement

The use of antibiotics as growth promoters in the poultry industry has been prohibited due to concerns about antibiotic resistance and presence of antibiotic residues in chicken products intended for human consumption (Song et al., 2022). Despite the growing adoption of a variety of alternative growth enhancers such as prebiotics, probiotics, nanotechnology, and plants containing bioactive compounds, they are still plagued with inconsistent reports compared to the relatively consistent effects of antimicrobial growth performance promoters (AGPs) (Alonge et al., 2017; El Sabry et al., 2018).

Furthermore, there are emerging concerns regarding the bioavailability limitations of antioxidants such as vitamins C and E when supplemented in diets containing high polyunsaturated fatty acids, while large doses of synthetic antioxidants have been linked to deleterious effects on the liver and possible carcinogenic issues (Mitterer-Daltoé et al., 2020). Identifying naturally existing alternatives, such as phytogenic plants, is critical to the physiological performance and meat quality response of broiler chickens. *M. oleifera* leaf and seed extracts contain bioactive compounds that make them a viable option for increasing broiler performance.

1.3 Justification

Broiler chickens drink twice as much water as feed, and they keep drinking even if their feed consumption has ceased due to illness. Water administration of additives is significantly superior to feed because it avoids non-delivery through different complexes generated in the small intestine (Younis and Elbestawy, 2017). Broilers generally rely on their gut to obtain nutrients from their feeds however, water administration of additives increases nutritional bioavailability by diffusing the nutrient straight into the blood system.

M. oleifera leaf and seed contain carbohydrates, protein, minerals, vitamins, flavonoids, and phenols (Adejumo et al., 2016) which are potential enhancers of the physiological performance and meat quality of broiler chickens. However, the high fibre content and condensed tannins are limiting their feed utilization efficiency, and there is a paucity of information with inconsistent data regarding its application to the physiological performance and meat quality of broiler chickens via their drinking water. Given that the use of AGPs has been outlawed in several countries, such information is required for finding feeding options to boost broiler performance. Although plant supplements are frequent dietary additions for humans (Mashayekhi et al., 2018), they are rarely employed in animal diets due to a lack of understanding about their mode of action and administration (Ahmed et al., 2019).

1.4 Research Aims

The purpose of this study was to explore how *M. oleifera* extracts affect the physiological and meat quality responses of broiler chickens as an alternative to AGPs. The extracts were compared to a commercial probiotic, a standard additive in broiler chicken.

1.4.1 Specific Research Objectives

The specific objectives of this study were to evaluate:

1. The effect of *M. oleifera* leaf extracts (MLE) on physiological and meat quality responses of Cobb 500 broiler chickens,
2. The effect of *M. oleifera* seed extracts (MSE) on physiological and meat quality responses of Cobb 500 broiler chickens,

3. The effect of *M. oleifera* leaf + seed extracts (MLSE) on physiological and meat quality responses of Cobb 500 broiler chickens.

1.5 Hypotheses

This study tested the alternative hypotheses:

1. The administration of MLE improves the physiological and meat quality responses of Cobb 500 broiler chickens,
2. The administration of MSE improves the physiological and meat quality responses of Cobb 500 broiler chickens,
3. The administration of MLSE improves the physiological and meat quality responses of Cobb 500 broiler chickens.

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

With a rising global population comes an increased need for food. Broiler chicken fits the current consumer demand for low-fat meat with a high degree of unsaturated fatty acids. This growing demand for broiler chicken has resulted in pressure on nutritionists and farmers to enhance their physiological performance and meat quality with antibiotic growth promoters (AGPs) that have proven relative beneficial effect. However, with the concerns around AGPs, producing antibiotic-free products for humans using alternative measures is currently posing a challenge for food producers. Antibiotics have been banned in several countries, necessitating the search for suitable alternatives such as prebiotics, probiotics, and phytogenics, to name a few in poultry production (Cheng et al., 2019).

Alternatives such as phytogenic plant supplementation have a physiological enhancing effect on broiler chickens (Movahhedkhah et al., 2019). Also, various herbs and their extracts have been previously applied as replacements for AGPs in poultry production (Sigolo et al., 2021). He et al. (2021) further indicated that researchers are currently applying unconventional feed additives, which may have the capability to enhance the physiological performance and meat quality of broiler chickens. Thus, researchers are searching for potential feed additives that will be sustainable and safe for human consumption (Liu et al., 2020).

2.2 Broiler Nutrition

Nutrition affects the physiological performance and meat quality of broiler chickens. Nutrition in broiler chickens normally varies depending on the age (stages of production). The science of nutrition involves providing a balance of nutrients that best meet the broiler chickens' physiological needs. This supply of nutrients should be at least cost and from organic sources for economic and welfare reasons. So, we must supply enough for requirements without there being any major excesses. It is very difficult and expensive to supply all nutrients at the exact nutrient needs, rather we must oversupply some nutrients in practical situations to meet the limiting nutrients. In broiler diets, these limiting nutrients are usually energy and some of the essential amino acids such as methionine and lysine. In formulating diets, the following nutrients are considered: energy, protein, fat, vitamins, minerals, and water (Świątkiewicz et al., 2014).

Energy is the most expensive nutrient in a broiler diet and its sources include everything in the diet except minerals. Energy is important because it governs feed intake. Proteins are required for the synthesis of body tissue particularly muscle, and physiological molecules such as enzymes, hormones, feathers, and egg production. Proteins also provide a small amount of energy (Sigolo et al., 2017). The major animal protein sources are fishmeal and soybean. Fats provide energy and essential fatty acids that are required for some bodily processes. Vitamins are the organic chemicals containing carbon, which help control body processes and are required in small amounts for normal health and growth. Minerals are the inorganic chemicals not containing carbon that help control body processes such as growth and development, immunity, and reproduction (Paiva et al., 2014).

2.3 *Moringa oleifera*: Chemical Composition

M. oleifera is famed for its nutritional components such as carbohydrates, especially dietary fibers; proteins; vitamins; minerals; lipids; and water (Abbas et al., 2018). The unique features of *M. oleifera* are its richness in proteins, carbohydrates, and fibers with low fat (Mahfuz and Piao, 2019). *M. oleifera* leaves have been reported to enclose a range of essential amino acids and are a good source of alpha-linolenic acid (Moyo et al., 2011). *M. oleifera* leaves are rich in vitamin A, C, and E (Hekmat et al., 2015). The relative bioavailability of folate originating from *M. oleifera* leaves was about 82% in a rat model, which confirmed the fact that *M. oleifera* leaves exhibit a rich source of dietary folate (Saini et al., 2016).

Studies by Adejumo et al. (2016) reported the nutritional composition of *M. oleifera* leaves and seeds on dry matter (DM) bases of about 93.63% to 95.0%, crude protein (CP) 17.01% to 22.23%, carbohydrate 63.11% to 69.40%, crude fibre 6.77% to 21.09%, ether extract (EE) 2.11% to 6.41%, ash (total mineral) 7.96% to 8.40%, gross energy 14.790 (MJ/kg), and fatty acid 1.69% to 2.31%. Also, estimated calcium (Ca) 1.91%, potassium (K) 0.97%, sodium (Na) 192.95, iron (Fe) 107.48, manganese (Mn) 81.65, zinc (Zn) 60.06, and phosphorus (P) 30.15 parts per million (ppm), magnesium (Mg) 0.38%, and copper (Cu) 6.1%, tannins 21.19%, phytates 2.57%, trypsin inhibitors 3.0%, saponins 1.60%, oxalates 0.45%, and cyanide 0.1% (Ogbe and Affiku 2012). The leaves are enriched with methionine, phosphorus, calcium, and iron (Gupta et al., 2018). Gopalakrishnan et al. (2016) reported that the leaves of *M. oleifera* contain more calcium and twice as much protein as milk, higher vitamin C than oranges, higher potassium, and iron than bananas, and higher vitamin A than carrots, and thus the plant is considered unique (Abdull et al., 2014). Niaziridin, an active component that was identified in

M. oleifera, can improve the absorption of different vitamins, minerals, and other micronutrients in the gastrointestinal tract of the host (Stohs and Hartman, 2015).

On the other hand, moringa contains different anti-nutritional factors, such as tannins, phytates, oxalates, and cyanide, which may affect the normal digestion and metabolism of nutrients in animals (Moreki and Gabanakgosi, 2014). In moringa, tannins and phytates are 12 g and 21 g kg-1 of DM, respectively, which can be neutralized by different feed processing techniques, including chopping, soaking, heat steaming, and fermentation with beneficial organisms (Nouman et al., 2014). Considering the health benefit effects of moringa, it is a unique plant due to its enriching minerals with lower anti-nutritional components (Nouman et al., 2014).

2.3.1 Antimicrobial effects of the bioactive compounds in *Moringa oleifera*

Plants are often a potential source of bioactive substances that can be used to replace AGPs; however, the main challenge is that they exist as complex combinations rather than single compounds (Altemimi et al., 2017). One significant advantage of bioactive substances is their interaction with the body's biochemistry (Hou and Tako, 2018). Individual bioactive chemical synergy in *M. oleifera* leaves/seeds may be a valuable quality that influences wide components of physiology, nutrients assimilation, and immunity (Ashour et al., 2020; Bhalla et al., 2021).

M. oleifera extract contains tannins, flavonoids, and glycosides, all of which have therapeutic effects. These compounds have demonstrated effective antioxidant, anti-carcinogenic, and antimicrobial properties (Akinyeye et al., 2014) due to their ability to inactivate lipid free radicals in phenolic compounds, which act as primary antioxidants, as well as to prevent the decomposition of hydroperoxides into free radicals due to their redox properties (Waheed et al., 2017). *M. oleifera's* antimicrobial capabilities have been confirmed following inhibitory action against pathogenic bacteria such as *Salmonella spp* (Elbushra et al., 2019). Extracts of *M. oleifera* leaf were reported to be inhibitory against various pathogenic bacteria (Fouad et al., 2019).

2.3.2 Antioxidant characteristics of *Moringa oleifera*

M. oleifera is abundant in nutraceuticals, which have antioxidant characteristics and can help with a variety of ailments such as diarrhea, asthma, and cancer (Gupta et al., 2018). *M. oleifera* leaf and seed are also high in phytonutrients such as carotenoids, tocopherols, and ascorbic acid, all of which are effective antioxidants (Unuigbe et al., 2014). *M. oleifera* leaf extracts showed a considerable increase in superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and a decrease in lipid peroxide (LP) concentration (Hussein and Jassim, 2019). Also, *M. oleifera* leaf extract enhanced SOD, CAT, GST, and LP levels while decreasing lipid peroxidation in albino mice (Sharma and Singh, 2010).

Phenols are present in both edible and inedible plants and have been linked to a variety of biological effects, including antioxidant activity (Alikwe and Omotosho, 2013). The concentration of phenols in plants increases as the plant matures (Moyo et al., 2016). Phenol has been found in *M. oleifera* leaves and seeds, as reported by Adejumo et al. (2016). Flavones are phenolic compounds with one carbonyl group that, when combined with a 3-hydroxyl group, creates flavonol, whereas flavonoids are hydroxylated phenolic substances that arise as a C_6-C_3 unit connected to an aromatic ring (Abd Rani et al., 2018). Extracts of *M. oleifera* have been shown to contain a variety of bioactive chemicals, including flavonols and other phenolics (Godinez-Oviedo et al., 2016). Flavonols are the derivatives of flavonoid with a 3-hydroxy flavone backbone (IUPAC name: 3-hydroxy-2-phenylchromen-4-1). Flavonoids, one of the key components of phytochemicals present in the *M. oleifera* plant, are said to exhibit most of the antioxidant effect (Mitterer-Daltoé et al., 2020).

2.3.3 Immunological effect of *Moringa oleifera*

Studies have shown the immune-stimulating characteristics of *M. oleifera* (AbouSekken, 2015; Hassan et al., 2018). The extract of several parts of *M. oleifera* has been shown to have anti-inflammatory bioactive substances such as quercetin, glycosides, isothiocyanate, kaempferol, and glycosides (Stohs and Hartman, 2015). The presence of different proteins and peptides (isothiocyanates, glycoside cyanides, etc.) in *M. oleifera* leaf extracts positively influence the immunological response of chickens (Gupta et al., 2018). An experiment was conducted to evaluate *M. oleifera's* immunomodulatory activities in mice. Chronic dosing of *M. oleifera* elevated white blood cell (WBC) count and neutrophil percentage in mice (Gupta et al., 2018). However, the precise method by which moringa leaves stimulate humoral and cellular immunity is unknown (Gupta et al., 2018).

2.4 Effect of *Moringa oleifera* on Broiler Chicken Physiological Performance

In most of the poultry feeding investigations, the fresh, green, and undamaged mature *M. oleifera* leaves were air-dried, and the dried leaves crushed to a fine powder in a hammer mill and considered moringa leaf powder or leaf meal. Similarly, fresh mature moringa seeds were air-dried and ground into a moringa seed meal. In certain investigations, the crushed particles were then soaked in distilled water for 24 hours, and the filtered aqueous solution was designated moringa extract. As a result of their high nutrient content, notably crude protein

(CP), vitamins, and minerals, *M. oleifera* leaves can be used as a dietary supplement for chickens (Mulaudzi et al., 2019; Bhalla et al., 2021; Sugiharto and Toana, 2021).

In layers and Japanese quail, Mabusela et al. (2018) and Ashour et al. (2020) documented that moringa leaf and seed meal might be utilized as a nutritional supplement resulting in improved egg quality and performance. However, there is still inconsistency in past research about the performance of chickens with varying dosages of *M. oleifera*. There are also several variations in dosages and plant components utilized, such as leaves, extract, sods, or seeds. Researchers believed that the *M. oleifera* plant may have beneficial effect on enhancing chicken productivity and health.

2.4.1 *Moringa oleifera* effect on broiler chicken growth performance

Alabi et al. (2017) investigated the effects of aqueous *M. oleifera* leaf extracts on the performance of broilers. Results showed that in the 120 mL/L extract-supplemented groups, average daily body weight and final body weight (BW) were higher than those in the control group. Feed intake was higher for birds fed positive control (antibiotics) compared to those administered 90 mL/liter leaf extracts. Whereas the feed conversion ratio (FCR) was better in birds administered 90 mL/L and 120 mL/L leaf extracts of moringa. Moringa leaf extracts up to 90 mL/L can be administered to broiler chicks for optimal performance, according to the authors. The increased BW and better FCR were attributed to the presence of several bioactive components in moringa leaf extracts, which may contribute to enhanced nutrient utilization in supplemented birds. Similarly, Khan et al. (2017) found that using moringa leaf powder as a dietary supplement at 1.2% levels in broiler resulted in higher BW. Also, Ochi et al. (2015) tested moringa seed meal supplementation in broilers and discovered that supplemented diets might improve growth performance. The authors concluded that moringa seed meal may be used as a natural source of protein in broiler diets. Similarly, including *M. oleifera* leaves in broiler diets at 15% and 20% increased growth rate and improved health status (Alnidawi et al., 2016). Furthermore, supplementation of *M. oleifera* leaves powder at from 5% to 20% levels resulted in improved broiler growth performance (Moreki and Gabanakgosi, 2014). A study of 35-day trial period, final live weight, weight gain, and FCR were better in diets supplemented with 10% moringa leaf meal than the control (Ebenebe et al., 2012). David et al. (2012) observed that feeding *M. oleifera* leaf powder might enhance broiler life weight, body weight gain, dressing percentage, and FCR.

In contrast, Onunkwo and George (2015) observed that the growth performance or economic indicators did not differ in broilers fed *M. oleifera* leaf meal. The authors indicated that *M. oleifera* leaf meal might be applied at a 10% concentration to reduce costs of production. Similarly, Gakuya et al. (2014) found that feeding moringa leaf meal to broilers resulted in decreased feed intake and poor FCR owing to the presence of anti-nutritional compounds in moringa leaves. Feeding moringa seed powder to broilers resulted in similar final live weight or dressing percentage (Ochi et al., 2015). Gadzirayi et al. (2012) utilized *M. oleifera* leaf meal as a supplement to traditional soybean meal in broiler diets at 0%, 25%, 50%, 75%, and 100% levels. The authors found no significant variations in feed intake or bodyweight increase between the control and moringa supplementation levels at 25%. FCR was shown to be considerably better in moringa leaf meal-fed groups. Finally, the research recommended 25% level of moringa leaf meal enhanced growth in broilers.

On the other hand, Ayssiwede et al. (2011) found that dietary inclusion of moringa leaf meal up to 24% level did not compromise weight gain, FCR, mortality, and organ weight in broilers when compared to the control diet. According to Olugbemi et al. (2010), the average daily growth rate was lower with *M. oleifera* leaf meal inclusion levels below 5% in diets, and the authors proposed adopting a maximum level of 5% with no compromise on growth performance and FCR in broilers. These results suggest that feeding moringa leaves had no negative impact on the experimental broilers' normal growth performance. However, other scientists stated that using *M. oleifera* leaf meal up to 10% inclusion level will not compromise the physiological performance of broilers (Abou-Elezz et al., 2011). Table 2.1 summarizes the primary findings on the effect of *M. oleifera* leaf and seed powder/meal or leaf extract on the physiological performance of broilers.

Table 2. 1. Effect of *Moringa oleifera* on broiler chicken physiological performance.

Forms of *M. oleifera*	**Methodology of study**	**Primary results**	**References**
Leaf meal	Japanese quail were served dosages of 0, 2.5, and 5 % for 35 days	Increased overall weight gain. No effect on feed intake, FCR, haematological, serum biochemical indices, weights of thigh, gizzard, liver, small intestine, HCW, CCW, dressing percentage,	Mulaudzi et al. (2019)

		meat pH, colour, drip loss, and WHC.	
Leaf powder	Hubbard broilers were served dosages of 6, 9, 12, and 15 g/kg for 35 days.	Increased pH, water holding capacity, weight, and diameter of breast muscle. increased weight length index, ash percentage, and no effect on tibia alkaline phosphatase	Rehman et al. (2018)
Leaf powder	Hubbard broilers were served dosages of 0, 0.6%, 0.9%, 0.12%, 0.15% for 35 days	There were no effects on feed intake, FCR, or bursa weight. increased final body weight, small intestine length, small intestine and ceca empty weight, villus height (duodenum, jejunum, ileum), villus height/crypt depth (ileum), goblet cell number (total) in the duodenum, and acidic mucin number in the duodenum, jejunum, and ileum	Khan et al. (2017)
Leaf extract	Hubbard broilers were served dosages of 0, 60, 90, 120, 150 mL/L for 42 days	Increased body weight gain, decreased FCR, no effect on internal organ weight and dressing percentage	Alabi et al. (2017)
Leaf meal	Broilers were served dosages of 0, 5%, 10% 15%, 20%, for 42 days	greater body weight, hemoglobin %, and RBC. reduced TC and LDL.	Alnidawi et al. (2016)
Seed powder	Broilers were served dosages of 0, 0.5%, 0.1%, and 2%, for 42 days	There were no differences in live weight, weight gain, FCR, dressing %, liver weight, or heart weight.	Ochi et al. (2015)

Leaf meal	Anik 2000 broilers were served dosages 0, 5%, 7.5%, 10 %, for 49 days	Increased dressing weight at 7.5 and 10% levels, elevated weight of liver, spleen, and gizzard There were no discernible impacts on body weight gain, feed intake, or FCR.	Onunkwo and George (2015)
Leaf meal	Cobb 500 broilers were served dosages starter (1, 3, and 5 g/kg); grower (3, 9, and 15 g/kg); and finisher (5, 15, and 25 g/kg), for 35 days	Increased Ca and P content in tibia bone, tibia weight, tibia length, weight-length index of the tibia bone, the ash content in the tibia, and bone-breaking strength.	Nkukwana et al. (2014b)
Leaf meal	Cobb 500 broilers were served dosages starter (1, 3, and 5 g/kg); grower (3, 9, and 15 g/kg); and finisher (5, 15, and 25 g/kg), for 35 days	Increased starter and finisher body weight, reduced FCR, no effect on feed intake, increased dressing %, thigh muscle weight, and bursa weight There were no effects on the digestibility of CP, CF, DM, EE, ash, NDF, or ADF.	Nkukwana et al. (2014a)
Leaf meal	Cobb 500 broilers were served dosages of 1%, 3%, and 5%, for 35 days	Thiobarbituric acid reactive values in breast muscle increased during storage, as did the fatty acid profile (C18:0, C15:0, C20:0, C20:3n6, and C22:6n3). There were no effects on the thrombogenic and atherogenic indexes in breast muscle.	Nkukwana et al. (2014c)
Leaf meal	Ross broilers were served dosages of 0, 3%, 5%, and 7%, for 49 days	Increased final body weight and weight gain, reduced FCR, higher feed intake, dressing %,	El-Tazi (2014)

		meat tenderness, and juiciness score	
Leaf meal	Broilers were served dosages of 0, 7.5%, 15%, and 30%, for 49 days	Reduced final body weight and weight gain. increased FCR, reduced dry matter digestibility, no effect on crude protein, crude fiber digestibility, lipid metabolic profile (HDL, TC, LDL), and increased meat color scores	Gakuya et al. (2014)
Leaf meal	Broilers were served dosages of 0, 10%, and 15%, for 35 days	Reduced FCR, increased body weight gain, final body weight, RBC, PCV, and HB %	Ebenebe et al. (2012)
Leaf meal	Hubbard broilers were served dosages of 0, 25%, 50%, 75 %, and 100%, for 42 days	There is no influence on feed intake, weight gain, and decreased FCR.	Gadzirayi et al. (2012)
Leaf powder	Broilers were served dosages of 0, 0.05%, and 0.10%, for 42 days	Reduced FCR, increased body weight gain, final body weight, and dressing percentage	David et al. (2012)
Leaf extract	Cobb broilers were served dosages of 0, 30, 60, and 90 mL/L, for 35 days	Increased live weight. reduced FCR, increased return on investment, and reduced feed intake	Portugaliza and Fernandez (2012)
Leaf meal	Cobb broilers were served dosages of 0, 5%, 10%, and 15%, from 14-42 days	Decreased weight gain and final body weight, increased FCR, no influence on dressing %, carcass weight, internal organs weight, meat CP and EE content, total cholesterol, HDL, LDL, total protein, glucose	Zanu et al. (2012)

FCR: feed conversion ratio; HDL: high-density lipoprotein cholesterol; TC: total cholesterol; LDL: low-density lipoprotein cholesterol; RBC: red blood cell; PCV: packed cell volume; HB: haemoglobin; CP: crude protein; CF: crude fibre; DM: dry matter; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber.

2.4.2 *Moringa oleifera*'s effects on meat quality and bone parameters of broiler chickens

Nutritional intervention in chickens is a significant method of enhancing meat quality (Akuru et al., 2020; Adeyemi et al., 2021; Matshogo et al., 2021a). Broiler chicken meat is a good source of protein, vitamins, minerals, and low fat, which has resulted in high customer demand (Mogire et al., 2021). Consumers place a high value on meat pH, tenderness, color (lightness, redness, and yellowness), and water holding capacity. Rehman et al. (2018) investigated the effects of *M. oleifera* leaf powder supplementation on the quality of meat and bone in broilers. The authors posited that a 12 g/kg supplementation of leaf powder increased pH, water holding capacity, and muscle fiber diameter in the breast muscle of experimental broilers. Furthermore, broilers given moringa leaf meal had increased weight, ash percentage, and tibia bone density (Rehman et al., 2018). The authors postulated in their study that increased muscle pH values in experimental groups were attributable to myofibril stability via activating antioxidant characteristics and avoiding free radicals. The authors agreed that the increased rate of protein deposition in moringa-supplemented groups were responsible for the enhanced breast muscle weight while phytoestrogen flavonoids found in moringa leaves powder increased tibia bone weight and ash percentage.

Nkukwana et al. (2014b) observed that while *M. oleifera* leaf meal had no effect on tibia bone traits, it did improve weight gain and FCR which they attributed to moringa inclusion levels and the form of application in broiler diets. However, it is widely held that dietary antioxidants can alter the color of meat, reduce rancidity, and slow lipid peroxidation, resulting in well-maintained meat quality (Xu et al., 2021). Meat muscle's oxidative state is closely connected to meat quality and has a negative impact on cooking loss, drip loss, meat color, and pH (Zhang et al., 2017). As a result, feed supplementation with antioxidant-rich moringa extracts might be a viable technique for improving meat quality in broilers. Furthermore, phytosterols were shown to lower malondialdehyde (MDA) levels and raise glutathione (GSH) concentration in the breast muscle of experimental broiler chickens (Naji et al., 2013). Moringa leaf meal increased fat content but had no influence on moisture, ash, or protein content in broiler meat (Nkukwana et al., 2015). The authors believed that the high-fat content was related to the concentration of saturated fatty acids in moringa leaves.

2.4.3 *Moringa oleifera*'s effects on the blood parameters and intestinal morphology of broiler chickens

Alnidawi et al. (2016) conducted an experiment to investigate the effects of *M. oleifera* leaf on broiler health. The study found that supplementing *M. oleifera* at 15% and 20% inclusion levels in broiler feeds reduced overall cholesterol concentration. Similarly, these levels of *M. oleifera* supplementation raised high-density lipoprotein cholesterol (HDL) concentration in serum while decreasing low-density lipoprotein cholesterol (LDL). Higher quantities of natural fiber in moringa leaves were suggested to play a function in decreasing cholesterol levels by enhancing lipid metabolism in the host body. Furthermore, at 20% supplementation levels, blood parameters such as hemoglobin percentage, total RBC count, and total PCV were shown to be higher than those in the control (Alnidawi et al., 2016). *M. oleifera* leaf powder was applied as a dietary supplement in broilers at doses of 0.6%, 0.9%, 1.2%, and 1.5% on growth performance and intestinal microarchitecture (Khan et al., 2017).

The anatomical traits of the chicken intestine are critical for nutrient absorption and may imply good physiology. Small intestine length and weight were observed to be greater in broilers fed 1.2% leaves powder. Furthermore, the 1.2% leaves powder fed group had increased villus height (duodenum, jejunum, ileum), villus surface area (duodenum), and villus height/crypt depth (ileum) than the control group. Higher villi indicate higher nutritional absorption owing to the increased surface area, which is a positive sign of the gastrointestinal system. Furthermore, the increased villus height and villus height/crypt depth ratio may be related to the high crude fiber content of moringa-supplemented meals. The study additionally revealed that total goblet cells of the duodenum were greater in broilers fed with all levels of *M. oleifera* leaf powder in comparison with the control group. The data show increased mucosal protection with *M. oleifera* addition in broiler diets. In chickens, goblet cells are critical components of the innate gut immune system. The researchers noted that a 1.2% *M. oleifera* feed supplementation might alter the intestinal structure and acidic mucin production without affecting broiler growth performance (Khan et al., 2017).

The extract from the leaves of *M. oleifera* exhibits antibacterial and antioxidant properties (Unuigbe et al., 2014; Chelliah et al., 2017). Ebenebe et al. (2012) investigated the effect of *M. oleifera* leaf meal inclusion at 10% and 15% on haematological parameters in broilers. Feeding moringa leaf meal to broilers improved red blood cells, packed cell volume, and haemoglobin levels in both diet levels. The authors concluded that *M. oleifera* leaf meal should be utilized in broiler diets at 10% inclusion level. *M. oleifera* contains antioxidants such as vitamins C and E, carotenoids, flavonoids, and selenium (Moyo et al., 2016). *M. oleifera* leaves contain a variety of phytochemicals (carotenoid, flavonoid, chlorophyll, phenolic, xanthin, cytokine, alkaloid) that may influence health status of birds (Falowo et al., 2014).

2.5 Plants Extraction Process

Extraction of medicinal plants is the separation of active plant components or secondary metabolites such as alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides from inactive or inert material using an appropriate solvent and standard extraction technique (Abubakar and Haque, 2020). Medicinal plants are extracted and processed for direct ingestion as herbal or traditional medicine, or for research purposes. Preparation of medicinal plants for experimental purposes entails correct and timely plant collection, validation by an expert, optimal drying, and grinding. If possible, the bioactive component is extracted, purified, and isolated. It also includes determining the quantity and quality of bioactive substances (Ingle et al., 2017).

Maceration, infusion, decoction, percolation, digestion and Soxhlet extraction, superficial extraction, ultrasound-assisted extraction, and microwave-assisted extraction were all utilized in the extraction of medicinal plants. Furthermore, secondary metabolites were separated and purified using thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), paper chromatography (PC), and gas chromatography (GC) (Ingle et al., 2017). The efficiency of the extraction process is dictated by the characteristics of the plant material, the solvent applied, the pH of the solvent, the temperature, and the solvent to sample ratio. It also hinges on how the eventual extracts will be applied. Any component that increases the diffusivity and solubility in the preceding phases will make the extraction easier (Zhang et al., 2018).

2.5.1 Extraction solvents

The menstruum is the solvent used in the extraction of medicinal herbs. The solvent used is determined by the type of plant, the part of the plant to be extracted, the nature of the bioactive substances, and the availability of the solvent. Polar solvents like water, methanol, and ethanol are commonly used in the extraction of polar compounds, whereas nonpolar solvents like hexane and dichloromethane are commonly employed in the extraction of nonpolar chemicals (Altemimi et al., 2017). The solvents used in extraction are categorized according to their polarity, with hexane being the least polar and water is the most polar. Table 2.2 outlines extraction solvents in increasing polarity order (Pandey and Tripathi, 2014).

Table 2. 2. The polarity of extraction solvents.

Solvents	Polarity
Hexane	0.009
Petroleum ether	0.117
Diethyl ether	0.117
Ethyl acetate	0.228
Chloroform	0.259
Dichloromethane	0.309
Acetone	0.355
Butanol	0.586
Ethanol	0.654
Methanol	0.762
Water	1.000

2.5.2 Characteristics of some extraction solvents

1. ***Water*:** It is the most polar solvent and is used to extract a variety of polar substances. It dissolves a wide spectrum of materials; it is inexpensive, safe, inert, and extremely polar. However, it increases bacterial development, may induce hydrolysis, and requires a significant quantity of heat to concentrate the extract (Tiwari et al., 2011).
2. ***Alcohol*:** It is also polar in nature, miscible with water and capable of extracting polar secondary metabolites. At concentrations greater than 20%, it is self-preservative. It is safe at low concentrations, and just a moderate quantity of heat is necessary to concentrate the extract. But it does not dissolve fats, gums, or wax, and it is combustible and volatile (Patel et al., 2021).
3. ***Chloroform*:** It is a nonpolar solvent that may be used to extract substances such as terpenoids, flavonoids, lipids, and oils. It is colourless, odourless, and soluble in alcohol. It is also easily absorbed and metabolized by the body. However, it is sedative and carcinogenic (Patel et al., 2021).
4. ***Ether*:** It is a nonpolar solvent that may be used to extract substances like alkaloids, terpenoids, coumarins, and fatty acids. It is miscible with water, has a low boiling point, and has no flavour. It is also an extremely stable substance that is unaffected by acids, bases, or metals. Nonetheless, it is extremely volatile and combustible in nature (Patel et al., 2021).
5. ***Green solvent*:** This is a one-of-a-kind extraction solvent that is highly polar and exceedingly stable under heat. It can remain liquid even at 3,000 °C and is utilized when

high temperatures are required. It is very miscible with water and other solvents, making it ideal for polar chemical extraction. It is good for microwave-assisted extraction because it contains a great solvent that attracts and transmits microwaves. It is non-flammable, extremely polar, and excellent for liquid-liquid extraction. Regardless, it is not excellent for tincture production (Bhan, 2017).

2.5.3 Methods often utilized in the extraction of plants

1. ***Maceration*****:** This is an extraction method in which coarsely powdered plant material, such as leaves or seeds, stem bark, or root bark, is placed within a container, and menstruum is poured on top until the plant is completely covered. After that, the container is sealed and stored for at least three days. The contents are shaken on a regular basis, and if placed within the bottle, they should be shaken occasionally to ensure complete extraction. The micelle is removed from the marc by filtering or decantation at the end of the extraction process. Following that, the micelle is separated from the menstruum by evaporation in an oven or on top of a water bath. This procedure is simple and ideal for thermolabile plant material (Ujang et al., 2013).
2. ***Infusion*****:** The herb is crushed to a fine powder before being put in a clean container. The extraction solvent, either hot or cold, is then poured on top of the powder, soaked, and set aside for a brief time. This approach is appropriate for extracting bioactive compounds that are easily soluble. Furthermore, it is a good procedure for preparing fresh extract before usage. Depending on the intended usage, the solvent to sample ratio is commonly 4:1 or 16:1 (Pandey and Tripathi, 2014).
3. ***Digestion*****:** It entails using mild heat throughout the extraction procedure. The extraction solvent is placed into a clean container, followed by powdered herbs. At a temperature of around 50 °C, the mixture is put over a water bath or in an oven. Throughout the extraction process, the heat was used to reduce the viscosity of the extraction solvent and improve the removal of secondary metabolites. This approach works well with plant materials that are easily soluble (Pandey and Tripathi, 2014).
4. ***Decoction*****:** This is a continuous hot extraction method that employs a predetermined volume of water as a solvent. A clean container is filled with powdered plant material. After that, water is poured and mixed. Heat is then used throughout the extraction process to speed up the extraction. The procedure takes roughly 15 minutes. The solvent to crude herb ratio is commonly 4:1 or 16:1. It is used to extract the water-soluble and heat-resistant plant material (Majekodunmi, 2015).

5. ***Percolation*****:** A percolator is a device used in this procedure. It's a glass vase with a small cone form with openings on both ends. In a clean container, finely powdered plant material is soaked with the extraction solvent. A larger amount of solvent is added, and the mixture is kept for 4 hours. The material is then put into a percolator with the lower end closed and left to stand for 24 hours (Majekodunmi, 2015). The extraction solvent is then poured from the top down until the herb is thoroughly saturated. The percolator's lower section is then opened, and the liquid is allowed to trickle gently. A constant amount of solvent was introduced, and the extraction was carried out by gravity force, which pushed the solvent downward through the plant material (Majekodunmi, 2015). The solvent addition was halted when the volume of solvent added reached 75% of the total amount of preparations. Filtration and decantation are used to separate the extract. The marc is then expressed, and the needed volume is obtained by adding the final amount of solvent (Majekodunmi, 2015).
6. ***Soxhlet extraction*****:** This method is also known as continuous heat extraction. The glass equipment is known as a Soxhlet extractor. It has a spherical bottom flask, an extraction chamber, a siphon tube, and a condenser on top. Powdered plant material is carefully packed into a porous bag (thimble) composed of clean cloth or strong filter paper (Hossain et al., 2014). The extraction solvent is poured into the bottom flask, and then the thimble is inserted into the extraction chamber. The solvent is then heated from the bottom flask, evaporates, and runs down to the extraction chamber, where it condenses and extracts the bioactive compounds by coming into contact. As a result, when the amount of solvent in the extraction chamber reaches the siphon's top, the solvent and the extracted plant material flow back to the flask (Hossain et al., 2014). The procedure is continued until the bioactive compounds are completely extracted, at which point a solvent running from the extraction chamber leaves no residue. This approach is appropriate for plant materials that are partly soluble in the selected solvent as well as plant materials containing insoluble substances. A high variety of bioactive compounds may be extracted using less solvent. It is also relevant to heat-resistant plant materials. There is no need for filtering, and a large amount of heat might be used. Regardless, regular shaking is not achievable, and the approach is not appropriate for thermolabile materials (Hossain et al., 2014).
7. ***Microwave-assisted extraction*****:** This is one of the most sophisticated extraction methods used in the preparation of medicinal herbs. The approach employs a dipole rotation and ionic transfer process based on the displacement of charged ions contained

in the solvent and plant material. This approach is appropriate for extracting flavonoids. It entails the use of electromagnetic radiation with frequencies ranging from 300 MHz to 300 GHz and wavelengths ranging from 1 cm to 1 m (Altemimi et al., 2017). Microwaves at a frequency of 2450 Hz produced energy ranging between 600 and 700 W. Microwave radiation is used to blast a plant, which may absorb electromagnetic energy and convert it to heat. As a result, the heat generated accelerates the passage of solvent into the plant (Ingle et al., 2017). Also, dipole rotation and ion migration occur when a polar solvent is utilized, increasing solvent penetration and aiding the extraction process. However, when a nonpolar solvent is applied, the emitted microwave radiation produces very minimal heat; hence, this procedure does not encourage the use of nonpolar solvents (Altemimi et al., 2017). Microwave-assisted extraction offers many benefits, including reduced solvent and extraction time, as well as improved extraction results. However, this approach is only applicable to phenolic chemicals and flavonoids. Because of the high temperature involved, compounds such as tannins and anthocyanins may be destroyed (Ingle et al., 2017).

8. ***Ultrasound-assisted extraction*:** The use of sound radiation at a very high frequency of more than 20 kHz to disrupt plant cells and increase the surface area for solvent penetration is involved in this procedure. As a result, secondary metabolites will be produced. Plant material should be dried first, then ground into a fine powder, and sieved thoroughly. The prepared sample is then combined with an extraction solvent and loaded into the ultrasonic extractor (Azwanida, 2015). The high sound energy is used to speed up the extraction process by reducing the amount of heat needed. Ultrasound-assisted extraction is appropriate for tiny samples; it minimizes extraction time and solvent consumption while increasing yield. Unfortunately, this approach is difficult to replicate; also, the quantity of energy used may damage the phytochemical by creating free radicals (Altemimi et al., 2017).

2.6 Conclusion

Conclusively, *M. oleifera* leaves and seeds can be extracted with water and administered as an excellent natural growth promoter as well as an immune-boosting substance in broiler chicken feed. Although *M. oleifera* leaves, seed powder, and extracts have been included in broiler experimental diets at different levels, more research on the level that will produce optimum performance and clinical health in chickens has been proposed by several researchers. Nonetheless, evidence on the use of moringa extracts on the physiology and meat quality of broilers is still limited. It may be safe to conclude that *M. oleifera* extracts can be employed as a long-term feed additive in broiler chicken diets.

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CHAPTER 3: EFFECT OF MORINGA OLEIFERA LEAF EXTRACT ON PHYSIOLOGICAL AND MEAT QUALITY RESPONSES OF COBB 500 BROILER CHICKENS

Abstract

Harnessing plant materials rich in phytochemicals would be vital as we progress in our hunt for antibiotic alternatives. The objective of the current study was to investigate the effect of oral administration of *M. oleifera* leaf extract (MLE) on growth performance, nutrient digestibility, carcass yield, visceral organs, blood parameters, meat quality, intestinal morphology, and bone parameters of broiler chickens. A total of 250 one-day-old Cobb 500 male chicks (57.92 ± 0.08 g live weights) were randomly allocated into 5 treatments with 5 replicates of 10 birds each. The treatments included a negative control containing drinking water only (NC), positive control with 5 g of multi-strain probiotic/L of drinking water (PC), 60 mL of MLE/L of drinking water (MLE_{60}), 90 mL of MLE/L of drinking water (MLE_{90}), and 120 mL of MLE/L of drinking water (MLE_{120}). The experiment lasted for 42 days and commercial starter (1–21 days) and finisher (22–42 days) diets were fed to the birds. Oral administration of MLE increased ($p < 0.05$) the overall final body weight (FBW), body weight gain (BWG), average daily weight gain (ADWG), and improved feed conversion ratio (FCR) while, decreasing feed intake (FI) and average daily feed intake (ADFI) compared to PC and NC. MLE administration increased the digestibility of dry matter, crude protein, ether extract, neutral detergent fibre (NDF), and acid detergent fibre (ADF), while MLE_{90} and MLE_{120} had the highest similar calcium and phosphorus digestibility. Birds administered NC and PC had comparable lower haematocrit, haemaglobin, and reticulocyte count. The NC and MLE administered groups had similar higher mean corpuscular haemoglobin (MCH) and mean cell haemoglobin concentration (MCV). Birds administered MLE presented a comparable higher serum calcium concentration. The slaughter weight (SLWT), dressing percentage, hot carcass weight (HCW), and cold carcass weight (CCW) were elevated with the oral administration of MLE. The bursa, spleen, and thymus weight were decreased with the administration of NC and PC. MLE administration decreased the meat pH at 24hr post-mortem (pH_{24hr}). Water holding capacity (WHC) was similar ($p > 0.05$) in PC and MLE administered groups, drip loss and cooking loss were similar ($p > 0.05$) in NC and MLE administered groups while MLE administration decreased meat shear force. The meat moisture and crude protein content were similar in NC and PC while crude fat content decreased in the MLE groups. NC and MLE groups presented similar ($p > 0.05$) meat ash content. Birds on NC and PC had shorter ($p <$

0.05) duodenum, jejunum, and ileum length than those in the MLE administered groups. At the duodenum, villus height and villus width were similar in NC and PC. Muscularis mucosa thickness was similar in the NC and MLE administered groups. MLE administration elevated villus height/crypt depth ratio. At the jejunum, MLE_{90} increased villus height, villus width, and crypt depth while NC and PC had similar decreased means. PC and MLE groups presented a similar villus height/crypt depth ratio. At the ileum, MLE administration increased ($p < 0.05$) villus height and crypt depth. MLE_{90} had the highest villus width when compared with the similar lower means of NC and PC. Acidic and total goblet cell counts were similarly lower ($p < 0.05$) in NC and PC when compared to the MLE groups while NC, PC, and MLE_{90} had similar mixed goblet cell counts at the duodenum. Acidic and total goblet cell counts were similarly higher ($p < 0.05$) in MLE administered groups at the jejunum. NC and PC administration presented similar lower acidic goblet cell counts when compared to the MLE groups. NC and MLE groups had similarly mixed goblet cell counts while NC and PC had similar total goblet cell counts at the ileum. MLE_{90} and MLE_{120} administration increased tibia weight while NC and PC increased tibia length. MLE_{120} had the highest bone breaking strength (BBS) when compared to the NC and PC that presented the least similar means. Tibia weight/tibia length index (TW/TL), the thickness of the medial wall (TMW), and the thickness of the lateral wall (TLW) were similar ($p > 0.05$) in MLE_{90} and MLE_{120}. MLE decreased ($p < 0.05$) the tibia medullary canal diameter (MCD). MLE elevated the tibiotarsal index while decreasing the robusticity index. NC and PC had similar lower tibia calcium composition and Ca: P. MLE_{60} and MLE_{90} had comparable higher magnesium when compared to NC. Iron was higher in PC and MLE administered groups. MLE administration increased ($p < 0.05$) zinc in the tibia. It was concluded that all the levels of MLE improved the physiological and meat quality responses of Cobb 500 broilers.

Keywords: Growth performance, Nutrient digestibility, Blood parameters, Carcass yield, Internal organs, Intestinal morphology, Bone parameters

3.1 Introduction

The poultry sector is continuously challenged by the prevalence of bacterial infections, which compromises productivity, revenue, and sustainability (Erinle et al., 2022). According to Chapman and Jeffers (2014), the global average annual economic cost of these infections in the poultry industry is \$3 to \$6 billion. Antibiotic growth promoters have been used effectively in poultry production at subtherapeutic levels to increase bird performance and meat quality (Waheed et al., 2017; Paul et al., 2018). Antibiotic growth promoters boost growth rates by improving gut health, leading to higher nutrient digestibility and an improved FCR. Furthermore, medication in water helps birds recover from diseases due to birds drinking twice the amount of feed they consume (Gultepe et al., 2019). However, there are concerns about continued subtherapeutic antibiotic usage due to the development of antibiotic-resistant microbes that may harm humans (Long et al., 2019).

As a result, attempts are being undertaken in several regions of the world to prohibit the use of all forms of antibiotics in animal production. With the European Union's ban on antibiotics (EC Regulation No. 1831/2003) and increasing pressure on livestock farmers throughout the world, antibiotic-free alternative products and techniques for animal growth promotion and disease prevention are being researched (Landy et al., 2011). As a result, prebiotics, probiotics, and organic phytogenic additives such as herbs have been adopted as safe alternatives (Alonge et al., 2017; Abd El-Hack et al., 2020). The *M. oleifera* plant is readily accessible in many tropical nations, and its leaf contains bioactive compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids, which also have probiotics properties (Sugiharto et al., 2020).

However, studies on the physiological and meat quality traits of broiler chickens to *M. oleifera* leaf meal inclusion in the diet are few and conflicting. Mulaudzi et al. (2019) reported no effect on feed intake, FCR, haemo-serum biochemical parameters, and meat quality of Japanese quails. Zanu et al. (2012) noted decreased weight gain and poor FCR when *M. oleifera* leaf meal was included beyond 5% level in the feed of broilers. This poor feed utilisation might be because of the high fibre and condensed tannin content of the leaf meal (Chowdhurya et al., 2018). When not processed such as extraction the birds rely only on their digestive system to obtain the plant's therapeutic compounds. According to Abbas et al. (2021), aqueous extractions may remove large amount of anti-nutritional components, notably fibre, condensed tannins and saponins. The objective of the current study was to investigate the effect of administering MLE in drinking water on growth performance, nutrient digestibility, blood parameters, carcass yield, visceral organs weight, meat quality, intestinal morphology, and bone parameters of broiler chickens.

3.2 Materials and Methods

The procedures used to conduct the feeding trial and slaughter the chickens were reviewed and approved by the Animal Production Research Ethics Committee (approval no. NWU-02002-20-A5) of North-West University (North West, South Africa).

3.2.1 Study site

This study was conducted during summer at Rooigrond Farm (25° 55' 0'' South and 25° 48' 0'' East) located 16 km southeast of Mafikeng (North West, South Africa). The temperatures ranged from 19°C to 37°C with an annual average rainfall of 450 mm at an elevation of 1224 m above sea level.

3.2.2 Sources of *Moringa oleifera* and preparation of leaf extract

The *M. oleifera* leaf powder used to prepare the experimental extracts was supplied by Supa Nutri (Johannesburg, South Africa). The leaf powder was soaked in distilled water at a ratio of 1:5 for 24 hours. The mixture was then filtered with a muslin cloth to separate the debris from the filtrate, and the extracts were stored in clean containers in a cold room at 4°C. The extracts were then administered into the fresh drinking water that was offered to the birds daily during the study as follows: negative control with drinking water only (NC), positive control with 5g probiotic per litre of drinking water (PC), 60 mL of MLE per litre drinking water (MLE_{60}), 90 mL of MLE per litre of drinking water (MLE_{90}), and 120 mL of MLE per litre of drinking water (MLE_{120}). Commercial starter (1–21 days) and finisher (22–42 days) diets from De Heus (Pty) Ltd. (Pietermaritzburg, South Africa) were used during the feeding trial. The probiotic (5×10^8 CFU/g) was acquired from QBLabs, (MO, USA), and contained beneficial bacteria (*Aspergillus oryzae, Bacillus subtilis, Lactobacillus acidophilus,* and *Enterococcus faecium*), which were identified *via* 16S rRNA gene sequencing. Dry matter, crude protein, crude fibre, and ash were analysed according to the methods of the Association of Official Analytical Chemists (2005). Minerals were analysed using the guidelines from AgriLASA (1998). Metabolizable energy (ME) was calculated using the following equation by Khalil et al. (1986):

$$Metabolizable\ Energy = 0.821 \times Digestible\ Energy\ (Mega\ Joule)$$

The nutritional composition of the diets is shown in Table 3.1.

Table 3. 1. Nutritional composition (g/kg as fed basis, unless stated otherwise) of the starter and finisher diets.

[1]Nutritional compositions	Starter (1-21d)	Finisher (22-42d)
Dry matter	884.6	885.1
Calculated ME (MJ/kg)	12.87	13.12
Crude protein	205.7	181.8
Crude fat	33.69	46.64
Crude fiber	44.68	50.35
Ash	35.34	34.85
Available phosphorus	3.80	3.10
Calcium	9.00	7.40
Chloride	2.00	2.00
Sodium	1.70	1.70
Potassium	6.80	5.40
Total phosphorus	5.89	5.21

ME = Metabolizable energy.

3.2.3 Experimental design and birds' management

A total of 250, one-day-old Cobb 500 male chicks were purchased from Poultry Ranch (Pty) Ltd. (Pretoria, South Africa). In a completely randomized design, the chicks were allocated to 25 pens (experimental units) to which the five experimental treatments were randomly assigned (Steel and Torrie, 1980). The treatments were replicated five times and each pen (experimental unit) had 10 birds and the trial lasted for 42 days. The birds were housed in floor pens measuring 2.55 m L × 1.0 m W × 5.0 m H and were partitioned using a galvanized wire net. The pens were covered with dried sunflower husks as bedding. For the first two weeks, infrared electric lights were used to maintain the temperature at 34°C and subsequently reduced by 2°C every other week. The poultry house was cleaned two weeks before the birds arrived by washing all equipment with biogel (detergent) while a disinfectant Verocid was used to disinfect the ceiling, walls, and floor. Finally, a formalin-and-salt solution was applied to the floors, wall junctions, and base posts to ensure that the house was safe from infectious pathogens. For biosecurity, disinfectant was regularly added to the foot dip. The birds were given free access to fresh water and were fed daily.

3.2.4 Chemical analysis of *Moringa oleifera* leaf extract treatments

The MLE treatments (MLE_{60}, MLE_{90}, and MLE_{120}) were analysed for alkaloids, carbohydrate, flavonoids, phenols, protein, saponins, steroids, tannins, and terpenoids using the standard methods by Ijarotimi et al. (2013) and Nathaniel et al (2020), as shown in Table 3.2. Alkaloids were quantified by adding 1 mL of the sample (W) to 20 mL of 10% acetic acid in ethanol, shaking, allowing it to stand for 4 hours, and filtering. The filtrate was allowed to evaporate to about a quarter of its original volume and one drop of concentrated ammonia was added. The precipitate formed was filtered through a weighed filter paper (W1). The filter paper was dried in the oven at 60°C and weighed until it has attained a constant weight (W2).

$$\text{Alkaloid} = \frac{W2 - W1}{W}$$

The flavonoids were determined by adding a volume of 0.5 mL of 2% $AlCl_3$ methanol solution to a 0.05 mL sample solution. After 1 hour at room temperature, the absorbance was measured at A_{420}. The flavonoid content was determined with the aid of a calibration graph. The determination of tannins was conducted by transferring 1 ml of the sample to 25 ml of the solvent mixture of 80:20 acetone to 10% glacial acetic acid for 5 hours. The supernatant was filtered and the absorbance of the filtrate as well as the reagent blank was measured at 500 nm absorbance. A standard graph was produced with 10, 20, 30, 40, and 50 mg/100g of tannic acid. The concentration of tannin was read off taking into consideration dilution factors. For the determination of proteins, in a 1.5 mL microcentrifuge tube, 900 µL of water was added to 100 µL of each sample. In clean 13×100 mm test tubes, each duplicate of 5, 20, or 50 µL portions of each sample was combined with water to yield a total sample volume of 100 µL. 5.0 mL of Bradford reagent was added to each tube, the solutions were inverted, and the tubes were allowed to sit at room temperature for 5 minutes. The absorbance at 595 nm of each tube is then measured using a blank or reference solution having no protein. The analysis was then performed on tubes having absorbance values within the range of the standard curve. A conversion factor calculated from the standard curve was used to convert the absorbance readings to a protein quantity. The protein concentration in the diluted extract was calculated by dividing the quantity by the volume examined. When many distinct volumes provide useable readings, they are averaged to yield a single value for the diluted solution, which is then multiplied by 10 to produce the protein concentration of the original extract.

For carbohydrate determination, in a 1.5 mL microcentrifuge tube, 900 µL of water was added to 100 µL of each sample. In clean 13×100 mm test tubes, each duplicate of 5, 20, or 50 µL portions of each sample was combined with water to yield a total sample volume of 500 µL. Each tube received 2.5 mL of cold anthrone reagent, and the solutions were mixed before being heated for 10 minutes at 95°C and cooled in a pan of water for 10 minutes. The absorbance of each tube at 620 nm is then measured using a carbohydrate-free blank or reference solution. For the analysis, only tubes with absorbance values within the range of the standard curve were considered. A conversion factor calculated from the standard curve was used to convert the absorbance readings to a specified amount of glucose "equivalents." The carbohydrate content in the diluted extract is then calculated by dividing this quantity by the volume examined. When many distinct volumes produce usable values, they are averaged to get a single value for the diluted solution, which is then multiplied by the dilution factor to produce the extract's carbohydrate content. The saponins were determined by adding 1 mL of sample to 5 mL of 20% ethanol in a conical flask and placing it in a water bath at 55°C for 4 hours. This was followed by filtering and washing the residue with 20% ethanol twice and reducing the extract to about 5 mL in the oven. The extract was further treated successively with petroleum ether, butanol, and 5% sodium chloride.

The steroids were determined by adding 5 g of the sample to 100 mL of water and adding drops of 0.1M ammonium hydroxide to bring the pH to 9.1. Then 2 mL petroleum ether, 3 mL acetic anhydride, and concentrated H_2SO_4 were added, and the absorbance was measured at 420 nm. For terpenoids, 1.5 ml of 95% (vol/vol) methanol was added to the reddish-brown precipitate and thoroughly vortexed until the precipitation was completely dissolved. Then the sample was transferred from the assay tube to a colorimetric cuvette [95% (Vol/Vol) methanol was used as a blank] to read the absorbance at 538 nm. A standard curve was calculated from the blank-corrected wavelength of 538 nm of the Linalool standard. The total terpenoid concentration of the sample was calculated as Linalool equivalents using the regression equation of the Linalool standard curve.

$$y = 0.012x + 0.011$$

Where: y = concentration of terpenoids in mg, x = absorbance at 538 nm

Table 3. 2. Phytochemical content (mg/L) of *Moringa oleifera* leaf extract treatments.

	[1]Treatments		
Phytochemicals	**MLE_{60}**	**MLE_{90}**	**MLE_{120}**
Alkaloids	3.22	3.33	3.80
Carbohydrates	35.67	37.68	39.50
Flavonoids	3.28	3.62	3.76
Phenols	3.56	3.80	4.30
Protein	18.58	21.00	22.40
Saponins	1.26	1.36	1.52
Steroids	3.16	3.36	3.52
Tannins	8.72	9.10	9.54
Terpenoids	4.00	4.46	4.80

[1]Treatments: MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water.

The calcium, magnesium, phosphorus, potassium, zinc, iron, and sodium content of MLE treatments (MLE_{60}, MLE_{90}, and MLE_{120}) were determined by the samples being treated with 2 mL of 5 N hydrochloric acid before being transferred to 100 mL volumetric flasks and topped up to the mark with distilled water. Before analysis, the mixture was thoroughly mixed with a vortex. Then, a solution of 2% nitric acid was used to dilute the samples before being analysed through an Inductively Coupled Plasma Mass Spectrometry (Perkin Elmer Nexion 300Q, Johannesburg, South Africa).

The MLE treatments (MLE_{60}, MLE_{90}, and MLE_{120}) were analysed for vitamin B1, B2, B3, B6, B12, C, A, D3, E, K3, and β-carotene according to the methods of Sami et al. (2014) (Table 3.3). They were analysed by adding 2 mL of sample to 25 mL of H_2SO_4 (0.1 N) solution and incubating for 30 min at 12°C. Then, the contents were cooled and maintained at a pH of 4.5 with 2.5M sodium acetate, and 50 mg of Takadiastase enzyme was added. The preparation was stored at 35°C overnight. The mixture was then filtered through a Whatman No-4 filter paper, and the filtrate was diluted with 50 mL of distilled water and filtered again through a micropore filter (0.45 μm). Then 20 μL of the filtrate was injected into the high-performance liquid chromatography (HPLC) system supplied by Chemetrix Export (Pty) Limited (Midrand, South Africa). The quantification of vitamin B complex content was achieved by comparison to vitamin B standards. Standard stock solutions for thiamine, riboflavin, niacin, pyridoxine, and cobalamin were prepared. Chromatographic separation was achieved on a reversed-phase (RP) HPLC column (Agilent ZORBAX Eclipse Plus C18; 250 × 4.6mm, 5 μm) through the isocratic delivery mobile phase (A/B 33/67; A: MeOH, B: 0.023M H3PO4, pH = 3.54) at a flow rate of

0.5 mL/min. Ultraviolet (UV) absorbance was recorded at 270 nm at room temperature. Vitamin C was extracted with 10 mL of the sample being homogenized with an extracting solution containing metaphosphoric acid (0.3 M) and acetic acid (1.4 M). The mixture was placed in a conical flask and agitated at 10,000 rpm for 15 minutes. The mixture was then filtered through a Whatman No-4 filter paper, and samples were extracted in triplicate. The ascorbic acid standard was prepared by dissolving 100 mg of l-ascorbic acid in a metaphosphoric acid (0.3M)/acetic acid (1.4 M) solution at a final concentration of 0.1 mg/mL. The calibration line was converted to a linear range based on four measured concentration levels. The quantification of ascorbic acid content was performed on an Agilent HPLC system (Chemetrix Export (Pty) Limited, Midrand, South Africa). Chromatographic separation was achieved on an RP-HPLC column through isocratic delivery of a mobile phase (A/B 33/67; A: 0.1M potassium acetate, pH = 4.9; B: acetonitrile: water [50:50]) at a flow rate of 1 mL/min. At room temperature, UV absorbance was recorded at 254 nm. Vitamin A, D3, E, K3, and β-carotene were determined when 10 mL of sample was added to 1 mL of pyrogallic acid, plus 70 mL of ethanol, and 30 mL of 50% potassium hydroxide were added. Then the mixture was stirred and refluxed for 40 min using a water bath at 50±2°C. Extracts were obtained three times using various ether concentrations (50 mL, 30 mL, and 20 mL). Double-distilled water was used to neutralize the extract, which was dehydrated using anhydrous sodium sulfate. The extract was concentrated to approximately 5 mL by using a water bath (50±2°C), diluted to 10 mL by using methanol, filtered using a 0.45 μm membrane, and finally subjected to HPLC analysis.

RP-HPLC analysis was performed with the Agilent 1100 series HPLC system, including a diode array detector. The column was made of stainless steel. For β- carotene quantification, the Agilent TC-C18 column was used (5 μm, 4.6 × 250 mm) with an acetonitrile-methyl alcohol ethyl acetate (88: 10: 2) solvent, and UV absorbance was recorded at 453 nm. For fat-soluble vitamins, the Agilent Eclipse XDB-C18 column was used (5 μm, 4. 6 × 150 mm), the solvent was methanol, and UV detection was recorded at 325 nm for vitamin A, 265 nm for vitamin D3, 290 nm for vitamin E, and 244 nm for vitamin K3. The separation of all vitamins was based on isocratic elution, and the solvent flow rate was maintained at 1 mL/min. 20 μL of sample oil was directly injected into the HPLC column. Fat-soluble vitamins were identified by comparing their retention times with those of authentic standards. All procedures were carried out under subdued light conditions. Standard solutions of vitamins were prepared by serial dilution to concentrations of 0.1, 1, 2, 5, and 10 mL/L of vitamins D3, E, K3, A, and β-

carotene, respectively. Standard solutions were prepared daily from a stock solution, which was stored in the dark at –20 °C. 20 μL of the standard solution was injected, and peak areas were determined to generate standard curves.

Table 3. 3. Mineral and vitamin composition (mg/L) of *Moringa oleifera* leaf extract treatments.

	[1]Treatments		
Micronutrients	**MLE_{60}**	**MLE_{90}**	**MLE_{120}**
Calcium	1264	1313	1362
Magnesium	280.2	294.6	309.4
Phosphorus	207.8	222.6	230.2
Potassium	1628	1668	1696
Zinc	2.52	2.70	2.88
Iron	17.40	17.61	17.79
Sodium	98.00	102.8	107.0
Vitamin A	15.04	15.25	15.32
Vitamin B1	1.93	1.98	2.02
Vitamin B2	16.97	17.12	17.22
Vitamin B3	7.10	7.39	7.56
Vitamin B6	5.21	5.38	5.59
Vitamin B12	2.03	2.07	2.13
Vitamin C	15.15	15.38	15.56
Vitamin E	49.40	50.20	51.40

[1]Treatments: MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water.

3.2.5 Growth performance

The initial weights (57.92 ± 0.08 g) of the chicks were taken on the day of arrival and subsequently weighed weekly using digital weighing scales (VidaXL® Frugo Cumbria, Ulverston, United Kingdom). The feed offered was weighed before feeding and refusals were collected before the next feeding and weighed. The difference was FI. Drinking water was measured before being presented to birds, and refusals were withdrawn and measured before the next drinking and the difference was the WI. The ADWG, ADFI, ADWI and feed conversion ratio (FCR) were calculated as:

$$ADWG\ (g/bird/day) = \frac{final\ weight - initial\ weight}{age\ (number\ of\ days)}$$

$$ADFI\ (g/bird/day) = \frac{feed\ offered - feed\ refusal}{number\ of\ birds\ x\ number\ of\ days}$$

$$ADWI\ (g/bird/day) = \frac{water\ offered - water\ refusal}{number\ of\ birds\ x\ number\ of\ days}$$

$$FCR = \frac{feed\ intake}{weight\ gain}$$

3.2.6 Apparent nutrient digestibility trial

At 34 days old, 5 birds per replicate pen were randomly selected into clean and disinfected metabolic cages (0.50 m L × 0.50 m W × 0.34 m H) for measurement of nutrient digestibility. The cages were fitted with feed troughs, water nipples, and perches for excreta collection. The birds were assigned the same treatments as in the growth trial. The birds were acclimatized for three days and thereafter measurements commenced for five days. The excreta obtained per day starting from the fourth day were carefully screened for spilled feed and feathers, then air-dried at room temperature, ground finely, and thereafter, used for the proximate determination for ash, crude protein, and ether extract according to the methods of the Association of Official Analytical Chemists (2005). Calcium and phosphorus were analysed following guidelines from the AgriLASA (1996). The acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined using the detergent methods described by Van Soest et al. (1991). The apparent ash, CP, EE, ADF, NDF, calcium, and phosphorus digestibility values were then calculated as:

$$Apparent\ nutrient\ digestibility = \frac{nutrient\ intake - excreta\ nutrient}{nutrient\ intake} \times 100$$

3.2.7 Proximate analysis

Prior to chemical analysis, samples of starter diet, finisher diet, excreta, and meat were dried in a forced-air oven at 55°C for 72 hours, followed by fine grinding through Kinematica Polymix PX-MFC 90D supplied by Labex Pty. Ltd. (Johannesburg, South Africa). The moisture content was determined by weighing the sample in a crucible and drying it in an oven at 105°C until a constant weight was obtained (AOAC, 2005; method no. 930.15). Ash content was determined by ashing at 550°C for about 6 hours (AOAC, 2005: method no. 924.05). Nitrogen was determined using the Kjeldahl method (AOAC, 2005: method no. 984.13). Crude protein was calculated by multiplying the percentage of nitrogen by 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by refluxing 0.45 g of samples with neutral detergent and acid detergent solutions, respectively, for 1 hour using the ANKOM^{2000} fibre analyser (ANKOM Technology, NY, USA).

The ashed samples were then allowed to cool in desiccators for 6 hours and then weighed to obtain the ash weight. Then 2 mL of 5 N hydrochloric acid was added to the samples and allowed to dissolve, then transferred to 100 mL volumetric flasks and topped up to the mark with distilled water. After the sediments were settled, the fluid was filtered into 14mL centrifuge tubes, and the fluid was further vortexed before analysis. A solution of 2% nitric acid was used to dilute the samples which were analysed through an Inductively Coupled Plasma Mass Spectrometry (Perkin Elmer Nexion 300Q, Johannesburg, South Africa).

3.2.8 Blood collection and analysis

On day 42 of age, blood samples were randomly collected from five birds from each replicate. The branchial veins of the birds were punctured with a set of sterilized needles and 5 mL syringes to draw out 4 mL of blood into two sets of sterilized tubes for haematological and serum biochemical analysis. The collected blood samples for haematology were placed in a cooler box with ice and determined within 48 hours after collection (Mulaudzi et al., 2019). Samples for serum biochemical analyses were centrifuged at room temperature for 10 min at 3000rpm to obtain serum.

The haematological parameters (basophils, eosinophils, haematocrits, haemoglobin, lymphocytes, mean corpuscular haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), mean corpuscular volume (MCV), monocytes, neutrophils, platelets, reticulocytes, and red cell distribution width (RDW)) were determined using an automated IDEXX LaserCyte Hematology Analyzer (Johannesburg, South Africa). The serum biochemical indices (albumin/globulin (ALB/GLOB), alkaline phosphatase (ALKP), alanine transaminase (ALT), albumin, amylase, cholesterol, calcium, creatine, gamma-glutamyl transferase (GGT), globulin, glucose, lipase, phosphorus, symmetric dimethylarginine (SDMA), total bilirubin, total protein, and urea) were analyzed using the automated IDEXX Vet Test Chemistry Analyzer (Johannesburg, South Africa).

3.2.9 Carcass yield and internal organs weight

On day 42 of age, after the feed was withheld for 12 hours all the birds were weighed to determine the slaughter weight (SLWT) then moved to the abattoir where they were sacrificed by cutting the jugular vein following stunning. All the carcasses were immediately weighed to determine hot carcass weight (HCW) and then reweighed after chilling in a cold room for 24 hours to determine cold carcass weight (CCW), dressing percentage was ascertained as the proportion of HCW on SLWT. Weights of all carcass parts (breast, drumstick, thigh, wing) and internal organs (cleaned gizzard, liver, heart, bursa, spleen, and thymus) were weighed with a digital scale (ADAM scale, readability 0.001 g to 0.01 g, Adam Equipment S.A. PTY, Johannesburg, South Africa).

3.2.10 Meat quality

For the meat quality parameters, all the carcasses were used. Breast pH was recorded at 45 minutes and 24 hours post-mortem with the aid of Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA, USA), and after each experimental unit was calibrated using standard pH solutions provided by the supplier. According to the methods of Association of Official Analytical Chemists (2005), proximate analysis of the breast samples was determined 24 hours post-mortem. The colour coordinates (L^* = lightness, a^* = redness, and b^* = yellowness) were determined 24 hours post-mortem in triplicate on the surface of the breast muscle with the aid of a colour spectrophotometer (CM 2500c, Konika Minolta, Osaka, Japan) which was set and calibrated following the manufacturer's prescription according to the Commission Internationale de I'Eclairage (1976) The breast meat water holding capacity was determined according to the method of Whiting and Jenkins (1981). Two Whatman No- 1 filter papers were weighed and designated as (A). (C) was designated as 500 mg of breast meat. The breast meat sample (C) was placed in between the two filter papers (A), then on a rigid flat surface of the glass plate. A weight of 2.81kg was applied to it as pressure for 5 minutes. The weight was removed, then the breast sample was weighed and designated as (D), while the filter papers were dried and weighed as (B). The following were observed:

Where: *A* = weight of two Whatman filter papers before press; *B* = weight of filter papers after drying; *C* = weight of the meat sample 500mg before press; *D* = weight of the breast meat sample after press; *E* = amount of protein attached to the filter paper (B – A); *F* = actual weight of meat sample after pressure treatment (E + D).

$$WHC = \frac{C - F}{2} \times 100$$

The breast meat drip loss was determined according to the method by Shen et al. (2019), whereby the breast muscles weighing 2 g were hooked with wire steel in a bottle properly sealed in such a way that the samples did not have contact with the sides of the bottle. The hooked samples were then stored in a cold room at 4°C for 72 hours, and then reweighed to obtain their weight after drip. The difference between the weight of meat samples before and after the drip was used to determine the drip loss percentage using the following formula:

$$Drip\ loss = \frac{W1 - W2}{W1} \times 100$$

Where: *W1* = initial weight; *W2* = weight after drip.

The breast meat samples were stored overnight at 4°C and then weighed to obtain the initial weight of the meat samples after thawing. The samples were then placed on a foil plate and cooked in the oven for 30 minutes at 180°C. The cooked meat samples were removed from the oven and left to cool at room temperature for 20 minutes. The cooled samples were then weighed to obtain the weight after cooking. The difference between the raw and cooked meat samples was then used to determine the cooking loss using the following equation:

$$Cooking\ loss = \frac{W1 - W2}{W1} \times 100$$

Where: *W1* = initial weight; *W2* = weight after cooking.

Thereafter, the cooked breast meat samples were sheared using a Meullenet-Owens Razor Shear Blade (A/MORS) mounted on a Texture Analyzer (TA.XT plus, Stable Micro Systems, Surrey, UK) to determine shear force (N), which is a measure of meat tenderness.

3.2.11 Intestinal morphology

Five birds per replicate were selected from the slaughtered birds. Then, the duodenum was separated from the pylorus to the distal section of the duodenal loop, the jejunum was separated from the distal portion of the duodenal loop to Meckel's diverticulum, and the ileum was separated from Meckel's diverticulum to the anterior portion of the ileal caecal junction and fixed in a mixture of 10% buffered formalin solution, processed with the paraffin embedding technique, and stained with haematoxylin and eosin (H&E) staining technique (Biasato et al., 2018). The lengths of the duodenum, jejunum, and ileum were measured using a tape measure (cm). For the three segments of the small intestine, five villus crypt units with intact lamina propria were selected and placed on slides. The villus height, villus width, crypt depth, villus height:crypt depth ratio, the thickness of lamina propria, muscularis mucosa, and muscularis externa were measured with a light microscope (Olympus CX31, Olympus, Hicksville, New York, USA) at a 4× magnification, supported with digitalized live image analysis program (Olympus DP20, Olympus, USA). The crypt depth was measured from the base of the crypt up to the zone of transition be-tween the crypt and the villus. The villus height was measured from the tip of the villus to the villus crypt junction as described by Sikandar et al. (2017).

Earlier prepared and processed slides were submitted to alcian blue periodic acid Shiff (AB-PAS) staining. Three sections were obtained from each intestinal segment and goblet cells were counted as described by Pawlina and Ross, (2018) in 5 villi per section. Thus, an average of 15 values was measured for each sample. The histochemical differentiation on the basis of acidic and mixed (acidic and neutral) mucin was observed according to the methods described elsewhere by Ashraf et al. (2013). Goblet cells containing acidic mucin were stained blue by the AB, while mixed mucin was stained purple by periodic acid Shiff staining.

3.2.12 Bone parameters

The left and right tibia of nine carcasses from each replicate were removed as drumsticks, thereafter de-fleshed and dried at room temperature for 24 hours. The tibia weight was determined using a weighing scale (ADAM scale, readability 0.001 g to 0.01 g, Adam Equipment S.A. PTY, Johannesburg, South Africa), while the tibia length (from its proximal to distal end), diaphysis diameter (midpoint of the tibia), femoral and metatarsal side proximal head thickness were measured using an RS PRO digital Vernier caliper (©RS Components, Midrand, South Africa). The bone breaking strength was measured with a force gauge using a Texture Analyzer (TA.XT plus, Stable Micro Systems, Surrey, UK). The MAC AFRIC dial gauge vernier caliper (Adendorff Machinery Mart, Johannesburg, South Africa) was used to measure at midpoint the thickness of the medial and lateral walls. The medullary canal diameter was calculated by subtracting the thicknesses of the medial and lateral walls from the diaphysis diameter (midpoint). The tibia weight/tibia length index was obtained by dividing the tibia weight by its length (Muszyński et al., 2018). The tibiotarsal and robusticity indexes were calculated using the following formulas, respectively:

$$Tibiotarsal\ index = \frac{diaphysis\ diameter - medullary\ canal\ diameter}{diaphysis\ diameter} \times 100$$

$$Robusticity\ index = \frac{tibia\ length}{cube\ root\ of\ bone\ weight}$$

The tibia samples were collected in triplicates into crucibles and weighed to obtain their fresh weight. The samples were dried in an oven at 106°C for 16 hours to obtain dry weight (AOAC, 2005). The samples were incinerated in a muffle furnace for 16 hours at 800°C to obtain ash weight. Then, 2 mL of 5 N hydrochloric acid was added to the ash samples and allowed to dissolve, before being transferred to 100 mL volumetric flasks and topped up to the mark with distilled water. After the sediments settled, the fluid was filtered into 14 mL centrifuge tubes and then mixed with a vortex before analysis. For analysis, a solution of 2% nitric acid was used to dilute the samples before being analysed through an inductively coupled plasma mass spectrometry (Perkin Elmer Nexion 300Q, Johannesburg, South Africa).

3.3 Data Analysis

The growth performance, nutrient digestibility, carcass yield, internal organs weight, meat quality, intestinal morphology, goblet cell count, tibia morphology, and tibia mineral content data were analyzed using a one-way analysis of variance by means of general linear model procedure of SAS (2010), where treatment was the main factor. The model of the statistics is shown below:

$$Yij = \mu + Ti + \mathcal{E}ij$$

Where:

Yij = dependent variable,

μ = population mean,

Ti = effect of the treatments,

$\mathcal{E}ij$ = random error associated with observation ij.

For all the measured parameters, significance was considered at $p < 0.05$ and the least-squares means were separated using the probability of difference options in SAS.

3.4 Results

3.4.1 Growth performance

The results of the starter phase performance of broilers orally administered with varying levels of MLE is presented in Table 3.4. The water intake and ADWI were not significantly ($p > 0.05$) influenced by the oral administration of MLE. Observations showed that the MLE supplemented groups had the highest mean values for final weight, weight gain, and ADWG while those on the NC and PC had the least values. Birds administered MLE_{120} consumed more ($p < 0.05$) at the end of the starter phase as well as on daily basis, but they were statistically similar ($p > 0.05$) to those offered MLE_{90}, MLE_{60}, and PC, while NC group had the least ($p < 0.05$) values. The results showed that the FCR was significantly ($p < 0.05$) enhanced in birds offered MLE when compared to the PC and NC groups.

Table 3. 4. Starter phase performance in Cobb 500 broilers orally administered Moringa leaf extract (MLE) through drinking water.

[2]Parameters	[1]Treatment					[3]SEM	*p* value
	NC	PC	MLE_{60}	MLE_{90}	MLE_{120}		
Initial weight (g/bird)	57.95	58.00	57.85	57.90	57.95	0.070	0.458
Final weight (g/bird)	835.0^{c}	896.8^{b}	979.8^{a}	984.8^{a}	997.6^{a}	13.48	0.005
Weight gain (g/bird)	777.0^{c}	838.8^{b}	922.0^{a}	926.9^{a}	939.6^{a}	13.48	0.007
ADWG (g/bird)	37.00^{c}	39.94^{b}	43.90^{a}	44.14^{a}	44.74^{a}	0.640	0.004
Feed intake (g/bird)	1475^{b}	1519^{ab}	1519^{ab}	1538^{ab}	1583^{a}	27.60	0.011
ADFI (g/bird)	70.25^{b}	72.31^{ab}	72.33^{ab}	73.25^{ab}	75.40^{a}	1.320	0.005
Water intake (mL/bird)	2388	2344	2450	2481	2500	50.30	0.624
ADWI (mL/bird)	111.6	113.7	116.7	118.2	119.0	2.400	0.684
FCR	1.90^{a}	1.81^{b}	1.65^{c}	1.66^{c}	1.69^{c}	0.020	0.001

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water, [2]Parameters: ADWG = average daily weight gain; ADFI = average daily feed intake; ADWI = average daily water intake; FCR = feed conversion ratio. [3]SEM: standard error of mean.

During the finisher phase (Table 3.5), weight gain, ADWG, water intake, and ADWI were not influenced ($p > 0.05$) by the oral administration of MLE. It was observed that the final weights were higher ($p < 0.05$) by MLE administration compared to those that received NC and PC. The study showed that birds administered MLE consumed less ($p < 0.05$) with enhanced FCR compared to those on NC and PC groups.

Table 3. 5. Finisher phase performance in Cobb 500 broilers orally administered Moringa leaf extract (MLE) through drinking water.

[2]Parameters	[1]Treatment					[3]SEM	*p* value
	NC	PC	MLE_{60}	MLE_{90}	MLE_{120}		
Final weight (g/bird)	1897^{c}	1941^{b}	2044^{a}	2059^{a}	2028^{a}	13.86	0.012
Weight gain (g/bird)	1062	1044	1064	1074	1031	14.51	0.348
ADWG (g/bird)	50.58	49.73	50.66	51.15	49.08	0.690	0.418
Feed intake (g/bird)	2026^{a}	1934^{a}	1798^{b}	1787^{b}	1775^{b}	32.00	0.008
ADFI (g/bird)	96.47^{a}	92.10^{a}	85.63^{b}	85.08^{b}	84.50^{b}	1.520	0.009
Water intake (mL/bird)	3304	3333	3210	3165	3147	141.1	0.168
ADWI (mL/bird)	157.3	158.7	152.9	150.7	149.9	6.720	0.244
FCR	1.91^{a}	1.85^{a}	1.69^{b}	1.67^{b}	1.73^{b}	0.040	0.021

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water. [2]Parameters: ADWG = average daily weight gain; ADFI = average daily feed intake; ADWI = average daily water intake; FCR = feed conversion ratio. [3]SEM: standard error of mean.

Results presented in Table 3.6 show that the oral administration of MLE did not influence (p > 0.05) the overall WI and ADWI of the birds. It was observed that birds in the MLE supplemented groups had the highest ($p < 0.05$) FBW, BWG, and ADWG compared to those in the NC and PC groups. The results also indicated that the oral administration of MLE reduced the overall FI, ADFI, and enhanced FCR when compared to control groups (NC and PC).

Table 3. 6. Overall growth performance in Cobb 500 broilers orally administered Moringa leaf extract (MLE) through drinking water.

	[1]Treatment						
[2]Parameters	NC	PC	MLE_{60}	MLE_{90}	MLE_{120}	[3]SEM	*p* value
Initial BW (g/bird)	57.95	58.00	57.85	57.90	57.95	0.070	0.458
FBW (g/bird)	1897^{c}	1941^{b}	2044^{a}	2059^{a}	2028^{a}	13.86	0.012
BWG (g/bird)	1839^{c}	1883^{b}	1986^{a}	2001^{a}	1970^{a}	13.86	0.012
ADWG (g/bird)	43.79^{c}	44.84^{b}	47.28^{a}	47.65^{a}	46.91^{a}	0.330	0.005
FI (g/bird)	3501^{a}	3453^{a}	3317^{b}	3325^{b}	3358^{b}	30.20	0.008
ADFI (g/bird)	83.36^{a}	82.21^{a}	78.98^{b}	79.16^{b}	79.95^{b}	0.720	0.009
WI (mL/bird)	5691	5677	5660	5647	5647	128.2	0.841
ADWI (mL/bird)	135.5	135.2	134.8	134.4	134.5	3.050	0.478
FCR	1.90^{a}	1.83^{b}	1.67^{c}	1.66^{c}	1.71^{c}	0.020	0.001

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water. [2]Parameters: ADWG = average daily weight gain; ADFI = average daily feed intake; ADWI = average daily water intake; FCR = feed conversion ratio, [3]SEM: standard error of mean.

3.4.2 Nutrient digestibility

The results presented in Table 3.7 indicated that dry matter digestibility was highest ($p < 0.05$) in birds supplemented with MLE, while those that were offered NC and PC were comparatively lower. Crude protein digestibility was significantly higher ($p < 0.05$) in the MLE groups when compared with those on NC and PC. Ether extract digestibility was lowest ($p < 0.05$) in birds offered NC and PC compared to those that received MLE treatments. The enhanced digestibility of NDF and ADF was observed in the MLE supplemented groups. Birds offered the MLE_{90} and MLE_{120} had the highest ($p < 0.05$) calcium and phosphorus digestibility when compared to those in PC, NC, and MLE_{60} groups.

Table 3. 7. Nutrient digestibility (%) in Cobb 500 broilers (n = 125) orally administered Moringa leaf extract (MLE) through drinking water.

[2]Parameters	[1]Treatments					[3]SEM	*p* value
	NC	PC	MLE_{60}	MLE_{90}	MLE_{120}		
Dry matter	63.72[b]	63.85[b]	65.29[a]	66.04[a]	66.20[a]	0.310	0.013
Crude protein	65.91[b]	66.22[b]	67.51[ab]	70.29[a]	68.86[ab]	0.920	0.010
Ether extract	61.00[b]	61.92[b]	63.94[a]	64.37[a]	65.33[a]	0.540	0.005
NDF	61.89[b]	62.16[b]	63.65[a]	64.28[a]	64.42[a]	0.420	0.008
ADF	62.99[b]	62.89[b]	64.94[a]	64.69[a]	64.53[a]	0.390	0.021
Calcium	58.98[c]	55.84[d]	63.51[b]	64.88[a]	64.61[ab]	0.410	0.005
Phosphorus	58.07[c]	54.90[d]	62.61[b]	64.26[a]	63.85[a]	0.360	0.005

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water. [2]Parameters: NDF = neutral detergent fibre; ADF = acid detergent fibre. [3]SEM: standard error of mean.

3.4.3 Blood parameters

The results in Table 3.8 showed that the oral administration of MLE had no influence ($p > 0.05$) on the basophils, eosinophils, lymphocytes, MCHC, monocytes, neutrophils, platelets, and RDW concentrations of the broiler chickens. Birds administered MLE and NC had comparable higher ($p < 0.05$) haematocrits than those offered PC. Haemoglobin concentrations were increased in those administered the MLE, while those on the PC had the lowest ($p < 0.05$) value of 8g/dL. Birds on NC presented the highest MCH and MCV concentrations, which were comparable to the values obtained for MLE_{60}, MLE_{90}, and MLE_{120}. Birds orally administered MLE had the highest ($p < 0.05$) reticulocytes when compared to those on NC and PC.

Table 3. 8. Haematological parameters of Cobb 500 broilers (n = 225) orally administered Moringa leaf extract (MLE) through drinking water.

[2]Parameters	[1]Treatment					[3]SEM	*p* value	[4]Reference range
	NC	PC	MLE_{60}	MLE_{90}	MLE_{120}			
Basophils (10^9/L)	0.14	0.15	0.15	0.17	0.15	0.020	0.214	0.10 - 2 × 10^9/L
Eosinophils (10^9/L)	0.35	0.44	0.31	0.40	0.30	0.060	0.521	0.20 - 4 × 10^9/L
Haematocrits (%)	27.10^{ab}	22.90^{b}	31.62^{a}	31.54^{a}	32.62^{a}	1.850	0.005	28 - 48 %
Haemoglobin (g/dL)	9.76^{bc}	8.00^{c}	11.40^{ab}	10.92^{ab}	11.80^{a}	0.620	0.006	7 - 13 g/dL
Lymphocytes (10^9/L)	67.23	60.60	63.75	59.30	59.12	5.960	0.471	50 – 300 × 10^9/L
MCH (pg)	42.38^{a}	32.64^{b}	38.90^{ab}	35.17^{ab}	40.52^{ab}	2.510	0.011	30 – 80 pg
MCHC (%)	36.10	34.80	36.40	34.60	36.20	0.090	0.235	32 – 42 %
MCV (fL)	117.8^{a}	93.50^{b}	106.6^{ab}	101.8^{ab}	112.0^{ab}	6.640	0.034	80 – 120 fL
Monocytes (10^9/L)	9.24	8.83	8.64	8.64	9.42	1.650	0.348	1 – 30 × 10^9/L
Neutrophils (10^9/L)	2.99	2.70	2.86	2.71	2.93	0.090	0.614	1 – 4 × 10^9/L
Platelets (K/µL)	1837	2030	2049	1898	1824	83.60	0.084	100 – 2400 K/µL
Reticulocytes (10^{12}/L)	2.33^{b}	2.45^{b}	2.99^{a}	3.10^{a}	2.91^{a}	0.130	0.023	1.5 – 5 × 10^{12}/L
RDW (%)	24.08	26.18	27.16	26.50	26.72	1.480	0.078	20 – 40%

^{a,b,c} Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water. [2]Parameters: MCH = mean corpuscular haemoglobin; MCHC = mean cell haemoglobin concentration; MCV = mean corpuscular volume; RDW = red cell distribution width. [3]SEM: standard error of mean. [4]Reference range: Talebi et al 2005; Oyeagu et al. (2019); Iyaode et al. (2020); Matshogo et al. (2020); Matshogo et al. (2021).

The data presented in Table 3.9 disclosed that all the serum biochemical constituents considered were not influenced ($p > 0.05$) by different dosages of MLE, except for calcium, which was impacted ($p < 0.05$) by the varying dosages of MLE supplementation. The lowest ($p < 0.05$) mean values for calcium concentration in the blood were observed when the NC and PC were given, while the MLE administered groups presented the highest comparable means.

Table 3. 9. Serum biochemical parameters of Cobb 500 broilers (n = 225) orally administered Moringa leaf extract (MLE) through drinking water.

[2]Parameters	[1]Treatment					[3]SEM	*p* value	[4]Reference range
	NC	PC	MLE_{60}	MLE_{90}	MLE_{120}			
ALB/GLOB	0.65	0.64	0.65	0.65	0.64	0.020	0.355	0.40 - 0.70
ALKP (U/L)	191.2	166.6	148.0	110.6	172.8	30.30	0.921	100 – 900 U/L
ALT (U/L)	11.60	10.00	12.00	13.20	10.80	1.140	0.062	5 – 20 U/L
Albumin (g/L)	20.08	21.48	21.96	21.95	20.37	0.990	0.058	10 – 30 g/L
Amylase (U/L)	488.6	460.4	489.0	486.4	478.0	13.06	0.084	300 – 700 U/L
Cholesterol (mmol/L)	3.26	3.12	3.02	3.05	2.85	0.190	0.534	1.68 - 5.81 mmol/L
Calcium (mmol/L)	1.14^{b}	1.31^{b}	2.39^{a}	2.69^{a}	2.60^{a}	0.130	0.005	1.95 - 2.83 mmol/L
Creatine (µmol/L)	10.67	10.97	10.44	10.69	10.47	0.200	0.258	5 – 40 µmol/L
GGT (U/L)	11.60	12.40	12.80	12.00	12.00	1.210	0.369	5 – 30 U/L
Globulin (g/L)	31.16	33.95	33.91	33.45	31.89	1.080	0.741	28 – 51 g/L
Glucose (mmol/L)	5.170	6.80	5.81	3.93	6.76	1.110	0.321	4.11 - 8.84 mmol/L
Lipase (U/L)	124.0	117.0	106.0	122.0	115.4	6.240	0.789	100 -1000 U/L
Phosphorus (mmol/L)	2.86	2.81	2.69	2.80	2.62	0.130	0.654	1 – 3 mmol/L
SDMA (µg/L)	8.00	6.80	9.00	9.00	7.60	1.480	0.452	0 – 14 µg/L
Total bilirubin (µmol/L)	4.40	2.40	3.00	3.80	3.00	0.960	0.246	0 – 15 µmol/L
Total protein (g/L)	51.24	55.43	55.87	55.40	52.26	1.940	0.357	45 – 89 g/L
Urea (mmol/L)	0.70	0.71	0.70	0.71	0.68	0.010	0.159	0 - 5.70 mmol/L

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water. [2]Parameters: ALB/GLOB = albumin/globulin ratio; ALKP = alkaline phosphatase; ALT = alanine transaminase; GGT = gamma-glutamyl transferase; SDMA = symmetric dimethylarginine. [3]SEM: standard error of mean. [4]Reference range: Chikumba et al. (2013); Matshogo et al. (2020); Matshogo et al. (2021).

3.4.4 Carcass yield and internal organs weight

The mean values for breast weight, drumstick weight, thigh, gizzard, heart, and liver weight were similar ($p > 0.05$) across the treatment groups. However, SLWT, dressing percentage, HCW, and CCW were statistically ($p < 0.05$) influenced (Table 3.10). Birds on the MLE_{60}, MLE_{90}, and MLE_{120} had the highest ($p < 0.05$) SLWT value, while those offered the NC had the lowest ($p < 0.05$) value. The dressing percentage was increased in the MLE supplemented groups, while those on the NC and PC had the least. Higher ($p < 0.05$) HCW and CCW were recorded for the birds offered MLE supplementation groups compared to NC and PC groups. It was observed from the presented results that the administration of MLE increased ($p < 0.05$) the weight for bursa compared to those offered NC and PC. The weight of the spleen and thymus was heavier in the MLE_{90} and MLE_{120} supplemented groups than those offered NC and PC groups.

Table 3. 10. Carcass yield and internal organs weight (g, unless stated otherwise) of Cobb 500 broilers (n = 225) orally administered Moringa leaf extract (MLE) through drinking water.

[2]Parameters	[1]Treatment					[3]SEM	*p* value
	NC	PC	MLE_{60}	MLE_{90}	MLE_{120}		
SLWT	1897^{c}	1941^{b}	2044^{a}	2059^{a}	2028^{a}	13.86	0.012
Dressing percentage (%)	69.17^{b}	69.33^{b}	71.12^{a}	71.62^{a}	71.44^{a}	0.190	0.025
HCW	1312^{c}	1346^{b}	1454^{a}	1475^{a}	1449^{a}	11.21	0.014
CCW	1289^{b}	1315^{b}	1426^{a}	1446^{a}	1420^{a}	11.67	0.031
Breast weight	627.0	710.2	671.5	644.6	590.0	41.20	0.741
Drumstick weight	128.9	132.9	128.1	136.9	125.9	6.930	0.124
Thigh weight	162.0	167.5	166.2	164.1	156.4	7.500	0.326
Gizzard	44.00	46.60	44.50	44.90	43.30	3.390	0.546
Heart	12.00	12.40	12.80	12.20	12.40	0.720	0.759
Liver	46.90	53.70	50.90	52.50	53.60	2.680	0.079
Bursa	4.41^{b}	4.28^{b}	4.69^{a}	4.80^{a}	4.78^{a}	0.070	0.014
Spleen	3.13^{c}	3.04^{c}	3.43^{b}	3.60^{a}	3.54^{ab}	0.050	0.032
Thymus	9.74^{bc}	9.54^{c}	9.91^{b}	10.58^{a}	10.58^{a}	0.110	0.006

^{a,b,c} Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water. [2]Parameters: SLWT: slaughter weight; HCW: hot carcass weight, CCW: cold carcass weight. [3]SEM: standard error of mean.

3.4.5 Meat quality

Most of the parameters considered for meat quality were significantly ($p < 0.05$) influenced by the supplementation levels of MLE, with parameters such as pH_{45min}, redness (a*), and yellowness (b*) being similar (p > 0.05) across the treatments (Table 3.11). The meat pH_{24hr} (6.89) for the NC and PC groups was comparable to those administered MLE_{60} (6.79) and MLE_{90} (6.81). Lightness (L*) was highest ($p < 0.05$) for MLE administered groups, while those offered PC had the lowest ($p < 0.05$) value. On the other hand, WHC was the lowest ($p < 0.05$) for breast meat of birds administered MLE_{60} compared to other groups. Meat samples from MLE_{60} had the lowest ($p < 0.05$) mean drip loss as compared with other (NC, MLE_{90}, and MLE_{120}) groups. The meat from the MLE_{90} had the lowest ($p < 0.05$) cooking loss of 25.88%, while birds offered NC (32.18%), MLE_{60} (33.54), and MLE_{120} (32.35) had comparable higher ($p < 0.05$) mean values. The NC and PC groups had the highest ($p < 0.05$) shear force when compared to the MLE administered groups. The meat samples from NC and PC had the highest ($p < 0.05$) moisture content, while those from the MLE administered group had the lowest ($p < 0.05$) mean value. Crude protein was increased ($p < 0.05$) for breast meat from the MLE supplemented groups, while NC and PC had the lowest ($p < 0.05$) crude protein value. Also, crude fat was significantly ($p < 0.05$) reduced in the breast meat samples from the MLE supplemented groups compared to those in the NC and PC groups. The ash mean value was increased ($p < 0.05$) in meat samples from the MLE supplemented and NC groups when compared to the PC.

Table 3. 11. Meat quality parameters of Cobb 500 broilers (n = 225) orally administered with varying levels of Moringa leaf extract (MLE) in drinking water.

	[1]Treatments						
[2]Parameters	NC	PC	MLE_{60}	MLE_{90}	MLE_{120}	[3]SEM	*p* value
pH_{45min}	6.70	6.70	7.01	7.00	7.00	0.010	0.274
pH_{24hr}	6.89^{a}	6.89^{a}	6.79^{ab}	6.81^{ab}	6.74^{b}	0.050	0.005
L^* (lightness)	49.36^{bc}	48.65^{c}	51.70^{ab}	52.29^{a}	51.30^{abc}	0.870	0.008
a^* (redness)	-1.15	-1.28	-0.85	-0.85	-0.88	0.230	0.629
b^* (yellowness)	11.75	13.18	13.53	13.61	13.73	0.620	0.415
WHC (%)	16.40^{a}	12.90^{ab}	12.00^{b}	16.00^{ab}	15.60^{ab}	1.260	0.034
Drip loss (%)	4.79^{ab}	5.82^{a}	3.75^{b}	4.54^{ab}	4.98^{ab}	0.690	0.042
Cooking loss (%)	32.18^{ab}	35.23^{a}	33.54^{ab}	25.88^{b}	32.35^{ab}	2.750	0.017
Shear force (N)	3.81^{a}	4.13^{a}	2.54^{b}	2.84^{b}	2.73^{b}	0.150	0.026
Moisture (%)	74.72^{a}	75.31^{a}	72.61^{b}	72.71^{b}	72.36^{b}	0.260	0.021
Crude protein (%)	21.06^{b}	20.59^{b}	23.72^{a}	23.53^{a}	23.95^{a}	0.310	0.007
Crude fat (%)	1.01^{b}	1.37^{a}	0.65^{c}	0.60^{c}	0.58^{c}	0.080	0.035
Ash (%)	0.82^{ab}	0.67^{b}	0.78^{ab}	0.94^{a}	0.91^{a}	0.060	0.018

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water. [2]Parameters: pH_{45min} = pH at 45mins post-mortem; pH_{24hr} = pH at 24hr post-mortem; WHC = water holding capacity. [3]SEM: standard error of mean.

3.4.6 Intestinal morphology

The varying levels of oral administration of MLE had a significant ($p < 0.05$) influence on the morphometric parameters (Table 3.12) of the gut sections. It was observed that MLE groups had longer ($p < 0.05$) duodenum, jejunum, and ileum lengths, while those from NC and PC groups were shorter ($p < 0.05$). At the duodenum, villus height was higher ($p < 0.05$) for birds administered MLE than those from NC and PC groups. Villus samples from MLE_{90} had the highest ($p < 0.05$) villus width when compared to other treatments. Muscularis mucosa thickness was lowest ($p < 0.05$) for villus samples from PC when compared to the other groups. Villus samples from the NC and PC groups had the lowest ($p < 0.05$) comparable values for villus height/crypt depth ratio when compared to MLE_{90} and MLE_{120} supplemented groups. At the jejunum, villus samples from MLE_{90} had the highest ($p < 0.05$) villus height, width, and crypt depth when compared to other treatments. Villus samples from the PC and MLE supplemented groups had the highest ($p < 0.05$) comparable values for villus height/crypt depth ratio compared to those from the NC group. At the ileum section, villus samples from the NC and PC groups are similar and they had a lower ($p < 0.05$) villus heights when compared with the MLE supplemented groups. The villus width value from the MLE_{90} group was the highest ($p < 0.05$) when compared to other treatments. The crypt depth value for villus samples from the NC group was the lowest ($p < 0.05$), while those from the MLE supplemented groups were the highest ($p < 0.05$).

Table 3. 12. Intestinal morphometric parameters of Cobb 500 broilers (n = 125) orally administered with varying levels of Moringa leaf extract (MLE) in drinking water.

	[1]Treatments						
[2]Parameters	**NC**	**PC**	**MLE_{60}**	**MLE_{90}**	**MLE_{120}**	**[3]SEM**	***p* value**
Duodenum length (cm)	29.38^{b}	29.05^{b}	30.56^{a}	30.90^{a}	30.42^{a}	0.180	0.001
Jejunum length (cm)	74.54^{b}	72.60^{b}	77.62^{a}	78.31^{a}	78.81^{a}	0.770	0.005
Ileum length (cm)	74.60^{b}	73.05^{b}	78.46^{a}	78.04^{a}	79.10^{a}	0.650	0.005
Duodenum							
Villus height (μm)	1803^{b}	1819^{b}	1939^{a}	1983^{a}	1962^{a}	20.98	0.010
Villus width (μm)	132.4^{d}	135.4^{cd}	139.4^{c}	172.5^{a}	155.1^{b}	1.570	0.022
Crypt depth (μm)	179.8	181.2	184.2	183.8	177.8	2.810	0.698
Lamina propria thickness (μm)	136.2	133.6	143.4	158.6	149.0	10.84	0.748
Muscularis mucosa thickness (μm)	34.00^{ab}	32.20^{b}	37.60^{a}	37.20^{a}	36.40^{ab}	1.410	0.029
Muscularis externa thickness (μm)	210.8	201.6	212.2	243.0	237.8	13.73	0.057
Villus height/Crypt depth ratio	10.04^{b}	10.04^{b}	10.53^{ab}	10.81^{a}	11.04^{a}	0.180	0.033
Jejunum							
Villus height (μm)	1378^{c}	1365^{c}	1515^{b}	1655^{a}	1515^{b}	16.19	0.004
Villus width (μm)	112.8^{d}	110.1^{d}	120.2^{c}	135.5^{a}	126.6^{b}	1.440	0.018
Crypt depth (μm)	142.6^{c}	139.0^{c}	153.0^{b}	165.0^{a}	151.4^{b}	1.450	0.027
Lamina propria thickness (μm)	116.0	109.4	124.4	135.0	133.0	8.420	0.487
Muscularis mucosa thickness (μm)	25.60	24.20	24.40	27.60	27.00	1.670	0.784
Muscularis externa thickness (μm)	201.2	184.2	185.2	211.2	197.0	12.34	0.089
Villus height/Crypt depth ratio	9.66^{b}	9.82^{ab}	9.90^{ab}	10.03^{a}	10.01^{a}	0.080	0.034
Ileum							
Villus height (μm)	1041^{b}	1025^{b}	1179^{a}	1244^{a}	1221^{a}	30.20	0.011
Villus width (μm)	105.3^{d}	107.3^{cd}	110.7^{c}	120.4^{a}	115.7^{b}	1.480	0.008
Crypt depth (μm)	100.0^{c}	106.8^{b}	116.6^{a}	121.2^{a}	117.8^{a}	2.160	0.005
Lamina propria thickness (μm)	110.8	106.2	104.6	122.6	119.4	8.020	0.117
Muscularis mucosa thickness (μm)	25.20	23.20	23.20	26.00	25.00	1.790	0.546
Muscularis externa thickness (μm)	181.4	172.2	178.2	197.8	196.6	14.07	0.147
Villus height/Crypt depth ratio	9.76	10.25	10.11	10.27	10.36	0.230	0.368

[a,b,c,d] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water. [3]SEM: standard error of mean

The results presented in Table 3.13 showed that in the three segments of the small intestine, the MLE supplemented groups had the highest ($p < 0.05$) acidic goblet cell count when compared to the control groups (NC and PC). The mixed goblet cell count was only influenced ($p < 0.05$) in the duodenum and ileum segments of the small intestine, with the PC and MLE_{60} having the lowest ($p < 0.05$) mean values in the duodenum, while those from the PC had the lowest ($p < 0.05$) mean in the ileum section compared to other treatments.

Table 3. 13. Goblet cell count (per 100 µm villus height) of Cobb 500 broilers (n = 125) orally administered with varying levels of Moringa leaf extract (MLE) in drinking water.

		[1]Treatment						
[2]Intestinal segment	**[3]Goblet cells**	**NC**	**PC**	**MLE_{60}**	**MLE_{90}**	**MLE_{120}**	**[4]SEM**	***p* value**
Duodenum	Acidic	50.63^{b}	50.00^{b}	55.89^{a}	60.24^{a}	57.96^{a}	1.520	0.036
	Mixed	48.58^{ab}	46.83^{b}	47.16^{b}	51.54^{a}	50.70^{ab}	1.320	0.025
	Total	99.20^{bc}	96.80^{c}	103.0^{b}	111.8^{a}	108.7^{a}	1.680	0.014
Jejunum	Acidic	66.90^{b}	66.15^{b}	78.11^{ab}	83.24^{a}	83.51^{a}	4.750	0.045
	Mixed	58.86	58.16	64.00	69.31	69.32	4.860	0.742
	Total	125.0^{b}	125.1^{b}	142.1^{ab}	152.5^{a}	152.8^{a}	6.600	0.026
Ileum	Acidic	55.90^{b}	52.91^{b}	64.45^{a}	66.69^{a}	66.30^{a}	2.060	0.015
	Mixed	49.19^{ab}	44.32^{b}	52.47^{ab}	58.17^{a}	58.27^{a}	3.360	0.011
	Total	105.1^{bc}	97.20^{c}	116.9^{ab}	124.9^{a}	124.6^{a}	4.740	0.031

^{a,b,c} Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water. [4]SEM: standard error of mean.

3.4.7 Bone parameters

The result from Table 3.14 showed no significant ($p > 0.05$) difference in diaphysis diameter, FPHT, and MPHT. Tibia weight was influenced ($p < 0.05$) by MLE administration, with the NC group having the lowest ($p > 0.05$) weight of 11.12g while those offered MLE_{90} and MLE_{120} had the highest ($p > 0.05$) tibia weight. Different levels of MLE in the drinking water affected ($p < 0.05$) the tibia length, with PC groups having the highest tibia length (104.89 mm), while those on MLE administration had the lowest. The BBS was affected ($p < 0.05$) by the administration of MLE, with those on MLE_{120} administration being the strongest, while birds administered NC and PC had the weakest ($p > 0.05$) BBS. Results indicated that the bone TW/TL index was higher ($p > 0.05$) in the tibia of MLE_{90} and MLE_{120}, while those of NC (0.11g/mm) had the lowest TW/TL index. The highest ($p > 0.05$) mean values for TMW and TLW were obtained when MLE_{90} and MLE_{120} were orally administered, whereas comparable lower mean values were observed in the tibia of NC and PC groups. The MCD of the tibia from the MLE supplemented groups was significantly ($p < 0.05$) lower compared with the controls.

Higher tibiotarsal index values were obtained in the tibia from the MLE groups when compared to the control groups (NC and PC). Similarly, the MLE groups presented the lowest robusticity index when compared to the control groups (NC and PC).

Table 3. 14. Tibia morphometric parameters of Cobb 500 broilers (n = 225) orally administered with varying levels of Moringa leaf extract (MLE) in drinking water.

	[1]Treatments						
[2]Parameters	**NC**	**PC**	**MLE_{60}**	**MLE_{90}**	**MLE_{120}**	**[3]SEM**	***p* value**
Tibia weight (g)	11.12^{d}	12.25^{c}	13.75^{b}	14.75^{a}	14.25^{ab}	0.200	0.005
Tibia length (mm)	101.1^{ab}	104.9^{a}	97.34^{bc}	94.78^{c}	95.12^{c}	1.460	0.009
Diaphysis diameter (mm)	8.58	8.90	8.84	8.81	8.562	0.160	0.258
FPHT (mm)	26.27	26.12	25.66	26.50	26.51	0.370	0.147
MPHT (mm)	19.52	19.96	19.31	19.55	19.49	0.360	0.369
BBS (N)	246.8^{d}	249.8^{d}	264.9^{c}	280.1^{b}	292.6^{a}	2.190	0.026
TW/TL index (g/mm)	0.11^{d}	0.12^{c}	0.14^{b}	0.16^{a}	0.15^{a}	0.010	0.014
TMW (mm)	1.49^{c}	1.52^{c}	1.69^{b}	1.79^{a}	1.74^{ab}	0.030	0.012
TLW (mm)	2.49^{c}	2.49^{c}	2.78^{b}	2.96^{a}	2.86^{ab}	0.040	0.032
MCD (mm)	4.60^{ab}	4.89^{a}	4.37^{bc}	4.06^{c}	3.96^{c}	0.140	0.021
Tibiotarsal index	46.34^{b}	45.26^{b}	50.64^{a}	53.91^{a}	53.77^{a}	0.760	0.005
Robusticity index	21.70^{ab}	22.23^{a}	21.16^{bc}	20.79^{c}	20.84^{c}	0.200	0.011

[a,b,c,d] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water. [2]Parameters: FPHT = Femoral side proximal head thickness; MPHT = Metatarsal side proximal head thickness; TBS = tibia breaking strength; TW/TL = tibia weight/tibia length index; TMW = thickness of the medial wall; TLW = thickness of the lateral wall; MCD = Medullary canal diameter. [4]SEM: standard error of mean.

Table 3.15 presents the tibia ash and mineral composition of the bones measured from carcasses of birds supplemented with MLE. All parameters considered were significantly ($p < 0.05$) influenced by the administration of different levels of MLE, except for the ash and phosphorus content of the bone. The highest ($p < 0.05$) mean values for calcium concentration were obtained when MLE_{120} and MLE_{90} were orally administered, whereas the lowest ($p < 0.05$) comparable mean values were observed in the NC and PC groups. Similarly, the treatment effect observed in the Ca:P indicates that Ca:P was highest ($p < 0.05$) in MLE oral administered groups. The NC group had the lowest ($p < 0.05$) mean values for magnesium when compared to other treatment groups. The PC and MLE supplemented groups recorded the highest ($p < 0.05$) iron content, while the zinc content of the tibia was reduced ($p < 0.05$) for NC and PC groups.

Table 3. 15. Tibia ash and mineral composition of Cobb 500 broilers (n = 225) orally administered with varying levels of Moringa leaf extract (MLE) in drinking water.

	[1]Treatments						
[2]Parameters	NC	PC	MLE_{60}	MLE_{90}	MLE_{120}	[3]SEM	*p* value
Ash (g)	48.26	49.85	51.37	53.41	54.75	0.440	0.683
Calcium (mg/g)	216.6^{d}	226.0^{cd}	241.5^{bc}	252.7^{ab}	264.1^{a}	5.540	0.005
Phosphorus (mg/g)	114.5	121.1	118.0	122.0	127.0	4.280	0.312
Ca:P (mg/mg)	1.90^{b}	1.87^{b}	2.05^{a}	2.08^{a}	2.09^{a}	0.040	0.024
Magnesium (mg/g)	7.74^{d}	8.34^{c}	8.67^{ab}	8.94^{a}	8.63^{b}	0.100	0.021
Iron (µg/g)	40.22^{b}	44.96^{ab}	45.37^{ab}	46.18^{ab}	55.03^{a}	3.740	0.034
Zinc (µg/g)	1.35^{b}	1.94^{b}	2.77^{a}	2.05^{ab}	1.99^{ab}	0.250	0.042

^{a,b,c,d} Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MLE_{60} = 60 mL moringa leaf extract/L drinking water; MLE_{90} = 90 mL moringa leaf extract/L drinking water; MLE_{120} = 120 mL moringa leaf extract/L drinking water. [3]SEM: standard error of mean.

3.5 Discussion

3.5.1 Growth performance

The nutrients and bioactive compounds contained in MLE as presented in Tables 3.2 and 3.3 can be leveraged to increase the efficiency of feed utilization and the performance of chickens. The earlier report of Sigolo et al. (2021), who noted a decreased feed intake when coriander extracts were supplemented in broiler drinking water, supports the reduced overall feed intake (FI) and average daily feed intake (ADFI) as influenced by the oral administration of MLE. This reduction in FI and ADFI may be due to the bioactive compounds in MLE as phytogenic feed additives possess organoleptic properties that affect dietary intake in poultry (Van der Aar et al., 2017). Generally, broilers eat to meet their metabolic requirements, which implies that the decrease in feed consumption could be because the birds received extra nutrients from the MLE and thus tended to reduce the feed intake since their nutritional requirements were met.

In this current study, the increased overall final body weight (FBW), body weight gain (BWG) and average daily weight gain (ADWG) observed in the MLE supplemented groups are not consistent with the earlier results of Nkukwana et al. (2014a), who reported no difference in the body weight of broilers after a 35-day trial of dietary inclusion of moringa leaf meal. Likewise, Mulaudzi et al. (2019) reported that the incorporation of moringa leaf meal above 2.5% in the diet of quail compromised their growth performance. These earlier results can possibly be attributed to the route of administration, the dosage administered, and stage of the harvest of the moringa leaves, and possibly the presence of antinutrients (oxalate, phytates, and fibre) in moringa leaves that may render growth-boosting compounds such as vitamins, minerals, and amino acids biologically unavailable (Igwilo et al., 2014). This suggests the need for processing moringa leaves before application to improve their nutrient uptake and utilization by chickens.

However, the observations documented in this study are supported by the findings of Alabi et al. (2017), who noted that the FBW and BWG of broilers increased with reduced FI when aqueous *M. oleifera* leaf extracts were administered as a replacement for the banned antibiotics. The report by Younis and Elbestawy (2017) also gives credence to the present observations. They posited that the supplementation of 3% *M. oleifera* leaf aqueous extract did increase BWG and decrease the FCR of broiler chicks for the whole period of the experiment. Paul et al. (2020) conducted a similar study where neem leaf extract was orally administered and elevated the performance of broilers in terms of BW, BWG, and reduction of FCR compared to the control groups.

The better performance of MLE over moringa leaf meal may perhaps be linked to the efficient nutrient absorption through water delivery by reducing the digesta viscosity (Alabi et al., 2017). However, there are other contrasting reports (Botsoglou et al., 2002; Tekeli et al., 2006) where the administration of plant extracts did not increase the growth performance of broilers which could be due to the difference in solvents used in extractions, concentration of bioactive compounds, and route of administration.

3.5.2 Nutrient digestibility

Nutrient digestibility is the percentage of feed nutrients taken into the digestive lumen that are absorbed for utilization by animals (Okpanachi et al., 2016). In this study, the oral administration of varying dosages of MLE increased the digestibility of dry matter, crude protein, ether extract, NDF, ADF, calcium, and phosphorus digestibility values of birds. These findings are in contrast with the report of Nkukwana et al. (2014a), who reported that the inclusion of moringa leaf meal in the diets of broilers had no effect on the apparent digestibility of ether extract and crude protein. The difference in results observed between the two studies may be attributed to the already dissolved nutrients embedded in the MLE and its subsequent increased permeability and absorption made possible through water delivery (Alabi et al., 2017).

Likewise, Nhlane et al. (2020) reported no linear or quadratic effects on dry matter, crude protein, or ADF digestibility when indigenous hens were fed a diet containing seaweed. The antimicrobial capability of MLE could decrease harmful gut microbes and impede microbial metabolism by preventing oxidative phosphorylation (Viuda-Martos et al., 2010). These may lead to increased bioavailability and absorption of nutrients in the intestinal tract (Hamady et al., 2015). In consonance with this study, Akuru et al. (2021) noted an increased digestibility of dry matter, ether extract, ADF and NDF of broilers supplemented with pomegranate peel powder. The elevated nutrient digestibility points to the fact that oral administration of MLE ensures digestive secretion, enzymatic functions, and beneficial activities within the intestinal lumen of broilers. These assertions were confirmed by earlier findings by Banerjee et al. (2013) and Murugesan et al. (2015).

Furthermore, phytogenic additives such as MLE control pre-caecal nutrient digestion by decreasing harmful bacteria colony count, fermentation, and reduction of gut-related lymphatic system actions (Amad et al., 2011). Also, Murugesan et al. (2015) noted that there was a significant increase in ash digestibility in birds when phytogenic additives were administered. The increased calcium and phosphorus digestibility observed in birds orally administered MLE may be a result of the increased bioavailability of calcium and phosphorus in MLE orchestrated through water extraction and enhanced absorption through water delivery (Rockwood et al., 2013).

3.5.3 Blood parameters

The blood haematological profile is a good indicator of good health status for broilers (Oyeagu et al., 2016). Matshogo et al. (2021a) opined that blood indices are significant in appraising the safety and quality of feed and other feed additives for broilers. Also, Oyeagu et al. (2019) agreed that the physiological responsiveness of broilers to their environment, such as feeds, is normally mirrored by their haematological indices. The increased haematocrit, haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and reticulocyte with all observed values in this study fall within the normal ranges for broilers as documented by Talebi et al. (2005), Oyeagu et al. (2019), Iyaode et al. (2020), Matshogo et al. (2020), and Matshogo et al. (2021a). This may imply that oral administration of MLE did not impair the health status of the birds. This is supported by the earlier report of Mulaudzi et al. (2019), who found that moringa leaf meal supplementation in the diets of quail did not compromise their health status. The normal blood level recorded indicated that the MLE dosages are optimal and not detrimental to the bird’s health.

The normal level of haematocrit shows that the supplemented MLE aided the normal production of red blood cells while the normal neutrophils, eosinophils, basophils, monocytes, and lymphocytes recorded are indicative of a balanced defence system. Increased haemoglobin concentration indicated higher oxygen-carrying capacity as well as the absence of anaemia in the MLE administered groups. Haemoglobin is a protein found in the red blood cells that carry oxygen and is responsible for the red colour of the blood. A low level of haemoglobin concentration in animals usually indicates anaemic condition. The earlier findings of Hassan et al. (2016) agree with this observation as they confirm that moringa leaf meal supplementation increases the haemoglobin count of birds.

The observed MCH, MCV, and reticulocyte counts in this present study are comparable to the findings of Rasouli et al. (2020), who reported increased MCV, MCH, and reticulocyte counts in birds administered with *Salvia officinalis L.* aqueous leaf extract. The MCH is the average amount of haemoglobin in each red blood cell, and a low MCH value indicates the presence of iron deficiency (anaemia). The MCV measures the average size of the red blood cells, and the size of the red blood cells is significant in the supply of oxygen from the lungs to every other cell in the body. A low MCV signifies microcytic anaemia, which may be caused by poor dietary intake of iron. The reticulocytes are newly produced, relatively immature red blood cells and reflect recent bone marrow activity. It, therefore, suggests that the increased MCV, MCH, and reticulocyte count further affirmed the normal blood oxygen-carrying capacity of MLE supplemented groups. This further implies that birds orally administered with MLE may be more efficient in performing respiratory functions. This gives credence to why birds from the MLE groups recorded better growth performance than the control groups because of the readily utilizable energy generated from aerobic respiration.

The nutritional value and toxicity of ingested feed nutrients in broilers are better understood by evaluating the serum indices of their blood, making serum biochemistry a useful tool in identifying sick birds among healthy ones (Mokone et al., 2022). This study observed no significant difference in the biochemical indices measured except for calcium concentration, and all the values were within the normal ranges for healthy birds reported by previous studies (Chikumba et al., 2013; Matshogo et al., 2020; Matshogo et al., 2021a). This could suggest that oral administration of MLE had positive post-digestion feedback.

Significant for measuring liver performance are the alkaline phosphatase (ALKP), alanine transaminase (ALT), and gamma-glutamyl transferase (GGT), which were within the normal range for clinically healthy chickens, indicating that MLE may possibly have potential as an oral additive for broiler chickens. The bioactive constituents in MLE may protect liver injury by resuscitating glutathione activity (Olugbemi et al., 2010). The serum albumin concentration mirrors the liver's performance, and the albumin values observed in this study are comparable to those found by Kumanda et al. (2019), implying good liver functioning in response to the MLE. Tóthová et al. (2019) noted that serum albumin does increase step by step with age, peaking on days between 32 and 42 of feeding, which could be due to maintaining metabolic balance during rapid growth. The serum protein determines the protein reserve in the body, implying that MLE supplies sufficient dietary protein (Matshogo et al., 2020). Additionally, the absence of any significant effects observed for total protein, urea, and creatinine suggests that MLE-administered groups promoted comparable renal function compared to the NC and PC groups.

The comparable glucose and amylase concentrations among birds in this study signify normal fat metabolism, inferring no detrimental effects suffered by the birds from MLE-administered groups. These findings agree with the study of Mulaudzi et al. (2019) who reported similar glucose concentrations in Japanese quails supplemented with moringa leaf meal. Glucose, an important energy nutrient, is responsible for regulating osmotic pressure in the blood of livestock. Serum phosphorus concentrations were lower in this study compared to the 4.55 – 5.09 mmol/L reported by Matshogo et al. (2021a) when fibrolytic enzyme treated green seaweed was supplemented in the diets of Cobb 500 broilers. However, the calcium concentrations in this study were within the range (2.05 – 2.74 mmol/L) observed by Nhlane et al. (2020) for clinically healthy chickens. The observed differences in calcium concentrations may be peculiar to the differences in dosage of polyphenol additive, route of administration, age, reproductive organs, and the mineral content of the MLE administered in this current study (Makola et al., 2021a). The findings of the present study contradict those observed by Mulaudzi et al. (2019), who found supplementation in quail diet does not affect growth performance parameters.

Symmetric dimethylarginine (SDMA) is a more reliable indicator of kidney function than creatine concentration because SDMA detects declining kidney function earlier and it is not impacted by muscle mass. The SDMA values in this study were lower compared with the 25.49 – 33.83 mg/L recorded by Matshogo et al. (2021a). The SDMA value in this study is within the reference interval and with the low creatine levels which may suggest that kidney function is likely good among the MLE administered groups (Iyaode et al., 2020).

3.5.4 Carcass yield and internal organs weight

Meat product grading and market prices are influenced by carcass yield. For the MLE supplemented groups, carcass yield observed mirrored the final and daily gains earlier noted in the present study. The oral administration of MLE as an additive improved the slaughter weight (SLWT), dressing percentage, hot carcass weight (HCW), and cold carcass weight (CCW). Our findings are in contrast with the previous studies by Nkukwana et al. (2014a), Mulaudzi et al. (2019) and Matshogo et al. (2021a) who supplemented broiler and quail diets with different levels of moringa leaf meal. CCW is a positive indicator of total edible meat after storage, suggesting that oral delivery of MLE did not reduce the amount of edible meat. The extraction process of moringa leaf into aqueous extract may have decreased the antinutrients such as phytates, fibre, and oxalates that ordinarily would have rendered the minerals, vitamins, and amino acids in moringa leaves. These available nutrients are absorbed by the chicken for increased tissue (flesh) deposit (Khanal et al., 2009).

The oral administration of MLE did not decrease the prime parts such as breast, drumsticks, and thighs, which is an index of greater economic returns, and these results are consistent with the findings of Nkukwana et al. (2014a), Mulaudzi et al. (2019), and Matshogo et al. (2020), who included moringa leaf and seaweed meal in the diets of broilers and Japanese quail, respectively. Raach-Moujahed and Haddad (2013) noted that there is a direct relationship between body weight and carcass yield. Also, carcass yield is impacted by sex, body weight at slaughter, genetics, feed, and slaughtering conditions.

The similar values observed for liver, heart, and gizzard were consistent with the findings of Nkukwana et al. (2014a). They reported no significant difference in the liver, gizzard, and heart weight of broilers supplemented with moringa leaf meal in their diet. Also, the lack of influence on the liver, heart, and gizzard agreed with the reports of Matshogo et al. (2020) as well as Kumanda et al. (2019), who recorded no difference between the control and treatments containing plant products on the organ weight of broilers. This infers that the extraction of moringa leaves into MLE did not interfere with proper functioning of the gastrointestinal tract and visceral organs of the birds. The B and T cells are types of white blood cells of the lymphocyte subtype, and their development and production of immunoglobulin are the responsibility of the bursa and thymus (Swann et al., 2014). A reduction in the development of these organs will lead to decreased immunological functions linked to B and T lymphocytes. Likewise, due to the poorly developed lymphatic vessels and nodes of birds, the spleen is more significant to their immune system than in mammals (Swann et al., 2014).

Suffice it to say that in clinically healthy animals, the increase in the size of lymphoid organs is correlated with enhanced immune responses in their bodies (Matshogo et al., 2020). The weights of the bursa, spleen, and thymus were increased in this study, which could be a result of the polyphenols and vitamins in MLE. In a similar study, Nkukwana et al. (2014a), found that, moringa leaf meal in broiler diets only increased the weight of the bursa but not the spleen and thymus weights. This disparity in data might be a result of the different broiler strains used in the study, non-processing and/or of the moringa leaves to minimize antinutrients, and the increased nutrient permeability through water delivery. The improved weights of the lymphoid organs observed in this study are consistent with the earlier report of Rasouli et al. (2020), who studied the effect of sage leaf aqueous extract supplementation on broilers.

3.5.5 Meat quality

Water holding capacity (WHC) and meat pH are critical qualitative characteristics of broiler meat which affect the appearance of the meat as well as its juiciness, cooking losses (Karthivashan et al., 2015), and meat tenderness, a quality that mainly determines consumer taste (Lomiwes et al., 2014). This present study noted that the meat from the birds orally administered MLE_{120} and MLE_{60} had decreased pH_{24hr} and WHC values, respectively, when compared to the control groups. These observations are not in agreement with the findings of Matshogo et al. (2021b), who reported no differences in meat quality traits of broilers fed a diet supplemented with seaweed treated with fibrolytic enzymes. This contrasting report may be attributed to the inability of the fibrolytic enzyme to reduce the lignin, cellulose, and hemicellulose content of green seaweed used in their study. Also, Mulaudzi et al. (2019) reported no differences in the meat quality of quails whose diet was supplemented with moringa leaf meal.

The results of the current study further showed that pH_{24h}r values in all experimental groups gradually decreased from pH_{45min}, an observation in conformity with that of Kadim et al. (2009), who studied the effect of ascorbic acid on the meat quality of broilers. Dyubele et al. (2010) noted that meat pH is impacted by the quantity of glycogen in meat tissues before slaughter and the rate of glycogen balance conversion to lactic acid after slaughter. Therefore, the normal range of pH_{24hr} of breast samples from the MLE-administered groups, suggests that

MLE did not decrease the glycogen levels of the broilers (Shakeri et al., 2020; Sugiharto et al., 2020).

Since MLE is rich in vitamin E, tannins, and phenols, we can assume that the oral administration of MLE might have stabilized the muscular membrane by activating antioxidants, preventing free radicals and bacterial degradation, thereby slowing microbial spoilage of the meat (Alabi et al., 2017). Also, increased pH_{45mins} values in MLE administered groups might have contributed to the stabilization of the volume of myofibrils by reducing protein denaturation, thus leading to the conservation of water inside the muscle cells (Sugiharto et al., 2020).

Janisch et al. (2012) posited that drip loss, cooking loss, colour, etc. could help ascertain the post-slaughter quality of meat. The significantly decreased drip loss, cooking loss, and shear force of meat samples from birds orally administered MLE_{60}, MLE_{90}, and MLE_{120}, respectively, may be ascribed to their respective pH_{24hr}, rigor state, and denaturation of water-binding proteins (Leygonie et al., 2012).

The appearance of raw meat is enhanced while the extracellular free water content is measured by drip loss and the output of the ready-to-eat is measured by cooking loss (Bozkurt et al., 2017). Results in this study suggest that MLE has a high water-binding capacity, which enhances WHC, thereby reducing drip loss and cooking loss. This finding is consistent with the report of Mahmmod (2014), who studied the effect of olive leaf extract on the meat quality of sheep. Also, the reduced cooking loss and drip loss in this study observed for breast meat samples from the MLE administered groups may be attributed to accelerated water retention and osmolality of the cell, which is consistent with the findings of Nutautaitė et al. (2020), who included betaine in the diets of broiler chickens. A low shear force will usually infer tender meat. The oral administration of MLE reduced the shear force of breast meat, which is consistent with Nkukwana et al. (2014b) who included moringa leaf meal in broiler diets, suggesting MLE administration increased meat quality.

The colour of the meat affects consumer acceptance during purchase, and as earlier reported by Francisco et al. (2015), there is a strong correlation between breast meat pH and colour. Likewise, the pH of lighter meat is low, and when the recorded lightness (L^*) value surpasses 59 (Petracci et al., 2015), the meat is said to be pale, soft, and exudative (PSE), while darker meat pH values are high and, in excessive cases, are typically dry, firm, and dark meat (DFD)

(Mir et al., 2017). In meat classification, the higher the lightness (L*) value, the paler the meat. However, observed lightness (L*) values in this study are within the typical range for standard broiler meat and are also in agreement with the findings of Akuru et al. (2020), who examined the effect of dietary supplementation of *Punica granatum L* on broilers.

The decreased moisture content of the breast meat as influenced by the oral administration of MLE agrees with the report of Sugiharto et al. (2020), who inoculated fermented moringa leaf into the diet of broilers. But in an earlier study, Sugiharto et al. (2020) did not note difference between feeding cassava pulp fermented with *Acremonium charticola* on the moisture content of broiler breast meat. Also, Ahmed et al. (2019) indicated no effect of chitosan oligosaccharide and valine on the moisture content of breast meat. Though there is no clear explanation for this discordant result, it might probably be due to broiler strains, differences in the method of phytogenic feed additive processing, bioactive compound content, experimental environment, and route of administration, which may elicit divergent responses by broilers.

The increased crude protein content of the broiler's breast meat is supported by earlier reports (Sugiharto et al., 2017; Marcinčák et al., 2018) when broilers were supplemented with fermented phytogenic additives. It is most likely that the water extraction of *M. oleifera* leaves into MLE increased the protein utilization (protein efficiency ratio), and its delivery through the birds' drinking water further increased protein bioavailability in the musculature (intramuscular protein anabolism) of broiler chickens (Hossain et al., 2013; Nie et al., 2015). Earlier studies (Nkukwana et al., 2015; Mir et al., 2017) posited that reduced crude fat and higher crude protein contents in breast meat may be attributed to decreased water losses from meat. Therefore, increased crude protein content possesses higher water affinity than reduced crude fat content. Thus, this justifies the reported reduced WHC, drip loss, and cooking loss in MLE administered groups.

The reduced crude fat content in the breast meat of broilers is consistent with the earlier documented report of Sugiharto et al. (2020) that fed a fermented mixture of cassava pulp and *M. oleifera* leaf meal to broilers. The reduction in crude fat in the breast meat samples from the MLE supplemented groups indicates its capacity to lower the intramuscular fat synthesis in broiler chickens (Abdurrahman et al., 2016). Similarly, in broilers, Camy et al. (2019) reported a variation in meat crude fat content due to neem leaf extract oral administration. Studies have shown that herbal extracts generally act as strong superoxide anion scavengers and natural antioxidants (Choudhary and Swarnkar, 2011; Thampi and Jeyadoss, 2015). Antioxidants reduce fat by preventing auto-oxidation process in the body. It is also possible that MLE may have accelerated the breakdown of tissue triglycerides and β-oxidation of fatty acids, leading to decreased fat deposition in the muscle of broilers (Sugiharto and Ranjitkar, 2019). However, several investigations into garlic, a rich source of antioxidants supplemented in broiler diets, noted no significant effect on meat crude fat content (Hashemi et al., 2020), which contradicts the findings of the present study. The difference between these earlier reports and this study might be linked to different dosages and routes of administration of the additives to the birds. The increased ash content of breast meat inferred that MLE increased the bioavailability of minerals and their absorption, thus resulting in their deposition in the muscle tissues (Wang et al., 2016).

3.5.6 Intestinal morphology

The ability of the enterocytes to absorb bioavailable nutrients may suggest that the anatomy of the small intestine is linked to broiler performance. In this study, it was noted that the length of the three segments of the small intestine was increased in birds administered MLE. These results are at variance with the results reported by Mohtashami et al. (2021), who observed that the relative length of the small intestine of partridges was reduced when insoluble fibre sources were incorporated into their diets. The increments recorded in this study for duodenum, jejunum, and ileum length may be a result of the elimination of insoluble fibre in moringa leaf through the extraction process, thereby reducing bulky digesta and transit time in the lumen (Jiménez-Moreno et al., 2019).

Improved growth performance in broilers may be correlated to their increased villus length (Ashraf et al., 2013). Results from this study on intestinal morphology indicate a significant increase in the villi height and width of the duodenum, jejunum, and ileum of birds administered varying levels of MLE. The increase in villi height and width in the current study could be attributed to the efficiency in the absorption and utilization of nutrients orchestrated by delivering them through the water. Paiva et al. (2014) noted that greater surface area and absorption capacity are normally associated with increased villi height, which results in mature enterocytes on the villus tips, whereas poor nutrient absorption and digestion are indicators of decreased villi height, which results in a decreased absorptive area and fewer mature enterocytes. The report of Long et al. (2019) is also in agreement with the current study, where they observed a significant increase in the jejunum and ileum villus height when *Forsythia suspense* extract was administered to pigs as a substitute for antibiotics.

Furthermore, Ashraf et al. (2013) observed that the increase in villus height with a corresponding increase in villus height/crypt depth ratio has a direct relationship with increased epithelial cell turnover and activated cell mitosis. This phenomenon was observed only in the duodenum and jejunum segments of the small intestine where the MLE-administered groups had the highest villus height/crypt depth ratio. These observations may have been due to controlled damage to epithelial cells that line the villi of the small intestine, thereby reducing the need for their turnover and stimulating enzyme production from the villi tip. Increments in the length of the villi imply the better absorptive ability of the small intestine due to improved surface area and indicate improved gut health (Dawood and Koshio., 2020).

In the current study, a significant difference was only observed for the muscularis mucosa thickness in the duodenum segment of the small intestine, which was at variance with Vidanarachchi et al. (2010). In their study, Vidanarachchi et al. (2010) orally administered seaweed extract composed mainly of fucose (55% w/w) and galactose (44% w/w), unlike MLE, which contained various bioactive compounds such as polyphenols, minerals, vitamins, etc. The lamina propria and muscularis mucosa have an important role in transporting absorbed nutrients to the blood and regulating the expulsion of secretions into the lumen (Khan et al., 2017). This result may suggest that the oral administration of MLE did not compromise the intestinal entities, which resulted in a better FCR observed in this study among the MLE-administered groups when compared with the NC and PC groups.

Goblet cells are significant constituents of the inherent immune system in the digestive system. They induce a mucus layer composed of mucin glycoproteins, which provides protection, lubrication, and transport between the epithelial lining and the luminal contents of the intestine (Pelaseyed, 2014; Sikandar et al., 2017). The oral administration of MLE increased acidic, mixed, and total goblet cell counts in the duodenum and ileum, while acidic and total goblet cell counts increased in the jejunum, which was consistent with an earlier report (Khan et al., 2017). These observations suggest increased mucosal protection with MLE administration. Although the precise method by which MLE boosted goblet cell count is unknown, it is possible that vitamin A, a constituent of MLE, aided goblet cell yield and development due to its demonstrated capacity to influence mucin production and differentiation (Fan et al., 2015).

3.5.7 Bone parameters

Morphometric parameters and tibia breaking strength have been used as indicators of bone quality in chickens (Güz et al., 2019; Stęczny and Kokoszyński, 2020; Njoku et al., 2021). The results obtained in this study indicated that birds administered with MLE had an increased tibia weight, BBS, TW/TL, TMW, TLW, and tibiotarsal index while reducing tibia length, medullary canal diameter (MCD), and robusticity index. These findings are not in agreement with Abbas and Khauoon (2021), who administered grape leaf extract at 2 mL/L of drinking water. They reported no effect on TBS and tibia morphometric parameters measured except for tibia length after 35 days. Nkukwana et al. (2014b) who supplemented moringa leaf meal in broiler diets reported no effect on morphometric parameters and BBS after 35 days. The variation in the results may possibly be due to the age of the birds, polyphenols in the plants used, the dosage administered, and the breed.

The biological unavailability of calcium causes blood hypocalcemia, which results in reduced bone strength (Yan et al., 2019). Likewise, Kim et al. (2017) inferred that BBS is a vital index employed to evaluate the bioavailability of calcium and phosphorus supplied in the diets of broilers. The TW/TL index alludes to bone density (Javid et al., 2022), and the higher the index, the denser the bone (Araujo et al., 2022). There is an inverse relationship between robusticity index and bone structure, with a high robusticity index implying a weak bone structure (Hafeez et al., 2020). Furthermore, there is a direct relationship between the tibiotarsal index and bone mineralization, with a low tibiotarsal index suggesting low bone mineralization (Ogunwole et al., 2016). It was noted that the tibia of birds orally administered with MLE had the highest tibiotarsal index compared with the NC and PC groups. These results imply that the oral administration of MLE increased the rate of mineralization and development of bones in the birds (Tomaszewska et al., 2018). In this study, TMW, TLW, and MCD of the tibia of birds offered MLE increased compared with the control groups. The TMW, TLW, and MCD have a direct relationship with the tibia breaking strength (Tomaszewska et al., 2017).

Observations showed that calcium content increased as the dosage of MLE increased, which is consistent with the findings of Makola et al. (2021b), who reported an increase in calcium content of the tibia as the level of nano-dicalcium phosphate substitution increased in broiler diets. Hamdi et al. (2015) concluded that tibia weight and mineralization of bone are increased by calcium levels in the diet, with the high-calcium content in the diet presenting the highest bone weight and ash content. The ash content of the tibia measured in the present study did not differ among the treatments and the present study is in contrast with the results of Nkukwana et al. (2014b) who reported higher tibia ash content for birds fed moringa leaf meal.

Also, in agreement with the present study, El-Husseiny et al. (2012) reported no difference in the ash content of tibia when organic sources of zinc, manganese, and copper were supplemented in the diets of broilers. Savaris et al. (2021) opined that calcium and phosphorus are related to the metabolism of magnesium. The highest magnesium level observed with the MLE administered groups could be explained by the findings of Shastak and Rodehutscord (2015), who reported that an increase in dietary calcium and phosphorus levels increases the amount of magnesium needed in the diet for maximal weight gain.

Nevertheless, absorption and homeostasis of calcium and phosphorus are assumed to be optimized at a 2Ca:1P ratio (González-Cerón et al., 2015). The mineral content of matured bone is believed to have a structure of calcium phosphate and hydroxyapatite, which has a molar Ca: P ratio of 1.67:1 (Pieniazek et al., 2017). The current study recorded the Ca:P ratio of 1.87:1 and 1.90:1 for PC and NC groups, respectively, these groups also recorded the lowest breaking strength, which was consistent with an earlier report of Nkukwana et al. (2014b). The authors supplemented broiler diets with moringa leaf meal at varying levels depending on the stage of feed: 1, 3, and 5 g (starter), 3, 9, and 15 g (grower), and 5, 15, and 25 g (finisher) per kg of feed. It could be inferred that the difference in results may be due to the extraction of moringa leaves into MLE and the route of administration applied in this recent study.

It was further noted that the content of magnesium, iron, and zinc was increased in the tibia of birds orally administered with MLE, which gives credence to the fact that these micronutrients were well catered for by MLE and that they were also bioavailable in the small intestine for absorption. These results are in tandem with those of Kwiecień et al. (2016), who in their study supplemented zinc glycine chelate at 25, 50, and 100 mg/kg in broiler diets. They suggested that the higher the intake of minerals through diet, the higher the content in the bones. Also, Nguyen et al. (2021) noted a gradual reduction in zinc content in the bones of the birds due to the lower zinc content in the feed. Likewise, in the studies conducted by De Marco et al. (2017), the concentration of zinc in the tibia was observed to increase along with the increasing level of zinc in the diet. The present study showed that the tibia of broilers orally administered MLE, a good source of organic minerals, was characterized by a higher content of minerals compared to those of broilers in the NC and PC groups, which is supported by the findings by other authors (Kwiecień 2016; Mwangi et al., 2017).

3.6 Conclusion

In this study, oral administration of moringa leaf extract increased weight gain, enhanced feed conversion ratio, nutrient digestibility, carcass yield, meat quality, intestinal morphology, and bone parameters, and did not compromise blood parameters.

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CHAPTER 4: EFFECT OF MORINGA OLEIFERA SEED EXTRACTS ON PHYSIOLOGICAL AND MEAT QUALITY RESPONSES OF COBB 500 BROILER CHICKENS

Abstract

Moringa oleifera seed extract (MSE) contains phytochemicals that can improve chicken production and health. Thus, this study determined the effect of MSE on growth performance, nutrient digestibility, blood parameters, carcass yield, internal organs weight, meat quality, intestinal morphology, and bone parameters in broilers. A total of 250 one-day-old male Cobb 500 broiler chicks (58.11 ± 0.23 live weight) were randomly assigned to five treatments, with five replicates per group and ten chicks per replicate. The chicks were fed commercial starter (1–21 d) and finisher (22–42 d) diets. The treatments were negative control containing drinking water only (NC), positive control with 5g multi-strain probiotic ($5×10^8$ CFU/g) per litre of drinking water (PC), 60 mL of MSE per litre of drinking water (MSE_{60}), 90 mL of MSE per litre of drinking water (MSE_{90}), and 120 mL of MSE per litre of drinking water (MSE_{120}). Treatments and commercial diets were offered ad libitum for 42 days. Oral administration of MSE reduced ($p < 0.05$) overall feed intake (FI) and feed conversion ratio (FCR). The nutrient digestibility of calcium and phosphorus was increased with the administration of MSE. Haematocrits, reticulocyte count, and serum calcium concentration varied significantly ($p <$ 0.05). Birds administered MSE had similar ($p > 0.05$) dressing percentage, hot carcass weight (HCW), cold carcass weight (CCW), bursa weight, and spleen weight, while MSE_{120} increased (p < 0.05) thymus weight. NC and MSE presented similar ($p > 0.05$) meat pH_{24hr} and redness (a^*), while PC and MSE had similar water holding capacity (WHC). NC and PC elevated (p < 0.05) meat shear force and moisture content, while PC had the highest crude fat content when compared to the MSE groups. Oral administration of MSE increased (p < 0.05) meat crude protein content. Duodenum and ileum lengths from NC and PC were shorter ($p < 0.05$) when compared to the MSE administered groups. Oral administration of MSE significantly ($p < 0.05$) improved the intestinal morphology and bone parameters of the birds. MSE has the potential to replace antibiotics in poultry production.

Keywords: Growth performance, Nutrient digestibility, Carcass yield, Internal organs, Intestinal morphology, Bone parameters.

4.1 Introduction

Sustainable intensification of broiler chickens as a major source of animal protein is currently restricted by many factors, including high feed cost (Abd EL Latif, 2022). The increase in feeding cost is caused by the over-reliance on maize and soybean, two major ingredients whose market prices are very high due to high demand by the food, feed, and biofuel sectors (Mohammed et al., 2017). Furthermore, the use of conventional antibiotics to promote growth and feed utilisation efficiency in broiler chickens increases production costs because antibiotics are expensive (Egbu et al., 2022a; Egbu et al., 2022b). This is not withstanding the fact that their usage has been outlawed in many countries due to traces of antibiotic residues in meat products and the risk of transmitting drug-resistant pathogenic bacteria to humans (Abbas and Khauoon, 2021; Yang et al., 2022). This has led to the use of probiotics as safe alternatives to conventional antibiotics (Elbushra et al., 2019). Indeed, the supplementation of broilers with probiotics has been reported to improve weight gain and feed utilisation efficiency (Akinyeye et al., 2014). Unfortunately, the utility of probiotics for large-scale poultry production can be prohibitive because they are also expensive.

The use of locally available phytogenic plant products as sources of nutrients and pharmaceuticals in place of conventional antibiotics and probiotics can deliver sustainable broiler production systems that can contribute to food and nutrition security in South Africa. *Moringa oleifera* seeds contain a variety of nutrients (calcium, phosphorus, protein, carbohydrates, and vitamins A, C, and E) and bioactive compounds (alkaloids, flavonoids, phenols, saponins, and tannins) with antimicrobial and antioxidant properties (Adejumo et al., 2016; Mune et al., 2016; Liang et al., 2019). The seed bioactive substances are reported to elicit probiotic effects, growth-stimulating and health-promoting properties, and immuno-modulatory actions, which result in improved broiler performance (Elbushra et al., 2019). However, there are limited and conflicting studies on the effects of including *M. oleifera* seed meal in the diets of broiler chickens. Ochi et al. (2015) reported that the inclusion of 5 g/kg M. oleifera seed powder in broiler diets reduced feed intake (FI) but had no effect on the feed conversion ratio (FCR) and body weight gain (BWG) of the birds. However, the supplementation of diets with 20 g/kg of M. oleifera seed meal reduced FCR and BWG (Sugiharto and Toana, 2021). This could be because the seed meal has high levels of fibre and condensed tannins (Ashour et al., 2021), which can be eliminated using moringa seed extracts (MSE) (Pandey and Tripathi, 2014). Alabi et al. (2017) noted that the administration of 120 mL of moringa leaf extract increased weight gain and enhanced feed efficiency in broilers.

Thus, the use of the extracts instead of the meal would reduce the antinutritional effects of dietary fibre and condensed tannins and ensure that the birds have full access to moringa's beneficial bioactive compounds. In addition, water extraction of phytogenic substances does not require expensive and sophisticated equipment and, as such, could easily resonate with resource-limited poultry farmers. However, there are no data on the effect of *Moringa oleifera* seed extract (MSE) on the performance and meat quality of broiler chickens. This study, therefore, evaluated the effect of oral administration of MSE on growth performance, nutrient digestibility, blood parameters, carcass yield, internal organs weight, meat quality, intestinal morphology, and bone parameters of Cobb 500 broiler chickens.

4.2 Materials and Methods

4.2.1 Study site

Same as described in Chapter 3.

4.2.2 Sources of *Moringa oleifera* and preparation of the seed extract

The *M. oleifera* seed powder used to prepare the experimental extracts was supplied by Supa Nutri (Johannesburg, South Africa). The seed powder was soaked in distilled water at a ratio of 1:10 for 24 hours. The mixture was then filtered with a muslin cloth to separate the debris from the filtrate, and the extracts were stored in clean containers in a cold room at 4°C. The extracts were administered into the fresh drinking water that was offered to the birds daily during the study as follows: negative control with water only (NC), positive control with 5g probiotic per liter of drinking water (PC), 60 mL of MSE per litre of drinking water (MSE_{60}), 90 mL of MSE per litre of drinking water (MSE_{90}), and 120 mL of MSE per litre of drinking water (MSE_{120}). The probiotic (5×10^8 CFU/g) was acquired from QBLabs (MO, USA) and contained beneficial bacteria (*Aspergillus oryzae*, *Bacillus subtilis*, *Lactobacillus acidophilus*, and *Enterococcus faecium*), which were identified *via* 16S rRNA gene sequencing. Commercial starter (1–21 days) and finisher (22–42 days) diets from De Heus (Pty) Ltd Pietermaritzburg South Africa were used during the feeding trial. Dry matter, crude protein, crude fibre, ash, calcium, and phosphorus were analysed as earlier described in Chapter 3. Metabolizable energy (ME) was calculated using the earlier stated equation in Chapter 3. The nutritional composition of the diets is same as presented in Table 3.1.

4.2.3 Experimental design and birds' management

Same as earlier described in Chapter 3.

4.2.4 Chemical analysis of *Moringa oleifera* seed extract treatments

Same as earlier described in Chapter 3.

The alkaloids, carbohydrates, flavonoids, phenols, protein, saponins, steroids, tannins, and terpenoids content of MSE are shown in Table 4.1.

Table 4. 1. Phytochemical content (mg/L) of *Moringa oleifera* seed extract treatments.

	[1]Treatments		
Phytochemicals	**MSE_{60}**	**MSE_{90}**	**MSE_{120}**
Alkaloids	8.42	8.84	9.30
Carbohydrates	2.77	3.21	3.45
Flavonoids	3.70	4.20	4.66
Phenols	17.84	18.34	19.52
Protein	32.36	33.28	34.52
Saponins	5.90	6.20	6.60
Steroids	4.30	4.80	5.30
Tannins	48.56	52.00	55.60
Terpenoids	18.34	18.90	19.40

[1]Treatments: MSE_{60} = 60 mL moringa seed extract/L drinking water; MSE_{90} = 90 mL moringa seed extract/L drinking water; MSE_{120} = 120 mL moringa seed extract/L drinking water.

The calcium, magnesium, phosphorus, potassium, zinc, iron, sodium, vitamin A, B1, B2, B3, B6, B12, C, and E are shown in Table 4.2.

Table 4. 2. Mineral and vitamin composition (mg/L) of *Moringa oleifera* seed extract treatments.

	[1]Treatments		
Micronutrients	**MSE_{60}**	**MSE_{90}**	**MSE_{120}**
Calcium	606.8	618.2	630.4
Magnesium	37.40	40.80	43.60
Phosphorus	376.0	395.4	407.0
Potassium	68.00	73.00	76.20
Zinc	1.07	1.16	1.25
Iron	6.16	6.47	6.73
Sodium	253.8	263.6	272.2
Vitamin A	4.03	4.49	4.65
Vitamin B1	0.15	0.18	0.24
Vitamin B2	0.24	0.32	0.36
Vitamin B3	0.22	0.33	0.38
Vitamin B6	0.27	0.32	0.36
Vitamin B12	0.10	0.13	0.18
Vitamin C	4.68	4.80	4.96
Vitamin E	548.0	598.0	620.0

[1]Treatments: MSE_{60} = 60 mL moringa seed extract/L drinking water; MSE_{90} = 90 mL moringa seed extract/L drinking water; MSE_{120} = 120 mL moringa seed extract/L drinking water.

4.2.5 Growth performance

Same as earlier described in Chapter 3.

4.2.6 Apparent nutrient digestibility trial

Same as earlier described in Chapter 3.

4.2.7 Proximate analysis

Same as earlier described in Chapter 3.

4.2.8 Blood collection and analysis

Same as earlier described in Chapter 3.

4.2.9 Carcass yield and internal organs weight

Same as earlier described in Chapter 3.

4.2.10 Meat quality

Same as earlier described in Chapter 3.

4.2.11 Intestinal morphology

Same as earlier described in Chapter 3.

4.2.12 Bone parameters

Same as earlier described in Chapter 3.

4.3 Data Analysis

Same as earlier described in Chapter 3.

4.4 Results

4.4.1 Growth performance

At the starter phase of the experiment, the initial weight, feed intake, ADFI, water intake, and ADWI were not influenced ($p > 0.05$) by the oral administration of MSE (Table 4.3). However, the final weight, weight gain, and ADWG of birds were significantly different ($p < 0.05$). The birds offered MSE_{120} had the highest ($p > 0.05$) final weight, weight gain, and ADWG, which was comparable to the MSE_{60} and MSE_{90}, while those offered the NC and PC recorded the lowest ($p > 0.05$) mean values. Birds orally administered with MSE_{60} and MSE_{120} had the best FCR ($p > 0.05$) values, which were comparable to those given PC and MSE_{90} supplementation, and birds on NC had a poor FCR value.

Table 4. 3. Starter phase performance in Cobb 500 broilers orally administered moringa seed extract (MSE) through drinking water.

[2]Parameters	[1]Treatment					[3]SEM	*p* value
	NC	PC	MSE_{60}	MSE_{90}	MSE_{120}		
Initial weight (g/bird)	58.35	58.25	58.15	57.85	57.95	0.230	0.624
Final weight (g/bird)	854.0^{c}	893.1^{bc}	940.0^{ab}	927.8^{ab}	967.3^{a}	19.55	0.021
Weight gain (g/bird)	795.6^{c}	834.8^{bc}	881.8^{ab}	869.9^{ab}	909.3^{a}	19.55	0.017
ADWG (g/bird)	37.89^{c}	39.75^{bc}	41.99^{ab}	41.42^{ab}	43.30^{a}	0.930	0.015
Feed intake (g/bird)	1481	1481	1468	1515	1555	47.50	0.341
ADFI (g/bird)	70.50	70.51	69.91	72.16	74.03	2.260	0.578
Water intake (mL/bird)	2573	2555	2532	2573	2531	45.80	0.159
ADWI (mL/bird)	122.5	121.6	120.6	122.5	120.5	2.180	0.348
FCR (g/g)	1.86^{a}	1.78^{ab}	1.66^{b}	1.74^{ab}	1.71^{b}	0.040	0.005

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MSE_{60} = 60 mL moringa seed extract/L drinking water; MSE_{90} = 90 mL moringa seed extract/L drinking water; MSE_{120} = 120 mL moringa seed extract/L drinking water. [2]Parameters: ADWG = average daily weight gain; ADFI = average daily feed intake; ADWI = average daily water intake; FCR = feed conversion ratio. [3]SEM: standard error of mean.

During the finisher phase, final weight and FCR were not affected ($p > 0.05$) by the administration of MSE (Table 4.4). However, weight gain and ADWG of birds administered MSE_{120} had the lowest ($p > 0.05$) value when compared with birds on NC. Similarly, the birds on NC had the highest ($p > 0.05$) feed intake and ADFI, which was comparable to those on PC, while those offered MSE recorded the lowest ($p > 0.05$) mean values. Also, water intake and ADWI were increased ($p > 0.05$) in birds that received NC, which was comparable to those on PC, MSE_{60}, and MSE_{120}, with those on MSE_{90} having the lowest ($p > 0.05$) value.

Table 4. 4. Finisher phase performance in Cobb 500 broilers orally administered moringa seed extract (MSE) through drinking water.

[2]**Parameters**	[1]**Treatment**					[3]**SEM**	***p* value**
	NC	**PC**	**MSE_{60}**	**MSE_{90}**	**MSE_{120}**		
Final weight (g/bird)	1918	1932	1956	1971	1939	21.00	0.462
Weight gain (g/bird)	1064^{a}	1039^{ab}	998^{ab}	1023^{ab}	952^{b}	32.20	0.011
ADWG (g/bird)	50.67^{a}	49.49^{ab}	47.52^{ab}	48.72^{ab}	45.31^{b}	1.530	0.015
Feed intake (g/bird)	2067^{a}	2005^{ab}	1935^{bc}	1882^{c}	1870^{c}	38.40	0.005
ADFI (g/bird)	98.41^{a}	95.50^{ab}	92.16^{bc}	89.61^{c}	89.05^{c}	1.830	0.005
Water intake (mL/bird)	3691^{a}	3624^{ab}	3670^{ab}	3540^{b}	3577^{ab}	45.70	0.005
ADWI (mL/bird)	175.8^{a}	172.6^{ab}	174.7^{ab}	168.6^{b}	170.3^{ab}	2.180	0.008
FCR (g/g)	1.95	1.93	1.95	1.84	1.97	0.040	0.287

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MSE_{60} = 60 mL moringa seed extract/L drinking water; MSE_{90} = 90 mL moringa seed extract/L drinking water; MSE_{120} = 120 mL moringa seed extract/L drinking water. [2]Parameters: ADWG = average daily weight gain; ADFI = average daily feed intake; ADWI = average daily water intake; FCR = feed conversion ratio. [3]SEM: standard error of mean.

The presented data showed that oral administration of MSE had no effect ($p > 0.05$) on initial BW, overall FBW, BWG, ADWG, WI, and ADWI (Table 4.5). However, FI, ADFI, and FCR were influenced ($p < 0.05$) by the treatments. The birds in the MSE_{90} supplemented groups had the lowest ($p < 0.05$) feed intake when compared with the treatment groups. Similarly, birds offered NC (97.63 g) recorded the highest ($p < 0.05$) ADFI when compared with MSE_{90} (87.65 g). However, the best FCR was recorded among the MSE_{90} supplemented groups, while the NC and PC were the poorest.

Table 4. 5. Overall growth performance in Cobb 500 broilers orally administered moringa seed extract (MSE) through drinking water.

[2]Parameters	[1]Treatment					SEM	*p* value
	NC	PC	MSE_{60}	MSE_{90}	MSE_{120}		
Initial BW (g/bird)	58.35	58.25	58.15	57.85	57.95	0.230	0.624
FBW (g/bird)	1918	1932	1956	1971	1939	21.00	0.462
BWG (g/bird)	1860	1874	1897	1913	1881	21.00	0.665
ADWG (g/bird)	44.28	44.62	45.18	45.55	44.78	0.500	0.472
FI (g/bird)	3547^{a}	3486^{b}	3404^{c}	3397^{c}	3425^{c}	19.96	0.005
ADFI (g/bird)	97.63^{a}	93.07^{ab}	88.84^{ab}	87.65^{b}	88.47^{ab}	2.880	0.005
WI (mL/bird)	6264	6178	6202	6113	6108	77.80	0.084
ADWI (mL/bird)	193.3	165.1	177.3	171.9	176.0	11.53	0.086
FCR (g/g)	1.91^{a}	1.86^{ab}	1.80^{bc}	1.77^{c}	1.82^{bc}	0.030	0.005

^{a,b,c} Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MSE_{60} = 60 mL moringa seed extract/L drinking water; MSE_{90} = 90 mL moringa seed extract/L drinking water; MSE_{120} = 120 mL moringa seed extract/L drinking water. [2]Parameters: ADWG = average daily weight gain; ADFI = average daily feed intake; ADWI = average daily water intake; FCR = feed conversion ratio. [3]SEM: standard error of mean.

4.4.2 Nutrient digestibility

Table 4.6 shows that there were treatment effects ($p > 0.05$) on the digestibility of calcium and phosphorus. Calcium digestibility was highest ($p < 0.05$) with the administration of MSE_{90} and MSE_{120} and lowest ($p < 0.05$) in the PC group. Birds reared on the MSE groups had the highest ($p < 0.05$) phosphorus digestibility, followed by those in NC, and the lowest ($p < 0.05$) phosphorus digestibility was in the PC group.

Table 4. 6. Nutrient digestibility (%) in Cobb 500 broilers (n = 125) orally administered moringa seed extract (MSE) through drinking water.

[2]Parameters	[1]Treatments					[3]SEM	*p* value
	NC	PC	MSE_{60}	MSE_{90}	MSE_{120}		
Dry matter	66.62	67.18	67.74	68.48	68.32	0.580	0.487
Crude protein	71.83	71.89	73.06	73.68	73.75	2.483	0.254
Ether extract	62.70	62.94	66.66	65.97	66.70	1.762	0.548
NDF	64.86	63.60	65.67	62.50	62.24	1.061	0.684
ADF	62.73	62.71	64.38	64.16	62.98	1.002	0.187
Calcium	57.48^{c}	55.49^{d}	62.47^{b}	64.20^{a}	63.81^{a}	0.401	0.031
Phosphorus	57.30^{b}	55.46^{c}	62.39^{a}	63.85^{a}	63.55^{a}	0.512	0.015

^{a,b,c} Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MSE_{60} = 60 mL moringa seed extract/L drinking water; MSE_{90} = 90 mL moringa seed extract/L drinking water; MSE_{120} = 120 mL moringa seed extract/L drinking water. [2]Parameters: NDF: neutral detergent fibre; ADF: acid detergent fibre. [3]SEM: standard error of mean.

4.4.3 Blood parameters

There were treatment effects ($p < 0.05$) on haematocrits and reticulocytes of the birds (Table 4.7). The birds that were administered with MSE (MSE_{60}, MSE_{90}, and MSE_{120}) had higher ($p < 0.05$) haematocrit counts than the birds in PC but did not vary ($p > 0.05$) with the birds in NC. The NC and PC groups had lower ($p < 0.05$) reticulocyte levels compared to all the MSE treatment groups (MSE_{60}, MSE_{90}, and MSE_{120}), whose reticulocyte levels did not differ ($p > 0.05$).

Table 4. 7. Haematological parameters of Cobb 500 broilers (n = 225) orally administered moringa seed extract (MSE) through drinking water.

	[1]Treatment							
[2]Parameters	NC	PC	MSE_{60}	MSE_{90}	MSE_{120}	[3]SEM	*P* value	[4]Reference interval
Basophils (10^9/L)	0.14	0.15	0.14	0.16	0.15	0.022	0.512	0.10 - 2 × 10^9/L
Eosinophils (10^9/L)	0.33	0.42	0.33	0.36	0.32	0.063	0.478	0.20 - 4 × 10^9/L
Haematocrits (%)	24.20^{ab}	19.84^{b}	27.16^{a}	26.38^{a}	26.72^{a}	1.912	0.014	28 - 48 %
Haemoglobin (g/dL)	8.50	7.08	9.30	8.42	9.10	0.686	0.247	7 - 13 g/dL
Lymphocytes (10^9/L)	68.12	61.50	64.59	60.16	60.04	5.474	0.741	50 – 300 × 10^9/L
MCH (pg)	36.88	29.35	31.05	29.83	29.10	3.202	0.941	30 – 80 pg
MCHC (%)	34.80	35.80	35.40	32.00	34.50	0.231	0.084	32 – 42 %
MCV (fL)	104.7	82.21	90.66	93.41	86.54	9.101	0.857	80 – 120 fL
Monocytes (10^9/L)	9.88	9.05	9.91	9.13	9.84	1.462	0.764	1 – 30 × 10^9/L
Neutrophils (10^9/L)	2.97	2.75	2.87	2.71	2.89	0.090	0.365	1 – 4 × 10^9/L
Platelets (K/µL)	1841	2070	2037	1905	1844	72.72	0.653	100 – 2400 K/µL
Reticulocytes (10^{12}/L)	2.35^{b}	2.42^{b}	3.00^{a}	2.83^{a}	3.19^{a}	0.122	0.003	1.5 – 5 × 10^{12}/L
RDW (%)	24.22	26.28	27.28	26.58	26.88	1.463	0.235	20 – 40%

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MSE_{60} = 60 mL moringa seed extract/L drinking water; MSE_{90} = 90 mL moringa seed extract/L drinking water; MSE_{120} = 120 mL moringa seed extract/L drinking water. [2]Parameters: MCH: mean corpuscular hemoglobin; MCHC: mean cell haemoglobin concentration; MCV: mean corpuscular volume; RDW: red cell distribution width. [3]SEM: standard error of mean. [4]Reference range: Talebi et al 2005; Oyeagu et al. (2019); Iyaode et al. (2020); Matshogo et al. (2020); Matshogo et al. (2021).

The GLM results show that there was a significant treatment effect on serum calcium concentrations only (Table 4.8). The birds that were administered with the MSE treatments (MSE_{60}, MSE_{90}, and MSE_{120}) had higher ($p < 0.05$) serum calcium.

Table 4. 8. Serum biochemical parameters of Cobb 500 (n = 225) broilers orally administered moringa seed extract (MSE) through drinking water.

[2]Parameters	[1]Treatment					[3]SEM	*p* value	[4]Reference interval
	NC	PC	MSE_{60}	MSE_{90}	MSE_{120}			
ALB/GLOB	0.60	0.62	0.63	0.63	0.61	0.033	0.355	0.40 - 0.70
ALKP (U/L)	191.2	166.6	148.0	110.6	172.8	30.30	0.604	100 – 900 U/L
ALT (U/L)	11.00	9.40	11.20	12.00	10.20	1.400	0.685	5 – 20 U/L
Albumin (g/L)	20.08	21.48	21.96	21.95	20.37	0.992	0.587	10 – 30 g/L
Amylase (U/L)	464.8	495.4	493.0	491.2	486.8	13.09	0.728	300 – 700 U/L
Cholesterol (mmol/L)	3.750	3.68	3.53	3.49	3.38	0.211	0.854	1.68 - 5.81 mmol/L
Calcium (mmol/L)	1.83^{b}	1.80^{b}	2.39^{a}	2.69^{a}	2.60^{a}	0.109	0.015	1.95 - 2.83 mmol/L
Creatine (µmol/L)	11.23	11.55	11.13	11.39	11.30	0.302	0.184	5 – 40 µmol/L
GGT (U/L)	13.60	14.40	14.80	14.00	13.60	0.967	0.285	5 – 30 U/L
Globulin (g/L)	33.42	34.64	34.71	34.63	33.60	0.618	0.541	28 – 51 g/L
Glucose (mmol/L)	5.72	7.35	6.37	5.75	7.19	0.749	0.845	4.11 - 8.84 mmol/L
Lipase (U/L)	130.0	124.0	118.0	126.0	121.2	6.577	0.432	100 -1000 U/L
Phosphorus (mmol/L)	3.10	3.07	2.96	3.07	2.92	0.276	0.087	1 – 3 mmol/L
SDMA (µg/L)	9.20	8.00	10.00	10.00	8.60	1.219	0.072	0 – 14 µg/L
Total bilirubin (µmol/L)	5.00	3.20	3.80	4.60	3.80	1.038	0.598	0 – 15 µmol/L
Total protein (g/L)	53.49	56.12	56.67	56.58	53.97	1.144	0.415	45 – 89 g/L
Urea (mmol/L)	0.62	0.62	0.67	0.64	0.62	0.038	0.847	0 - 5.70 mmol/L

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MSE_{60} = 60 mL moringa seed extract/L drinking water; MSE_{90} = 90 mL moringa seed extract/L drinking water; MSE_{120} = 120 mL moringa seed extract/L drinking water. [2]Parameters: ALB/GLOB: albumen/globulin; ALKP: alkaline phosphatase; ALT: alanine transaminase; GGT: gamma-glutamyl transferase; SDMA: symmetric dimethylarginine. [3]SEM: standard error of mean. [4]Reference range: Chikumba et al. (2013); Matshogo et al. (2020); Matshogo et al. (2021).

4.4.4 Carcass yield and internal organs weight

The GLM data showed that there were treatment effects ($p < 0.05$) on the dressing percentage, HCW, CCW, bursa, spleen, and thymus weights (Table 4.9). The MSE treatments (MSE_{60}, MSE_{90}, and MSE_{120}) resulted in a higher ($p < 0.05$) dressing percentage than the NC and PC treatments. The birds that were orally administered with the MSE (MSE_{60}, MSE_{90}, and MSE_{120}) treatments had higher ($p < 0.05$) HCW than the birds in PC but were statistically similar ($p > 0.05$) to the birds in NC and MSE_{120}. The NC and PC groups had lower ($p < 0.05$) CCW compared to all the MSE treatment groups, however, PC was statistically similar ($p > 0.05$) with the birds in MSE_{60} and MSE_{120}. Birds on MSE_{60}, MSE_{90}, and MSE_{120} treatments had the heaviest ($p < 0.05$) bursa weight, followed by those on NC, and the lowest ($p < 0.05$) bursa weight was from the PC group. Treatment MSE_{60}, MSE_{90}, and MSE_{120} promoted heavier ($p < 0.05$) spleen weight than the NC and PC groups. Birds on MSE_{120} had the heaviest ($p < 0.05$)

thymus weight, followed by those in MSE_{60} and MSE_{90}, then the NC, and the lowest ($p < 0.05$) was from the PC groups.

Table 4. 9. Carcass yield and internal organs weight (g, unless otherwise stated) of Cobb 500 (n = 225) broilers orally administered moringa seed extract (MSE) through drinking water.

[2]Parameters	[1]Treatment					[3]SEM	*p* value
	NC	PC	MSE_{60}	MSE_{90}	MSE_{120}		
SLWT	1918	1932	1956	1971	1939	21.00	0.154
Dressing percentage (%)	69.61^b	69.39^b	71.37^a	71.43^a	71.34^a	0.270	0.002
HCW	1335^b	1341^b	1396^a	1408^a	1383^{ab}	15.88	0.006
CCW	1295^c	1307^{bc}	1350^{ab}	1365^a	1335^{abc}	15.00	0.011
Breast weight	618.3	694.6	624.4	678.4	633.7	35.40	0.242
Drumstick weight	133.0	151.6	135.8	136.2	137.8	6.630	0.325
Thigh weight	156.5	142.2	160.2	160.7	161.7	16.12	0.415
Gizzard	43.00	44.40	45.90	44.80	45.90	1.140	0.542
Heart	12.20	12.40	12.30	12.80	12.80	0.620	0.652
liver	55.40	55.70	55.10	55.80	49.50	3.010	0.084
Bursa	4.34^b	4.24^c	4.44^a	4.45^a	4.45^a	0.010	0.009
Spleen	3.12^b	3.13^b	3.22^a	3.24^a	3.22^a	0.010	0.012
Thymus	9.62^c	9.57^d	9.67^b	9.68^b	9.71^a	0.010	0.015

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MSE_{60} = 60 mL moringa seed extract/L drinking water; MSE_{90} = 90 mL moringa seed extract/L drinking water; MSE_{120} = 120 mL moringa seed extract/L drinking water. [2]Parameters: SLWT: slaughter weight; HCW: hot carcass weight; CCW: cold carcass weight. [3]SEM: standard error of mean.

4.4.5 Meat quality

There were significant treatment effects on pH_{24hr}, *a**, WHC, shear force, moisture, crude protein, and crude fat of the breast meat (Table 4.10). The breast meat pH_{24hr} and *a** of birds on PC was lower ($p < 0.05$) than those on NC, MSE_{60}, MSE_{90}, and MSE_{120} treatments. Breast meat from MSE_{60} had lower ($p < 0.05$) WHC than those from NC but was similar ($p > 0.05$) to those from PC, MSE_{90}, and MSE_{120}. MSE treatments (MSE_{60}, MSE_{90}, and MSE_{120}) promoted lower ($p < 0.05$) breast meat shear force and moisture than those from NC and PC. The crude protein percentage of breast meat from MSE_{60}, MSE_{90}, and MSE_{120} was highest ($p < 0.05$), followed by those on NC and the lowest ($p < 0.05$) crude protein percentage was from those on PC. The PC treatment promoted the highest ($p < 0.05$) crude fat percentage, followed by NC, and the lowest ($p < 0.05$) crude fat was from the MSE treatments.

Table 4. 10. Meat quality parameters of Cobb 500 broilers (n = 225) orally administered moringa seed extract (MSE) through drinking water.

[2]Parameters	[1]Treatments NC	PC	MSE_{60}	MSE_{90}	MSE_{120}	[3]SEM	*p* value
pH_{45min}	6.84	6.89	6.87	6.86	6.97	0.060	0.315
pH_{24hr}	5.89^{ab}	5.87^{b}	5.96^{ab}	5.89^{ab}	6.01^{a}	0.040	0.022
L^* (lightness)	51.58	52.43	50.74	51.59	51.67	0.810	0.684
a^* (redness)	-0.93^{ab}	-1.22^{b}	-0.55^{ab}	-0.57^{ab}	-0.11^{a}	0.320	0.021
b^* (yellowness)	5.60	5.43	4.63	4.86	5.20	0.770	0.542
WHC (%)	16.40^{a}	12.90^{ab}	11.20^{b}	12.80^{ab}	12.80^{ab}	1.270	0.025
Drip loss (%)	4.79	5.82	4.53	5.19	5.44	0.870	0.485
Cooking loss (%)	38.14	36.59	32.22	32.46	30.48	2.510	0.842
Shear force (N)	3.19^{a}	3.27^{a}	2.240^{b}	2.28^{b}	2.28^{b}	0.190	0.005
Moisture (%)	74.07^{a}	75.93^{a}	72.76^{b}	72.99^{b}	73.00^{b}	0.280	0.004
Crude protein (%)	21.96^{b}	19.85^{c}	23.83^{a}	23.82^{a}	23.78^{a}	0.210	0.019
Crude fat (%)	0.86^{b}	1.58^{a}	0.69^{c}	0.66^{c}	0.69^{bc}	0.060	0.011
Ash (%)	0.73	0.71	0.83	0.76	0.80	0.070	0.621

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MSE_{60} = 60 mL moringa seed extract/L drinking water; MSE_{90} = 90 mL moringa seed extract/L drinking water; MSE_{120} = 120 mL moringa seed extract/L drinking water. [2]Parameters: pH_{45min}: pH at 45 minutes post-mortem; pH_{24hr}: pH at 24-hour post-mortem; WHC: water holding capacity. [3]SEM: standard error of mean.

4.4.6 Intestinal morphology

The GLM results showed that there were treatment effects ($p < 0.05$) on the lengths of the duodenum and ileum, duodenal (villus height, villus width, crypt depth, and muscularis mucosa thickness), jejunal (villus height, villus width, crypt depth, lamina propria thickness, and muscularis mucosa thickness), and ileal (villus height, villus width, muscularis mucosa thickness, and villus height/crypt depth) (Table 4.11). The MSE treatments (MSE_{60}, MSE_{90}, and MSE_{120}) resulted in longer duodenum and ileum lengths ($p < 0.05$) than the NC and PC treatments. The birds that were administered with the MSE treatments (MSE_{60}, MSE_{90}, and MSE_{120}) had higher ($p < 0.05$) duodenal villus height and crypt depth than the birds in PC but were statistically similar ($p > 0.05$) with the birds in NC, MSE_{90}, and MSE_{120}. The NC and PC groups had lower ($p < 0.05$) duodenal villus width and muscularis mucosa thickness compared to all the MSE treatment groups, whose duodenal villus width and muscularis mucosa thickness values did not differ ($p > 0.05$).

Treatment MSE_{60} promoted the highest ($p < 0.05$) jejunal villus height, followed by those in MSE_{90} and MSE_{120}, and the lowest ($p < 0.05$) jejunal villus height was from the NC and PC groups. Birds on MSE_{90} and MSE_{120} had the highest ($p < 0.05$) jejunal villus width, followed by those in MSE_{60}, and the lowest ($p < 0.05$) was from the NC and PC groups. Further, birds reared on NC and PC had lower crypt depth, lamina propria thickness, and muscularis mucosa thickness than those on MSE_{60}, MSE_{90}, and MSE_{120}.

Treatments NC and PC promoted lower ileal villus height than on the MSE_{60}, MSE_{90}, and MSE_{120} treatments, but PC, MSE_{90}, and MSE_{120} were comparable ($p > 0.05$). Birds on MSE_{90} and MSE_{120} had the highest ($p < 0.05$) ileal villus width, followed by those in MSE_{60}, and the lowest ($p < 0.05$) ileal villus width was from the NC and PC groups. The MSE_{60}, MSE_{90}, and MSE_{120} had higher ($p < 0.05$) muscularis mucosa thickness than the NC group. However, PC and MSE_{60} promoted similar ($p > 0.05$) ileal muscularis mucosa thickness. Birds on MSE_{60}, MSE_{90}, and MSE_{120} had the highest ($p < 0.05$) ileal villus height/crypt depth, followed by those on PC, and the lowest ileal villus height/crypt depth was from the NC group.

Table 4. 11. Intestinal morphometric parameters of Cobb 500 (n = 125) broilers orally administered moringa seed extract (MSE) through drinking water.

[2]Parameters	[1]Treatments NC	PC	MSE_{60}	MSE_{90}	MSE_{120}	[3]SEM	*p* value
Duodenum length (cm)	25.81^{bc}	25.34^{c}	26.55^{abc}	27.54^{a}	27.15^{ab}	0.440	0.023
Jejunum length (cm)	67.87	67.12	68.74	69.15	69.47	0.930	0.845
Ileum length (cm)	67.35^{bc}	66.36^{c}	69.52^{ab}	70.71^{a}	69.72^{ab}	0.920	0.034
Duodenum							
Villus height (µm)	1904^{b}	1890^{b}	2009^{a}	1968^{ab}	1955^{ab}	27.30	0.018
Villus width (µm)	162.2^{b}	160.8^{b}	173.3^{a}	181.5^{a}	179.8^{a}	3.400	0.009
Crypt depth (µm)	188.4^{b}	187.4^{b}	196.2^{a}	191.2^{ab}	192.4^{ab}	1.930	0.004
Lamina propria thickness (µm)	125.2	121.6	127.8	135.0	136.8	5.490	0.845
Muscularis mucosa thickness (µm)	49.00^{b}	48.80^{b}	51.70^{a}	52.40^{a}	51.90^{a}	0.960	0.001
Muscularis externa thickness (µm)	247.2	244.2	247.4	256.4	255.4	5.260	0.942
Villus height/Crypt dept ratio	10.21	10.08	10.24	10.30	10.17	0.120	0.584
Jejunum							
Villus height (µm)	1632^{c}	1620^{c}	1739^{a}	1684^{b}	1686^{b}	14.49	0.005
Villus width (µm)	123.4^{c}	126.2^{c}	143.4^{b}	154.6^{a}	151.4^{a}	2.160	0.009
Crypt depth (µm)	163.8^{b}	158.8^{b}	171.6^{a}	169.2^{a}	169.8^{a}	2.180	0.025
Lamina propria thickness (µm)	118.8^{b}	118.6^{b}	121.1^{a}	121.2^{a}	121.0^{a}	0.680	0.005
Muscularis mucosa thickness (µm)	39.20^{b}	38.80^{b}	40.80^{a}	41.00^{a}	40.60^{a}	0.490	0.006
Muscularis externa thickness (µm)	240.4	226.0	233.6	244.4	239.4	5.980	0.752
Villus height/Crypt depth ratio	9.98	10.21	10.13	10.20	10.13	0.120	0.681
Ileum							
Villus height (µm)	1209^{c}	1249^{bc}	1333^{a}	1286^{ab}	1278^{ab}	20.59	0.011
Villus width (µm)	120.7^{c}	123.0^{c}	132.7^{b}	142.9^{a}	142.1^{a}	2.200	0.008
Crypt depth (µm)	126.2	127.0	127.1	126.4	126.2	1.520	0.142
Lamina propria thickness (µm)	127.6	108.4	112.4	119.6	119.4	7.470	0.214
Muscularis mucosa thickness (µm)	34.00^{c}	34.40^{bc}	35.20^{ab}	35.60^{a}	35.80^{a}	0.320	0.001
Muscularis externa thickness (µm)	234.8	235.0	235.4	235.6	235.4	0.410	0.524
Villus height/Crypt depth ratio	9.57^{c}	9.78^{b}	10.13^{a}	10.18^{a}	10.13^{a}	0.060	0.010

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; MSE_{60} = 60 mL moringa seed extract/L drinking water; MSE_{90} = 90 mL moringa seed extract/L drinking water; MSE_{120} = 120 mL moringa seed extract/L drinking water. [3]SEM: standard error of mean.

There were treatment effects ($p < 0.05$) on all the goblet cell count, apart from the duodenal mixed goblet cell count (Table 4.12). Treatment PC promoted lower ($p < 0.05$) duodenal acidic and total goblet cell count than MSE_{90} and MSE_{120} treatments, which did not differ ($p > 0.05$). Birds on MSE_{60} had similar ($p > 0.05$) duodenal acidic and total goblet cell count as birds on NC. The PC treatment promoted lower ($p < 0.05$) acidic, mixed, and total goblet cell count when compared to the MSE treatment (MSE_{60}, MSE_{90}, and MSE_{120}) groups. Birds on MSE_{120} had higher acidic goblet cell count than those on PC and NC groups. Treatment MSE_{60} promoted the same ($p > 0.05$) acidic goblet cell count as PC and NC treatments. However, treatment PC resulted in lower ($p > 0.05$) mixed goblet cell count than treatments MSE_{60}, MSE_{90}, and MSE_{120}, which did not differ ($p > 0.05$). The total goblet cell count of the birds from the MSE treatment (MSE_{60}, MSE_{90}, and MSE_{120}) groups was higher ($p < 0.05$) than those from treatment PC.

Table 4. 12. Goblet cell count (per 100 µm villus height) of Cobb 500 (n = 125) broilers orally administered moringa seed extract (MSE) through drinking water.

		[1]Treatment						
[2]Intestinal segment	[3]Goblet cells	NC	PC	MSE_{60}	MSE_{90}	MSE_{120}	[4]SEM	*p* value
Duodenum	Acidic	46.01^{bc}	45.25^{c}	47.16^{b}	49.55^{a}	48.98^{a}	0.620	0.001
	Mixed	36.80	36.01	37.67	38.59	38.39	1.010	0.254
	Total	82.81^{bc}	81.25^{c}	84.83^{b}	88.15^{a}	87.38^{a}	0.720	0.015
Jejunum	Acidic	50.57^{b}	47.86^{c}	51.04^{ab}	52.33^{a}	51.84^{ab}	0.540	0.003
	Mixed	41.71^{b}	41.29^{b}	44.71^{a}	45.92^{a}	45.29^{a}	0.830	0.007
	Total	92.28^{b}	89.15^{c}	95.75^{ab}	98.25^{a}	97.13^{ab}	1.180	0.004
Ileum	Acidic	50.97^{bc}	49.71^{c}	51.74^{abc}	52.86^{ab}	53.31^{a}	0.700	0.031
	Mixed	43.37^{b}	43.06^{b}	45.30^{ab}	46.28^{a}	45.23^{ab}	0.750	0.005
	Total	94.34^{b}	92.77^{c}	97.04^{a}	99.14^{a}	98.55^{a}	1.110	0.002

[a,b,c] Values within the same row with different subscripts significantly differed ($p < 0.05$). [1]Treatments: NC = drinking water only; PC = 5 g probiotic/L of drinking water; MSE_{60} = 60 mL of moringa seed extract/L of drinking water; MSE_{90} = 90 mL of moringa seed extract/L of drinking water; MSE_{120} = 120 mL of moringa seed extract/L of drinking water. [4]SEM = standard error of the mean.

4.4.7 Bone parameters

There were treatment-induced effects ($p < 0.05$) on tibia morphological parameters, except on diaphysis diameter, femoral side proximal head thickness (FPHT), and metatarsal side proximal head thickness (MPHT) (Table 4.13). The tibial weight of birds in MSE (MSE_{60}, MSE_{90}, and MSE_{120}) groups was heavier ($p < 0.05$) than those on PC. Treatment PC promoted the longest tibia than MSE_{60}, MSE_{90}, and MSE_{120} treatments, which did not differ ($p > 0.05$). The MSE_{120} promoted the highest BBS (282.5 N) compared to PC (246.9 N). Birds on PC had a lower TW/TL index than those from the MSE treatment (MSE_{60}, MSE_{90}, and MSE_{120}) groups. Likewise, PC had lower TMW than the MSE administered groups but had the same ($p > 0.05$) TMW as the birds on MSE_{60}. Birds on NC had a higher TLW than birds on MSE_{60}, MSE_{90}, and MSE_{120}, which had statistically similar TLW values. Birds on PC had a higher ($p < 0.05$) MCD and robusticity index than those on MSE_{60}, MSE_{90}, and MSE_{120}. Birds on PC had a lower tibiotarsal index compared to those on MSE_{90} and MSE_{120}.

Table 4. 13. Tibia morphometric parameters of Cobb 500 broilers (n = 225) orally administered moringa seed extract (MSE) through drinking water.

[2]Parameters	[1]Treatments					[3]SEM	*p* value
	NC	PC	MSE_{60}	MSE_{90}	MSE_{120}		
Tibia weight (g)	10.56^{c}	11.56^{b}	13.50^{a}	14.06^{a}	13.94^{a}	0.330	0.003
Tibia length (mm)	101.5^{ab}	103.5^{a}	97.55^{bc}	95.49^{c}	96.11^{c}	1.500	0.011
Diaphysis diameter (mm)	8.56	8.90	8.64	8.64	8.40	0.180	0.087
FPHT (mm)	26.27	26.12	25.51	25.19	25.61	0.410	0.075
MPHT (mm)	19.52	19.96	19.51	19.30	19.23	0.450	0.168
BBS (N)	242.8^{d}	246.9^{d}	261.0^{c}	273.0^{b}	282.5^{a}	2.170	0.001
TW/TL index (g/mm)	0.10^{b}	0.11^{b}	0.14^{a}	0.15^{a}	0.15^{a}	0.010	0.014
TMW (mm)	1.33^{c}	1.38^{bc}	1.45^{ab}	1.52^{a}	1.50^{a}	0.030	0.009
TLW (mm)	2.24^{b}	2.31^{b}	2.47^{a}	2.55^{a}	2.51^{a}	0.030	0.029
MCD (mm)	5.01^{ab}	5.21^{a}	4.72^{bc}	4.57^{bc}	4.39^{c}	0.160	0.020
Tibiotarsal index	41.61^{c}	41.64^{c}	45.48^{b}	47.12^{ab}	47.81^{a}	0.740	0.003
Robusticity index	21.76^{ab}	22.03^{a}	21.19^{bc}	20.89^{c}	20.98^{c}	0.210	0.005

[a,b,c,d] Values within the same row with different subscripts significantly differed ($p < 0.05$). [1]Treatments: NC = drinking water only; PC = 5 g probiotic/L of drinking water; MSE_{60} = 60 mL of moringa seed extract/L of drinking water; MSE_{90} = 90 mL of moringa seed extract/L of drinking water; MSE_{120} = 120 mL of moringa seed extract/L of drinking water. [2]Parameters: FPHT: Femoral side proximal head thickness; MPHT: Metatarsal side proximal head thickness; BBS: bone breaking strength; TW/TL: tibia weight/tibia length index; TMW: thickness of the medial wall; TLW: thickness of the lateral wall; MCD: Medullary canal diameter. [3]SEM = standard error of the mean.

There were significant treatment effects ($p < 0.05$) on tibia ash and mineral content except for iron (Table 4.14). The tibia ash of birds on PC was lower ($p < 0.05$) than those on MSE_{90} and MSE_{120} treatments. Birds on PC had lower tibia calcium levels than those on MSE_{120}, but not comparable ($p < 0.05$) to MSE_{60} and MSE_{90}. The tibia phosphorus of birds in the PC group was similar ($p > 0.05$) to those in the MSE treatment groups. Birds reared on MSE_{60}, MSE_{90}, and MSE120 had higher ($p < 0.05$) Ca:P than those reared on NC and PC, for which the Ca:P did not differ ($p > 0.05$). Birds reared on MSE_{60}, MSE_{90}, and MSE_{120} had the highest ($p < 0.05$) tibial magnesium and zinc contents followed by those on PC, and the lowest tibial magnesium and zinc concentration were from those on NC.

Table 4. 14. Tibia ash and mineral content of Cobb 500 broilers (n = 225) orally administered with varying levels of moringa seed extract (MSE) in drinking water.

	[1]Treatments						
[2]**Parameters**	**NC**	**PC**	**MSE_{60}**	**MSE_{90}**	**MSE_{120}**	[3]**SEM**	***p* value**
Ash (g)	43.36^d	45.34^c	47.63^b	49.88^a	50.86^a	0.460	0.001
Calcium (mg/g)	214.0^e	222.5^d	238.6^b	236.0^b	240.5^a	0.830	0.001
Phosphorus (mg/g)	114.0^b	118.7^a	119.0^a	117.6^a	118.4^a	2.080	0.005
Ca:P (mg/mg)	1.88^b	1.87^b	2.01^a	2.01^a	2.03^a	0.040	0.010
Magnesium (mg/g)	4.66^c	6.09^b	6.90^a	7.02^a	6.95^a	0.680	0.007
Iron (µg/g)	44.51	46.34	41.51	48.37	50.42	3.480	0.174
Zinc (µg/g)	1.45^c	1.60^b	1.75^a	1.77^a	1.80^a	0.100	0.001

[a,b,c,d,e] Values within the same row with different subscripts significantly differed ($p < 0.05$). [1]Treatments: NC = drinking water only; PC = 5 g probiotic/L of drinking water; MSE_{60} = 60 mL of moringa seed extract/L of drinking water; MSE_{90} = 90 mL of moringa seed extract/L of drinking water; MSE_{120} = 120 mL of moringa seed extract/L of drinking water. [3]SEM: standard error of mean

4.5 Discussion

4.5.1 Growth performance

Tables 4.1 and 4.2 show that MSE contains nutrients, antioxidants, and antimicrobials that can be used for the benefit of efficiency in feed utilization and performance in poultry. The increment in the final weight, weight gain, and ADWG noted at the starter phase may be due to the oral MSE administration that improved the functioning of the gut. This explains the better FCR noted between birds administered MSE_{60} and MSE_{120}, suggesting enhanced nutrient digestibility because phytogenic plants are known to decrease the population of pathogenic bacteria in the chicken gut (Hassan et al., 2015). At the finisher phase, birds on MSE_{60} and MSE_{90} had increased weight gain and ADWG, which was achieved with reduced feed intake, and ADFI, which may imply better nutrient digestibility which led to improved FCR among the MSE groups. The observed similar water intake and ADWI for birds administered MSE_{60}, MSE_{120}, PC, and NC suggest that MSE administration did not compromise the birds' water intake.

The present study demonstrated that the oral administration of MSE gave similar overall final body weight (FBW) and body weight gain (BWG). This finding is supported by the earlier reports of Chamorro et al. (2013) and Farahat et al. (2017), who administered grape seed extracts to broiler chickens in their study. Wang et al. (2008) also noted an increased BWG when 5 to 80 ppm of grape seed extracts were administered to broilers. A contrasting result from An et al. (2015) documented a decreased growth performance when onion extracts at 0.3% or 0.5% were administered to white mini broilers. The differences in results from the oral administration of phytogenic plant extracts in the various trials could be attributed to the different route of administration, solvent of extraction, and concentration of bioactive compounds. Brenes et al. (2008) demonstrated that a higher dosage of polyphenols could interact with proteins (of either dietary or endogenous origin, including digestive enzymes) and form tannin–protein complexes that decrease the efficiency of nutrient absorption, thereby compromising broiler growth performance. The MSE used in this study was obtained through a water extraction process, and Diehl et al. (2020) opined that the extraction solvent has an impact on the phytochemical profile of the resulting extract; a water-based extract generally contains lower molecular weight phytochemicals, while organic extraction solvents tend to have higher molecular weight. Higher molecular weight bioactive compounds are thought to be less bioavailable in the intestine (Chalvon-Demersay et al., 2021). Therefore, the extraction solvent and the resultant phytochemical content of the MSE may play a substantial role in the biological activity of the extract. The concentration of polyphenols in the present study at the highest dosage of MSE_{120} did not decrease the growth of the broilers.

The reduced feed intake (FI) observed among MSE supplemented groups may possibly be related to water intake (WI), which was statistically the same among all treatments. Studies have shown that moringa seed is rich in amino acids (Mune et al., 2016), antioxidants such as ascorbic acids, carotenoids, and flavonoids (Liang et al., 2019), and broilers consume feed to meet their nutrient and metabolizable energy requirements. Hence, the WI of the MSE administered groups may possibly have met these daily requirements of broilers, which was the reason for the observed decreased FI among them. Furthermore, earlier reports have shown that seeds of phytogenic plants induce antibacterial and anti-coccidiosis actions (Panggabean et al., 2019) that promote the growth of beneficial bacteria, inactivate pathogenic bacteria, and facilitate bioavailability of nutrients and absorption in the gastrointestinal tract (Adu et al., 2020) to enhance the growth performance of broiler chickens.

4.5.2 Nutrient digestibility

The MSE showed a rich composition of minerals, vitamins, and bioactive compounds, and these concentrations increased with increasing levels of the dosage per litre of drinking water. The nutraceutical composition of MSE shows its potential as a feed additive. Nutrient digestibility is the portion of feedstuff that is absorbed into the body of an animal upon consumption. In this study, the administration of MSE in the drinking water of Cobb 500 broiler did not vary dry matter, crude protein, and ether extract digestibility, and these findings differ with the report of Rezvani et al. (2018), who observed that administering pomegranate seed extract increased ether extract but decreased dry matter and crude protein digestibility. Attia et al. (2017) also reported the increased dry matter, crude protein, and ether extract digestibility in their blend of plant extracts containing oregano, fenugreek, chamomile, and fennel when administered to broilers. Brenes et al. (2008) noted no variation in crude protein digestibility of broilers administered grape pomace concentrate and vitamin E. Generally, the binding of polyphenolic compounds to nutrients has been used to explain the differences in nutrient digestibility in polyphenol-containing additives. Indeed, there is a negative relationship that exists between polyphenols and dietary protein due to the interaction of their reactive hydroxyl groups with the carbonyl group (Attia et al., 2017). The differences in results observed between the present study and earlier reports could be attributed to different sources of the extracts as well as the concentration of polyphenols in the experimental extracts.

The increased calcium and phosphorus digestibility noted among the MSE groups compared to the control groups implies that phytogenic extracts can increase mineral (calcium and phosphorus) digestibility by stimulating bile secretion, increasing pancreatic and intestinal enzyme secretion, and/or decreasing harmful bacteria in the gut. They also increase the efficiency of mineral absorption because of the presence of bioactive compounds (Banerjee et al., 2013; Reddy et al., 2014). These bioactive compounds reduce the microbial load in the gut, hence decreasing the host-microbial competition for nutrients (Attia et al., 2017).

4.5.3 Blood parameters

The pathophysiological responses of broiler chickens to the environmental variations (feed, disease, temperature, etc.) are reflected by their blood profile (Ologhobo et al., 2014; Oyeagu et al., 2016). Thus, due to the presence of nutraceuticals in MSE, its oral administration was hypothesized to improve haematological and serum biochemical parameters of the birds. However, no treatment-induced changes were noted for all haematological parameters, except for haematocrits, and reticulocytes. The increased haematocrit and reticulocyte count were within the normal ranges for clinically healthy broilers (Talebi et al., 2005; Oyeagu et al., 2019; Iyaode et al., 2020; Matshogo et al., 2020; Matshogo et al., 2021). The finding of this study is not supported by that of Gultepe et al. (2019), who noted an increased neutrophil count with differences among treatments for other haematogical parameters measured when *Myrtus communis L* extract was administered in drinking water for laying hens. Alimohamadi et al. (2013) reported increased RBC, haemoglobin, and haematocrit counts while all other haematological parameters statistically remained the same among treatments when black seed and cumin seed extracts were administered to broilers.

This result perhaps suggests that oral administration of MSE favours red blood cell formation as well as packed cell volume. Findings from this study agreed with Hassan et al. (2018) that noted no significant variations among treatments for mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV). This shows the importance of MSE as a potential nutraceutical for poultry since it did not compromise the health status of the chicken.

Also, Hussein and Jassim (2019) noted increased PCV and haemoglobin when ethanol extracted moringa was administered to broiler chickens for 42 days. MSE contains iron required for normal physiological functions such as blood formation. Likewise, Abdulazeez et al. (2016) observed an increased RBC in broilers fed diets containing baobab seed meal for 56 days. It can therefore be inferred that the differences in observations noted between this present study and others may be attributed to different routes of delivery of the phytogenic additive, the concentration of the bioactive content, sex, and age of the birds.

The serum biochemical index values obtained in this study are within the normal blood range for clinically healthy chickens as reported in other documented similar studies (Chikumba et al., 2013; Matshogo et al., 2020; Matshogo et al., 2021). This result is not in agreement with the earlier report of Abdulazeez et al. (2016), who noted no statistical difference in serum biochemical parameters of broilers measured except for glucose concentration, which decreased with the inclusion of baobab seed meal. This variation in findings may be a result of the low level of soluble carbohydrates and high fibre content of baobab seed meal, which led to the hypoglycemic condition of the birds (Oladunjoye et al., 2014). Fu et al. (2021) reported no difference among treatments for serum biochemistry parameters measured for geese-fed diets containing rapeseed meals. Aguilar et al. (2011) reported a decrease in glucose concentration with the inclusion of squash seed meal in the diets of broilers for 49 days. Waheed et al. (2017) reported a decrease in cholesterol concentration when black cumin seed extracts were administered to broilers for 35 days. In relation to this study, Rafeeq et al. (2021) noted no variation among treatments for total protein, albumin, globulin, albumin/globulin, and alanine transaminase when cumin and jir seed extracts were administered to broilers for 42 days.

Furthermore, liver enzymes such as alkaline phosphatase (ALKP), alanine transaminase (ALT), and gamma-glutamyl transferase (GGT) concentrations were not elevated among the MSE-administered groups, suggesting normal liver functioning. Albumin and total protein concentration did not decrease among the MSE administered group, implying good nourishment and quality protein supply. The birds orally administered MSE had the same levels of creatinine as the NC and PC groups, which implies normal renal functioning and protein muscular metabolism. The same levels of creatinine were reported by Manyeula et al. (2019) when canola seed meal was included in indigenous chicken strain diets. The numerically elevated serum calcium in the present study inferred that moringa seeds are a relatively good source of essential minerals (Ijarotimi et al., 2013). Summarily, water extraction of MSE and subsequent administration through the drinking water allows nutrients' bioavailability and permeability into the bloodstream for onward utilization of the birds.

This study is at variance with earlier studies that reported reduced serum cholesterol when moringa seed meal was supplemented in the diets of quail (Ashour et al., 2020) and broilers (Sugiharto and Toana, 2021).

4.5.4 Carcass yield and internal organs weight

Carcass yield is an essential yardstick for determining the growth performance and economic returns of broiler meat (Yu et al., 2020). The increase in dressing percentage, hot carcass weight (HCW), and cold carcass weight (CCW) for MSE supplemented birds despite the similar slaughter weight values among all the treatments suggests better muscle and bone development than the control groups. This assumption can be made because the main components of the carcass are flesh (muscle) and bone, after the visceral organs and inedible portion must have been removed. This result was not consistent with the earlier report by Sugiharto and Toana (2021), who reported similarities among treatments for dressing percentage, HCW, and CCW when moringa seed meal was included in the broiler diet. Contrary to this study, the findings of Ashom et al. (2014) noted a similarity among dietary treatments for dressing percentage when processed roselle seed meal was supplemented in the diets of broilers at the finishing stage. Also, the study of Manyeula et al. (2019) reported a decrease in the HCW but not the CCW of indigenous chickens when 37.5 g/kg of canola seed meal was included in their diets. The difference in results can be attributed to the route of administering the phytogenic additives in the trials.

Suliman et al. (2021) found that increasing amounts of clove seeds supplemented in the diet of broilers for 39 days resulted in a lower dressing percentage. Contrarily, Tariq et al. (2015) noted an increase in the dressing percentage and breast weight of chickens fed clove seed meal. Azadegan et al. (2014) observed no significant increase in dressing percentage and HCW for broilers supplemented with clove oil. In their study, Dalkiliç and Güler (2009) reported increased carcass traits of broilers administered up to 0.04% clove seed extracts. The discrepancy in results can be attributed to the concentration of nutrients and bioactive compounds in the tested phytogenic plant, dosages administered, route of administration, and extraction process.

The increase in CCW observed in this study among the MSE-administered group suggests increased edible meat after storage, which is a positive trait. Also, prime portions like drumsticks, breasts, and thigh weight were not decreased by the administration of MSE, since they were comparable to the control groups. This finding is consistent with previous studies on phytogenic plant additives in broilers (Ashom et al., 2014; Akuru et al., 2021,).

In this study, the weights of the gizzard, heart, and liver were the same among treatments and it contradicts the results of Karayagız and Bulbul (2015) and Manyeula et al. (2019). The similarity recorded on the internal organs of birds may be attributed to the elimination of the fibre content of moringa seed through water extraction into MSE, as it has been noted by Matshogo et al. (2021b) that a direct relationship exists between the fibre content of the additive and the size of internal organs. The size of the internal organs is an essential determinant of organ physiological function, and the bioactive compounds of MSE did not decrease the organogenetic development of the birds (Mahfuz and Piao, 2019). The liver is generally responsible for the secretion of ALKT, ALT, and GGT enzymes. The similarities recorded for serum ALKT, ALT, and GGT among treatments obtained in this study justifies the lack of difference in the liver weight since there is a correlation between the size of the organ and the concentration of enzymes secreted into the blood (Matshogo et al., 2020). This finding is in tandem with that of Qin et al. (2017), who found no increase in the liver size of ducklings supplemented with rapeseed meals for up to 30% for 21 days. Ashom et al. (2014) observed increased gizzard weights for birds fed diets containing boiled and soaked roselle seed meal.

The weights of the bursa, spleen, and thymus were elevated in this study, which implies a positive immune response to the nutraceutical content of MSE, indicating that immune function was increased (Miao et al., 2020). Fu et al. (2021) in their study with geese observed an increase only in the bursa but not in the spleen and thymus weight, which may be attributed to the high fibre content of the rapeseed meal used in their study. Hajati et al. (2018) noted increased thymus weight but no variation in the bursa and spleen weight when grape seed extracts were administered to birds. The zinc, flavonoids, and phenols present in MSE promote lymphocyte proliferation and differentiation in the bursa, spleen, and thymus (Zafarnejad et al., 2017), which is important in both cellular and humoral immunity of broilers. It is the increased humoral immunity that may have led to the increased weight noted among the MSE groups (M'Sadeq et al., 2018).

4.5.5 Meat quality

The oral administration of MSE increased the pH_{24hr} and this finding is supported by Liu et al. (2020), who administered chestnut wood extract to broilers for 42 days. The pH_{24hr} values generally suggest the rate of carcass glycolysis and are linked with meat shelf life. The pH_{24hr} was ascertained by the amount of glycogen before slaughter and its conversion to lactic acid after slaughter. Hence, oxidation tends to occur quickly in meat with a reduced pH_{24hr} value (Ozturk et al., 2011). In the present study, the elevated pH_{24hr} of the meat reduced the loss of soluble nutrients, resulting in higher meat quality among the MSE-administered groups. Observation showed that meat samples from the PC treatment had the lowest pH_{24hr} and redness (a^*) values, which supports previous studies that light-coloured broiler meat samples had reduced pH_{24hr} (Yang et al., 2020). In contrast with the present study, Liu et al. (2020) observed comparable effects on meat colour in broilers offered chestnut extract in drinking water and diet. Also, Rezar et al. (2017) reported a similar meat colour profile when hydrolysable tannins were administered in drinking water and diets.

Some authors (Bak et al., 2012) have previously implied that meat colour is impacted by various factors such as pigment level, pigment oxidative status, postmortem glycolysis, and intramuscular fat (IMF) content. Considering this, bioactive compounds in MSE may decrease energy catabolism in meat. An elevated amount of glycolysis relates to reduced myoglobin concentration, which possibly results in paler meat, while decreased carbohydrate oxidation is associated with redder meat and muscle colour stability (Zhou et al., 2021), this justifies the increased redness value in MSE supplemented groups.

Meat producers value the water holding capacity (WHC) because it plays an essential part in determining the ultimate weight of the meat. The increased WHC through oral administration of MSE_{90} and MSE_{120} agrees with the findings of Shen et al. (2019), who administered bamboo leaf extracts to broilers. In contrast, the significant difference in WHC in meat observed in this study is not in agreement with some published reports (Moraes et al., 2016; Drażbo et al., 2019). Increased meat WHC is known to reduce lipid oxidation (rancidity) by inactivating free radicals present in stored meat (Huang and Ahn, 2019) WHC is impacted by rigor mortis, pH, osmotic pressure, and sarcomere length by changing the cellular and extracellular components (Gopinger et al., 2014), which leads to increased muscle tenderness and appearance. Barcenilla et al. (2022) reported that low WHC results in higher cooking losses and vice versa. It can therefore be inferred that administration of MSE supports increased WHC, hence more water is held between myofibrils, and this would be highly acceptable to the consumers.

Present findings show that shear force values reduced with MSE administration, suggesting that the meat became tenderer with the supplementation of MSE, and this observation was not consistent with the report of Park et al. (2014). They reported no linear or quadratic effect on the shear force when *Saposhnikovia divaricata*, *Lonicera japonica*, or *Chelidonium majus* extracts were administered for 35 days on broiler chickens. On the other hand, Shen et al. (2019) noted statistical variation among treatments for shear force, which agrees with this present study. The differences observed in data among these trials can be attributed to differences in various plants used and dosages of the phytogenic additive.

Generally, the proximate composition of meat will depend on the feed quality, the age of the birds, genetics, sex, management, and environmental conditions. In the present study, the significant moisture content is not consistent with the findings of Yang et al. (2020). Furthermore, Jang et al. (2008) found no difference in meat moisture, crude protein, crude fat, or ash content after 35 days of supplementing broiler diets with medicinal herb extract. The increased crude protein content observed agrees with Rahman and Kim (2016) and Xu et al. (2021). They reported increased crude protein in pork and chicken meat when proanthocyanidin extract and *Nigella sativa* seed were supplemented in pig and broiler diets, respectively. In this study, the reduced moisture and crude fat content from birds administered MSE is not consistent with the inverse relationship between meat moisture and fat content reported by (Ahmed et al., 2016), who supplemented 0.4% pomegranate seeds in natural or fermented form as part of the basal diet of grower-finisher pigs. The reduced moisture content of the meat in the MSE supplemented groups can be said to influence the high WHC of the meat observed.

4.5.6 Intestinal morphology

Structures of the intestinal morphology can to an extent ascertain the rate of digestion and absorption of nutrients in broiler chickens. A contrasting result to the increased duodenum and ileum length was documented by Hajati et al. (2015). They supplemented grape seed extract and vitamin C in the diets of broiler chickens for 42 days. The length of the duodenum and ileum segments of the small intestine might have increased in response to the oral administration of MSE considering it contains dissolved bioactive compounds that are permeable, which can increase the viscosity of the digesta. Accelerated viscosity of the digesta can lengthen the segments of the small intestine by exerting a large amount of pressure on the lining of the small intestine, prompting the muscle layers of the intestinal wall to relax. This relaxation leads to an increase in the length of the sarcomere and consequently makes the myofibrils of the muscle stiffer to make up for the pressure (Bederska-Łojewska et al., 2017; Jha et al., 2019). Also, the viscosity may have increased the length of the small intestine since a greater surface area is required to satisfy broiler chickens' need for nutrients (Kaczmarek et al., 2016; Rezaei et al., 2018).

Nutrient absorption from the small intestine is actualized by specialized structures known as the villi (Li et al., 2017; Groff-Urayama et al., 2019). The increased villus height in this study may suggest an increased surface area for efficient nutrient absorption (Marcos et al., 2019). The crypt can be regarded as the villus workshop, and deep crypts suggest fast tissue turnover and a high requirement for new tissue (Wawrzyniak et al., 2017; Kim et al., 2018). Therefore, the increased crypt depth in this study implies acceleration in the replacement of enterocytes, which make up the walls of the villi (Marcos et al., 2019). The increased duodenal villus height, width, and crypt depth in this study are consistent with Carboni et al. (2020), who administered teff seed extracts to broilers.

Likewise, the elevated jejunal villi height, width, and crypt depth did not agree with the report of Ao and Kim (2020), who reported that grape seed extract (0.02%) increased jejunal villi height and decreased crypt depth. The noted increased ileal villi height and width in this study is comparable to the findings of Rafeeq et al. (2021). They evaluated the effect of an ethanolic extract of cumin seed on Hubbard broilers. (Li et al., 2020) reported that *Macleaya cordata* extract administration on weaned pigs for 28 days had no effect on jejunal villi height and crypt depth but increased duodenal, ileal height, and crypt depth. Pereira da Silva et al. (2019) noted that the administration of chia seed soluble extracts, regardless of the concentration used, increased intestinal villus surface area, villus length, and width.

The absorption capacity of the small intestine is also determined by the villus height/crypt depth ratio (Kargopoulos et al., 2018). Optimum digestion and absorption are attained when the villus height/crypt depth ratio is increased (Dawood and Koshio, 2020). The increased ileal villus height/crypt depth ratio is not consistent with the observations of Li et al. (2020). Li et al. (2020) noted that villus height/crypt depth ratio was decreased at the duodenal, increased at the ileal, and had no effect at the jejunal section. Farahat et al. (2021) reported increased jejunal villus height/crypt depth ratio but no effect on the duodenal and ileal villus height/crypt depth ratio when plant extracts were administered to broilers. The increased villus height/crypt depth ratio induced an intestinal structure more oriented to digestion, with improved absorptive and hydrolysis potential, and requiring fewer nutrients to be directed towards intestinal maintenance (Dawood and Koshio, 2020). Therefore, with MSE administration, the intestinal structure of the small intestine was enhanced for the birds, which may help to explain the reduced overall feed intake and overall FCR observed for birds administered MSE.

Furthermore, observation indicated that administration of MSE elevated muscularis mucosa thickness in the three segments of the small intestine and lamina propria thickness in the jejunum, which is consistent with earlier reports (Pereira da Silva et al., 2019; Rafeeq et al., 2021). This further justifies the reduced FCR noted among the MSE-administered groups. The lamina propria and muscularis mucosa are reliable in moving digested and absorbed nutrients into the bloodstream. Therefore, MSE potentially increased butyrate production, which may have led to enterocyte proliferation (Bogucka et al., 2016). Additionally, the soluble extract of moringa seed contains phenolic compounds, which can influence intestinal morphology (Akbarian et al., 2013).

Maximizing broiler chicken physiological performance is impossible without considering their digestive tract health, which is a complex, multifaceted system that takes several small intestine features into consideration (such as the microbiota and mucin dynamics) (Qureshi et al, 2016). Broiler gastrointestinal health is influenced by inherent (age, sex, breed) and extraneous (diet, environment) variables, thereby affecting the birds' performance (Mogire et al., 2021). Except for the mixed goblet cell count in the duodenum, there was an increase in the goblet cell count in the small intestine that did not agree with an earlier report by Carboni et al. (2020). They reported that teff seed extract increased duodenal neutral and mixed goblet cell counts. However, Tan et al. (2018) noted an increased goblet cell count when dandelion root extract was administered to gold pompanos fish. Pereira da Silva et al. (2019) recorded increased acidic, neutral, and mixed goblet cell counts in all three segments of the small intestine.

These findings of the present study imply that MSE administration increased mucus production, which protects the intestinal lumens as supported by Celi et al. (2017). They reported an increased goblet cell count in broiler chickens supplemented with plant extracts.

As earlier suggested, this may increase the intestinal digestive and absorptive capacity and possibly indirectly increase the bioavailability of nutrients, as suggested by the effects noted on the morphometric parameters (Han et al., 2017; Hou and Tako, 2018; Ma et al., 2018). The increase in the acidic goblet cells, containing acidic mucin due to the administration of MSE_{90} and MSE_{120}, may have contributed to the reduction of intestinal pH. The administration of MSE_{90} and MSE_{120} may lead to increased solubilization and uptake of nutrients and an improved intestinal microbial profile (Tako et al., 2014).

4.5.7 Bone parameters

The structure of bones and their morphometric parameters as well as their structural properties are critical parameters in ascertaining bones' ability to perform their basic functions, which are to give structural support and ensure locomotor skills (Muszyński et al., 2019). Furthermore, bones are also an essential mineral source for metabolic activities (Shah et al., 2019). Nutritional elements are the dominant factors impacting bone development and mechanical strength. Although factors such age, disease, and gender are equally significant, they can predispose the bone to fractures (Damaziak et al., 2019).

The observed increase in tibia morphometric parameters and BBS is however not consistent with the report of Leskovec et al. (2017) who reported a negative effect of olive leaf and marigold extracts on tibia morphometric parameters and BBS. Abbas and Khauoon (2021) noted increased tibia length but no effect on other tibia morphometric parameters when grape seed extract was administered in broiler diets. The elevated bone weight, BBS, and tibiotarsal index in the current study may possibly be attributed to the presence of flavonoids, minerals, vitamins, and antioxidants in MSE. Similarly, Hohman and Weaver (2015) reported an increase in tibia morphometric parameters and BBS when grape seed extracts were administered to Sprague Dawley rats.

Shen et al. (2012) explained that the presence of antioxidants in plants could lead to low oxidative stress in the bones and potentially benefit bone health. Furthermore, orally administered proanthocyanidin, from grape seed extract increased the bone health of mice (Tenkumo et al., 2020). Shah et al. (2019) noted differences in the thickness of the wall, medullary canal diameter, and tibiotarsal index, which supports the findings of this study. Moringa polyphenols can positively affect tibia morphometric parameters and strength by protecting the bone through various mechanisms. Through their antioxidative and anti-inflammatory activities, polyphenols can improve osteoblast genesis, suppress osteo-clast genesis, and increase osteoimmunological action (Leskovec et al., 2017). Polyphenol-rich plants, like dried plums (Franklin et al., 2006), blueberries (Devareddy et al., 2008), green, black, red, and white tea (Shen et al., 2012; Tomaszewska et al., 2016), have demonstrated positive effects on bone health in different rat models.

The obtained tibia ash and mineral composition results are not in agreement with the report of Liu et al. (2015) who administered extracts of *Herba epimedii* and *Ligustri lucidi* in diets of Wistar rats and observed a lack of dietary effects in ash content and mineral contents (Ca, P, K, Mg, Mn, and Zn). Calcium and phosphorus are the most abundant minerals in bones, and their distribution influences the formation and mineralization of bone (Moyo et al., 2021). The observed increase in tibia ash and mineral composition could point to better mineral bioavailability due to the increased absorption of water-soluble nutrients from MSE. The increased concentration of tibia calcium, phosphorus, Ca:P ratio, magnesium, and zinc of birds administered with MSE may possibly be due to the increased mineral absorption. Abdullah et al. (2022) stated that magnesium enriches bone formation by activating osteoclasts, which increases the Ca:P ratio (Skřivan et al., 2020). The increase in zinc concentration could indicate the stimulation of DNA production in osteoblasts, thereby increasing bone weight and the absorption of calcium ions in the bones (Boskovic et al., 2020).

4.6 Conclusion

This study revealed that the oral administration of *Moringa oleifera* seed extracts has the potential for use as an alternative to prophylactic antibiotics in Cobb 500 broilers. The administration of *Moringa oleifera* seed extracts in the drinking water has a positive effect on feed conversion ratio, calcium and phosphorus digestibility, some blood parameters, carcass yield, meat quality, intestinal morphology, and bone parameters of Cobb 500 broilers.

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CHAPTER 5: EFFECT OF MORINGA OLEIFERA LEAF + SEED EXTRACTS ON PHYSIOLOGICAL AND MEAT QUALITY RESPONSES OF COBB 500 BROILER CHICKENS

Abstract

The effects of a combination of *M. oleifera* leaf and seed extract (MLSE) as an alternative to antimicrobial growth promoter (AGP) on Cobb 500 broiler chicken growth performance, nutrient digestibility, carcass yield, meat quality, intestinal morphology, and bone parameters were studied. 250 one-day-old male Cobb 500 broiler chicks (57.80 ± 0.17 live weight) were randomly allocated to one of five treatments, with five replicates per group and 10 chicks per replication. Commercial starter (1–21 d) and finisher (22–42 d) diets were provided to the chicks. The treatments were negative control with water only (NC), positive control with 5g multi-strain probiotic (5×10^8 CFU/g) per litre of drinking water (PC), 60 mL of MLSE per litre of drinking water ($MLSE_{60}$), 90 mL of MLSE per litre of drinking water ($MLSE_{90}$), and 120 mL of MLSE per litre of drinking water ($MLSE_{120}$). For 42 days, treatments and commercial feeds were available ad libitum. $MLSE_{90}$ and $MLSE_{120}$ both showed an increase in overall body weight gain (BWG). Oral administration of MLSE significantly ($p < 0.05$) decreased overall feed conversion ratio (FCR) and feed intake (FI). Birds on PC presented the lowest ash, calcium, and phosphorus digestibility, while NC and PC had comparable ether extract digestibility. Birds on NC and MLSE had similar haematocrits counts. MLSE administration increased ($p < 0.05$) reticulocyte count and serum calcium concentration. $MLSE_{90}$ and $MLSE_{120}$ increased ($p < 0.05$) slaughter weight (SLWT). MLSE groups had the highest dressing percentage and hot carcass weight when compared to the NC and PC groups, while $MLSE_{120}$ had the highest cold carcass weight (CCW), while NC and PC were the lowest. PC and MLSE had similar thigh weights. $MLSE_{90}$ and $MLSE_{120}$ increased ($p < 0.05$) bursa and thymus weights, while $MLSE_{90}$ had the highest spleen weight when compared with other treatments. Oral administration of MLSE increased meat crude protein while decreasing moisture and crude fat content. $MLSE_{90}$ and $MLSE_{120}$ had the longest ($p < 0.05$) duodenum, jejunum, and ileum lengths. The intestinal parameters were significantly ($p < 0.05$) enhanced with the administration of $MLSE_{90}$ and $MLSE_{120}$. $MLSE_{90}$ and $MLSE_{120}$ administration increased tibia weight. Bone breaking strength (BBS), tibia weight/tibia length index (TW/TL), and tibiotarsal index were all increased by MLSE. NC and PC had similar values for the thickness of the medial wall (TMW) and the thickness of the lateral wall (TLW), while $MLSE_{90}$ and $MLSE_{120}$ had similar medullary canal diameters (MCD). It was concluded that oral

administration of $MLSE_{90}$ improves growth performance, nutrient digestibility, blood parameters, carcass yield, meat quality, intestinal morphology, and bone parameters of Cobb 500 broiler chickens.

Keywords: Growth performance, Nutrient digestibility, Blood parameters, Carcass yield, Internal organs, Intestinal morphology, Bone parameters.

5.1 Introduction

One of the most prominent consumer concerns regarding meat production and consumption is the possible impact of antibiotic usage on animal farms on public health. Antibiotics' ability to stimulate growth and maintain health in animals and birds at subtherapeutic doses was discovered in the late 1940s (Yang et al., 2022). Antibiotic growth promoters (AGPs) have been regular practice throughout time (Anwar et al., 2017), contributing significantly to the profitability of poultry production (He et al., 2021). It was postulated that antibiotic growth promoters modify the intestinal microflora, resulting in an optimum microbiome for improved nutrient digestibility and hence improved physiological performance (Mohamed et al., 2021). However, the widespread use of antibiotics for prophylaxis and growth enhancement has aided in the formation of antibiotic-resistant bacteria (Attia et al., 2017). Antibiotics as an AGP for poultry have been banned in various countries, including the EU, the United States, Canada, Mexico, Japan, Hong Kong, and China (Elnesr et al., 2022). However, study results show that removing AGPs from chicken feed reduces performance in several regions (Shafiee et al., 2020). Unfortunately, because of the high frequency of pathogens in these places, the use of AGPs remains widespread in tropical nations, while research on safe alternatives continues to stagnate (Mahlake et al., 2021). As a result, for sustainable chicken production, safe and reliable plant therapeutics with physiological performance improvement, meat-enhancing, and antimicrobial properties must be researched and investigated (Pham et al., 2022).

Due to their natural, low-toxicity, and no-residue qualities, *M. oleifera* leaf and seed are being explored as potential antioxidant and antibiotic alternatives (Akinyeye et al., 2014; Adejumo et al., 2016; Mune et al., 2016) that can improve broiler performance and the quality of meat. *M. oleifera* leaf and seed can alter growth performance and intestinal morphology (Khan et al., 2017), meat quality and morphometric characteristics of bone (Rehman et al., 2018), carcass traits and meat cholesterol (Sugiharto and Toana, 2021). These favorable benefits might be attributed to the presence of bioactive chemicals in *M. oleifera* leaf and seed, such as flavonoids, phenols, and alkaloids (Rockwood et al., 2013). Furthermore, these leaves and seeds contain protein, carbohydrates, vitamins, calcium, phosphorus, magnesium, selenium, and zinc, which may be used for sustained broiler production (Liang et al., 2019; Bhalla et al., 2021). Notwithstanding the nutraceutical advantages of *M. oleifera* leaf and seed, no research has been conducted to study the influence of their combination on Cobb 500 broiler physiological and meat quality responses. As a result, this study from the standpoint of cost, using water as a solvent for the extraction of the bioactive substance in *M. oleifera* leaf and seed, and administering MLSE through drinking water, is based on *M. oleifera* leaf and seed rich in biologically active substances and biological functions. The purpose of this study was to assess the effects of MLSE on Cobb 500 broiler chicken growth performance, nutrient digestibility, blood parameters, carcass yield, meat quality, intestinal morphology, and bone parameters, as well as the feasibility of substituting antibiotics with MLSE.

5.2 Materials and Methods

5.2.1 Study site

Same as described in Chapter 3.

5.2.2 Sources of *Moringa oleifera* and preparation of the leaf + seed extract

The *M. oleifera* leaf and seed powder were sourced and individually prepared same as in Chapters 3 and 4 respectively. Commercial starter (1–21 days) and finisher (22–42 days) diets and probiotic were acquired from the same sources as in Chapter 3. The calculated metabolizable energy and analysed nutritional composition of the diets is shown in Table 3.1. The extracts were administered into the fresh drinking water that was offered to the birds daily during the study as follows: negative control with water only (NC), positive control with 5 g probiotic per liter of drinking water (PC), 30 mL of moringa leaf extract + 30 mL of moringa seed extract per litre of drinking water ($MLSE_{60}$), 45 mL of moringa leaf extract + 45 mL of moringa seed extract per litre of drinking water ($MLSE_{90}$), and 60 mL of moringa leaf extract + 60 mL of moringa seed extract per litre of drinking water ($MLSE_{120}$).

5.2.3 Experimental design and birds’ management

Same as earlier described in Chapter 3.

5.2.4 Chemical analysis of *Moringa oleifera* leaf + seed extract treatments

Same as earlier described in Chapter 3.

The alkaloids, carbohydrates, flavonoids, phenols, protein, saponins, steroids, tannins, and terpenoids content of MLSE are shown in Table 5.1.

Table 5. 1. Phytochemical content (mg/L) of *Moringa oleifera* leaf + seed extract treatments.

	[1]Treatments		
Phytochemicals	**MLSE$_{60}$**	**MLSE$_{90}$**	**MLSE$_{120}$**
Alkaloids	8.56	9.26	9.780
Carbohydrates	29.00	35.60	40.60
Flavonoids	3.82	4.52	5.18
Phenols	18.24	18.70	19.92
Protein	32.76	33.58	34.92
Saponins	6.18	6.60	7.04
Steroids	4.48	5.00	5.60
Tannins	50.20	53.00	56.80
Terpenoids	18.52	19.10	19.68

[1]Treatments: MLSE$_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; MLSE$_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; MLSE$_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water.

The calcium, magnesium, phosphorus, potassium, zinc, iron, sodium, vitamin A, B1, B2, B3, B6, B12, C, and E are shown in Table 5.2.

Table 5. 2. Mineral and vitamin composition (mg/L) of *Moringa oleifera* leaf + seed extract treatments.

	[1]Treatments		
Micronutrients	**MLSE$_{60}$**	**MLSE$_{90}$**	**MLSE$_{120}$**
Calcium	1376	1400	1450
Magnesium	239.8	244.8	253.0
Phosphorus	312.8	317.2	320.2
Potassium	1371	1380	1389
Zinc	2.14	2.19	2.22
Iron	14.53	14.72	14.96
Sodium	202.8	220.0	231.8
Vitamin A	10.75	11.03	11.64
Vitamin B1	1.42	1.54	1.73
Vitamin B2	14.40	14.70	14.86
Vitamin B3	4.56	4.84	5.10
Vitamin B6	3.06	3.22	3.44
Vitamin B12	1.66	1.72	1.88
Vitamin C	12.68	13.02	13.50
Vitamin E	464.0	482.0	500.0

[1]Treatments: MLSE$_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; MLSE$_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; MLSE$_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water.

5.2.5 Growth performance

Same as earlier described in Chapter 3.

5.2.6 Apparent nutrient digestibility trial

Same as earlier described in Chapter 3.

5.2.7 Proximate analysis

Same as earlier described in Chapter 3.

5.2.8 Blood collection and analysis

Same as earlier described in Chapter 3.

5.2.9 Carcass yield and internal organs weight

Same as earlier described in Chapter 3.

5.2.10 Meat quality

Same as earlier described in Chapter 3.

5.2.11 Intestinal morphology

Same as earlier described in Chapter 3.

5.2.12 Bone parameters

Same as earlier described in Chapter 3.

5.3 Data Analysis

Same as earlier described in Chapter 3.

5.4 Results

5.4.1 Growth performance

At the starter phase (Table 5.3), there were no treatment effects ($p > 0.05$) on feed intake, ADFI, water intake, and ADWI. The final weight, weight gain, and ADWG were increased ($p < 0.05$) among birds offered PC and the MLSE supplemented groups, while those on NC had the lowest mean value. Also, the best ($p < 0.05$) FCR was recorded for birds offered the MLSE while those in NC had poor ($p < 0.05$) FCR.

Table 5. 3. Starter phase performance in Cobb 500 broilers orally administered moringa leaf + seed extract (MLSE) through drinking water.

[2]Parameters	[1]Treatment					[3]SEM	*p* value
	NC	PC	$MLSE_{60}$	$MLSE_{90}$	$MLSE_{120}$		
Initial weight (g/bird)	57.65	57.75	57.80	57.90	58.00	0.170	0.084
Final weight (g/bird)	778.8^{b}	823.8^{ab}	853.2^{a}	868.8^{a}	893.1^{a}	23.80	0.023
Weight gain (g/bird)	721.1^{b}	766.0^{ab}	795.4^{a}	810.9^{a}	835.1^{a}	23.80	0.036
ADWG (g/bird)	34.34^{b}	36.48^{ab}	37.88^{a}	38.61^{a}	39.77^{a}	1.130	0.014
Feed intake (g/bird)	1381	1401	1355	1381	1389	49.40	0.259
ADFI (g/bird)	65.76	66.73	64.50	65.77	66.14	2.350	0.348
Water intake (mL/bird)	2525	2510	2458	2497	2482	50.20	0.471
ADWI (mL/bird)	120.2	119.5	117.0	118.9	118.2	2.390	0.692
FCR (g/g)	1.92^{a}	1.83^{b}	1.70^{c}	1.70^{c}	1.66^{c}	0.030	0.005

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; $MLSE_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; $MLSE_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; $MLSE_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water. [2]Parameters: ADWG: average daily weight gain; ADFI: average daily feed intake; ADWI: average daily water intake; FCR: feed conversion ratio. [3]SEM: standard error of mean.

At the finisher phase, weight gain, ADWG, water intake, and ADWI were not affected ($p > 0.05$) by the oral administration of MLSE (Table 5.4). The birds offered $MLSE_{90}$ and $MLSE_{120}$ had the highest ($p < 0.05$) final weights compared to those in other treatment groups. The birds offered MLSE treatment had the lowest ($p < 0.05$) feed intake and ADFI when compared to those on NC and PC groups. Birds offered $MLSE_{120}$ had the best ($p < 0.05$) FCR, which was comparable to the PC, $MLSE_{60}$, and $MLSE_{120}$ groups, while poor ($p < 0.05$) FCR was recorded for those supplemented with NC.

Table 5. 4. Finisher phase performance in Cobb 500 broilers orally administered moringa leaf + seed extract (MLSE) through drinking water.

[2]Parameters	[1]Treatment					[3]SEM	*p* value
	NC	PC	$MLSE_{60}$	$MLSE_{90}$	$MLSE_{120}$		
Final weight (g/bird)	1876^{c}	1898^{bc}	1930^{bc}	1943^{ab}	1991^{a}	17.72	0.015
Weight gain (g/bird)	1035	1008	997	994	1007	19.34	0.137
ADWG (g/bird)	49.29	47.98	47.32	47.47	47.96	0.920	0.753
Feed intake (g/bird)	2086^{a}	1977^{b}	1934^{bc}	1911^{c}	1885^{c}	19.82	0.005
ADFI (g/bird)	99.32^{a}	94.13^{b}	92.11^{bc}	90.98^{c}	89.76^{c}	0.940	0.005
Water intake (mL/bird)	3473	3458	3394	3366	3375	82.30	0.063
ADWI (mL/bird)	165.4	164.7	161.6	160.3	160.7	3.920	0.075
FCR (g/g)	2.02^{a}	1.96^{ab}	1.95^{ab}	1.93^{ab}	1.87^{b}	0.040	0.001

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; $MLSE_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; $MLSE_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; $MLSE_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water. [2]Parameters: ADWG: average daily weight gain; ADFI: average daily feed intake; ADWI: average daily water intake; FCR: feed conversion ratio. [3]SEM: standard error of mean.

Overall, WI and ADWI were not influenced ($p > 0.05$) by the treatment groups (Table 5.5). The overall FBW, BWG, and ADWG were highest ($p > 0.05$) when $MLSE_{90}$ and $MLSE_{120}$ were administered, while the lowest ($p > 0.05$) value was recorded for NC group. The highest ($p > 0.05$) overall FI and ADFI were recorded for birds offered NC and PC, while the other treatment groups had the lowest ($p > 0.05$) mean values. The birds offered NC and PC had poor ($p > 0.05$) overall FCR, while those offered $MLSE_{120}$ had a better ($p > 0.05$) FCR.

Table 5. 5. Overall growth performance in Cobb 500 broilers orally administered moringa leaf + seed extract (MLSE) through drinking water.

	[1]Treatment						
[2]Parameters	NC	PC	MSE_{60}	MSE_{90}	MSE_{120}	[3]SEM	*p* value
Initial BW (g/bird)	57.65	57.75	57.80	57.90	58.00	0.170	0.084
FBW (g/bird)	1876^{c}	1898^{bc}	1930^{bc}	1943^{ab}	1991^{a}	17.72	0.015
WG (g/bird)	1819^{c}	1840^{bc}	1872^{bc}	1885^{ab}	1933^{a}	17.72	0.015
ADWG (g/bird)	43.30^{c}	43.82^{bc}	44.58^{bc}	44.89^{ab}	46.01^{a}	0.420	0.011
FI (g/bird)	3467^{a}	3378^{ab}	3289^{b}	3292^{b}	3274^{b}	44.70	0.005
ADFI (g/bird)	100.6^{a}	95.36^{ab}	91.42^{bc}	88.92^{c}	89.63^{c}	1.810	0.009
WI (mL/bird)	5998	5968	5852	5863	5857	117.0	0.472
ADWI (mL/bird)	198.4	171.4	181.6	186.8	191.4	8.860	0.456
FCR (g/g)	1.91^{a}	1.84^{ab}	1.76^{bc}	1.75^{bc}	1.69^{c}	0.030	0.005

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; $MLSE_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; $MLSE_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; $MLSE_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water. [2]Parameters: ADWG: average daily weight gain; ADFI: average daily feed intake; ADWI: average daily water intake; FCR: feed conversion ratio. [3]SEM: standard error of mean.

5.4.2 Nutrient digestibility

The results presented in Table 5.6 showed that the supplementation of MLSE had no significant ($p > 0.05$) influence on dry matter, crude protein, NDF, and ADF digestibility. The birds on varying levels of MLSE supplementation had the best ether ($p < 0.05$) extract digestibility when compared to the control treatments. The digestibility of calcium was improved ($p < 0.05$) with the supplementation of $MLSE_{90}$ and $MLSE_{120}$, with the PC group having the lowest ($p < 0.05$) value. Birds in the MLSE groups had the highest ($p < 0.05$) phosphorus digestibility, while the PC depicted the lowest ($p < 0.05$) value.

Table 5. 6. Nutrient digestibility (%) in Cobb 500 broilers (n = 125) orally administered moringa leaf + seed extract (MLSE) through drinking water.

[2]Parameters	[1]Treatments NC	PC	MLSE60	MLSE90	MLSE120	[3]SEM	p value
Dry matter	64.53	64.27	65.29	65.53	65.21	0.450	0.064
Crude protein	65.81	66.02	67.17	68.73	67.98	1.000	0.405
Ether extract	60.11[b]	61.28[b]	63.73[a]	64.15[a]	65.15[a]	0.500	0.012
NDF	62.67	63.46	64.38	63.69	64.00	1.120	0.435
ADF	64.31	63.44	65.16	63.77	63.91	0.570	0.524
Calcium	57.81[c]	55.12[d]	62.96[b]	64.33[a]	64.12[a]	0.310	0.475
Phosphorus	56.52[b]	54.66[c]	61.24[a]	62.46[a]	62.86[a]	0.550	0.011

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; $MLSE_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; $MLSE_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; $MLSE_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water. [2]Parameters: NDF: neutral detergent fibre; ADF: acid detergent fibre. [3]SEM: standard error of mean

5.4.3 Blood parameters

Results presented in Table 5.7 showed that the various supplementation levels of MLSE had no influence ($p > 0.05$) on most haematological indices except haematocrits and reticulocyte concentrations of the broiler chickens. The birds on $MLSE_{120}$ presented the highest (27.80%) haematocrit concentrations, which were comparable to the mean values from birds on NC (24.44%), $MLSE_{60}$ (24.84%), and $MLSE_{90}$ (27.10%). The blood samples from the MLSE groups had higher ($p < 0.05$) mean values for the reticulocytes, while those from NC (2.31×10^{12}/L) recorded the lowest ($p < 0.05$) reticulocytes which was comparable to those from the PC group (2.46×10^{12}/L).

Table 5. 7. Haematological parameters of Cobb 500 broilers (n = 225) orally administered moringa leaf + seed extract (MLSE) through drinking water.

[2]Parameters	[1]Treatment					[3]SEM	*p* value	[4]Reference interval
	NC	PC	$MLSE_{60}$	$MLSE_{90}$	$MLSE_{120}$			
Basophils (10^9/L)	0.17	0.18	0.16	0.18	0.17	0.020	0.884	0.10 - 2 × 10^9/L
Eosinophils (10^9/L)	0.41	0.50	0.42	0.44	0.42	0.060	0.355	0.20 - 4 × 10^9/L
Haematocrits (%)	24.44^{ab}	20.52^{b}	24.48^{ab}	27.10^{a}	27.80^{a}	2.020	0.022	28 - 48 %
Haemoglobin (g/dL)	8.44	7.28	8.06	9.44	9.80	0.840	0.644	7 - 13 g/dL
Lymphocytes (10^9/L)	73.12	68.50	69.59	66.04	65.16	5.520	0.234	50 – 300 × 10^9/L
MCH (Wideman et al.)	37.20	29.72	28.58	31.26	31.75	3.240	0.119	30 – 80 pg
MCHC (%)	34.30	35.50	32.90	34.60	35.20	0.100	0.142	32 – 42 %
MCV (fL)	107.8	83.84	86.71	89.82	89.95	8.000	0.335	80 – 120 fL
Monocytes (10^9/L)	10.34	10.78	10.36	10.82	11.59	2.440	0.076	1 – 30 × 10^9/L
Neutrophils (10^9/L)	3.02	2.84	2.87	2.79	2.88	0.080	0.059	1 – 4 × 10^9/L
Platelets (K/µL)	1873	2035	2036	1935	1924	54.00	0.437	100 – 2400 K/µL
Reticulocytes (10^{12}/L)	2.31^{b}	2.46^{b}	2.83^{a}	3.04^{a}	3.08^{a}	0.110	0.005	1.5 – 5 × 10^{12}/L
RDW (%)	24.78	25.88	27.10	26.58	26.32	1.520	0.628	20 – 40%

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; $MLSE_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; $MLSE_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; $MLSE_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water. [2]Parameters: MCH: mean corpuscular haemoglobin; MCHC: mean cell haemoglobin concentration; MCV: mean corpuscular volume; RDW: red cell distribution width. [3]SEM: standard error of mean. [4]Reference range: Talebi et al 2005; Oyeagu et al. (2019); Iyaode et al. (2020); Matshogo et al. (2020); Matshogo et al. (2021).

Data presented in Table 5.8 showed that most of the serum biochemical constituents considered were not influenced ($p > 0.05$) by the different levels of MLSE, except for serum calcium concentration, which was impacted ($p < 0.05$) by the various levels of MLSE supplementation. The lowest mean ($p < 0.05$) mean values for calcium concentration in the blood was observed in birds offered PC and NC, while those offered $MLSE_{90}$ and $MLSE_{120}$ had the highest ($p < 0.05$) comparable means.

Table 5. 8. Serum biochemistry of Cobb 500 broilers (n = 225) orally administered moringa leaf + seed extract (MLSE) through drinking water.

[2]Parameters	[1]Treatment NC	PC	$MLSE_{60}$	$MLSE_{90}$	$MLSE_{120}$	[3]SEM	*p* value	[4]Reference interval
ALB/GLOB	0.62	0.63	0.63	0.66	0.62	0.020	0.159	0.40 - 0.70
ALKP (U/L)	200.8	187.2	174.0	131.2	192.8	27.70	0.147	100 – 900 U/L
ALT (U/L)	12.20	10.60	12.40	13.20	11.40	1.530	0.258	5 – 20 U/L
Albumin (g/L)	21.34	22.55	22.69	23.47	21.58	1.060	0.357	10 – 30 g/L
Amylase (U/L)	404.8	435.4	433.0	431.2	426.8	58.00	0.488	300 – 700 U/L
Cholesterol (mmol/L)	3.35	3.28	3.13	3.09	2.98	0.350	0.677	1.68 - 5.81 mmol/L
Calcium (mmol/L)	1.98^c	1.88^c	2.43^b	2.740^a	2.63^{ab}	0.080	0.005	1.95 - 2.83 mmol/L
Creatine (µmol/L)	9.93	10.13	9.83	10.06	10.00	1.520	0.742	5 – 40 µmol/L
GGT (U/L)	11.80	12.60	13.00	12.20	11.80	1.830	0.652	5 – 30 U/L
Globulin (g/L)	34.42	35.64	36.06	35.63	34.60	1.310	0.678	28 – 51 g/L
Glucose (mmol/L)	5.48	6.95	5.83	5.69	6.740	0.800	0.542	4.11 - 8.84 mmol/L
Lipase (U/L)	142.0	136.0	128.0	138.0	133.2	16.76	0.845	100 -1000 U/L
Phosphorus (mmol/L)	2.50	2.470	2.360	2.47	2.32	0.380	0.418	1 – 3 mmol/L
SDMA (µg/L)	10.00	8.80	10.60	10.20	9.40	1.060	0.624	0 – 14 µg/L
Total bilirubin (µmol/L)	6.60	4.80	5.40	6.20	5.40	1.400	0.234	0 – 15 µmol/L
Total protein (g/L)	55.76	58.19	58.75	59.10	56.18	2.130	0.438	45 – 89 g/L
Urea (mmol/L)	0.68	0.68	0.72	0.69	0.67	0.040	0.557	0 - 5.70 mmol/L

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; $MLSE_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; $MLSE_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; $MLSE_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water. [2]Parameters: ALB/GLOB: albumen/globulin; ALKP: alkaline phosphatase; ALT: alanine transaminase; GGT: gamma-glutamyl transferase, SDMA: symmetric dimethylarginine. [3]SEM: standard error of the mean. [4]Reference range: Chikumba et al. (2013); Matshogo et al. (2020); Matshogo et al. (2021).

5.4.4 Carcass yield and internal organs weight

The carcass yield of broilers orally administered with varying levels of MLSE was presented in Table 5.9. The breast weight and drumstick weight were not significantly ($p > 0.05$) influenced by the various levels of MLSE supplementation, while SLWT, dressing percentage, HCW, CCW, and thigh weight differ ($p < 0.05$) across the treatment groups. The carcass from $MLSE_{120}$ and $MLSE_{90}$ had the best ($p < 0.05$) SLWT, while the lowest ($p < 0.05$) mean value was obtained from the NC groups. The carcass from $MLSE_{90}$ (71.50%), $MLSE_{60}$ (71.49%), and $MLSE_{120}$ (71.35%) had higher ($p < 0.05$) dressing percentages, while PC (69.74%) and NC (69.97%) had similarly low values. The HCW was elevated ($p < 0.05$) in carcass from the MLSE supplemented groups compared to control groups (NC and PC). Also, the best ($p < 0.05$) CCW was observed among carcass from $MLSE_{120}$, while those from the NC and PC recorded the lowest ($p < 0.05$) CCW. Furthermore, the carcass from the PC and MLSE supplemented groups had the highest ($p < 0.05$) mean value for thigh weight, while those from NC had the lowest ($p < 0.05$) thigh weight.

Results presented in table 5.9 showed that the supplementation of MLSE did not influence ($p > 0.05$) the weights of the gizzard, heart, and liver measured. The supplementation of various levels of MLSE, however, influenced ($p < 0.05$) the weight of all the lymphoid organs measured (Table 5.9). It was observed that the administration of $MLSE_{90}$ recorded a higher ($p < 0.05$) bursa weight, which was comparable to those from $MLSE_{120}$, while those from other treatments recorded the lowest ($p < 0.05$) bursa weight. Similarly, higher ($p < 0.05$) spleen weight was recorded in the $MLSE_{90}$ supplemented groups, while those from NC and PC presented the least ($p < 0.05$) mean values. Also, the thymus weight for the control groups had the lowest ($p < 0.05$) mean values when compared to the oral administration of $MLSE_{90}$ and $MLSE_{120}$.

Table 5. 9. Carcass yield and internal organs weight (g, unless otherwise stated) of Cobb 500 broilers (n = 225) orally administered moringa leaf + seed extract (MLSE) through drinking water.

	[1]Treatment						
[2]Parameters	**NC**	**PC**	**$MLSE_{60}$**	**$MLSE_{90}$**	**$MLSE_{120}$**	**[3]SEM**	***p* value**
SLWT	1876^{c}	1898^{bc}	1930^{bc}	1943^{ab}	1991^{a}	17.72	0.007
Dressing percentage (%)	69.97^{b}	69.74^{b}	71.49^{a}	71.50^{a}	71.35^{a}	0.220	0.005
HCW	1313^{b}	1324^{b}	1380^{a}	1389^{a}	1420^{a}	13.21	0.011
CCW	1277^{c}	1287^{c}	1337^{b}	1344^{b}	1388^{a}	14.56	0.021
Breast weight	647.7	660.0	688.9	655.4	662.0	16.64	0.348
Drumstick weight	134.9	136.2	144.9	136.7	140.1	4.020	0.774
Thigh weigh	158.7^{b}	165.2^{ab}	170.7^{a}	170.9^{a}	167.8^{ab}	3.370	0.009
Gizzard	47.50	46.90	44.00	46.00	44.50	2.400	0.522
Heart	12.60	12.60	13.30	12.20	12.10	0.560	0.423
liver	56.40	55.90	53.30	50.50	52.40	2.790	0.478
Bursa	4.56^{b}	4.57^{b}	4.66^{b}	4.94^{a}	4.84^{a}	0.060	0.005
Spleen	3.48^{c}	3.51^{c}	3.56^{bc}	3.720^{a}	3.61^{b}	0.030	0.005
Thymus	9.19^{c}	9.24^{bc}	9.40^{b}	9.79^{a}	9.75^{a}	0.060	0.005

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; $MLSE_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; $MLSE_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; $MLSE_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water. [2]Parameters: SLWT: slaughter weight; HCW: hot carcass weight; CCW: cold carcass weight. [3]SEM: standard error of mean.

5.4.5 Meat quality

The supplementation of different levels of MLSE did not significantly ($p > 0.05$) influence pH_{45min}, pH_{24hr}, lightness (L^*), redness (a^*), yellowness (b^*), WHC, drip loss, cooking loss, shear force, and ash (Table 5.10). The breast samples from the PC and NC groups had the highest ($p < 0.05$) moisture content, while those from MLSE supplementation had the lowest ($p < 0.05$) mean value. Crude protein was highest ($p < 0.05$) for breast samples from MLSE administered groups, while the lowest ($p < 0.05$) mean value was recorded for the PC group. The breast samples from the PC presented the highest ($p < 0.05$) mean values for crude fat, while those on MLSE supplementation had the comparably lowest ($p < 0.05$) values.

Table 5. 10. Meat quality parameters of Cobb 500 broilers (n = 225) orally administered moringa leaf + seed extract (MLSE) through drinking water.

[2]Parameters	[1]Treatments NC	PC	$MLSE_{60}$	$MLSE_{90}$	$MLSE_{120}$	[3]SEM	*p* value
pH_{45min}	6.59	6.41	6.52	6.60	6.58	0.180	0.194
pH_{24hr}	5.96	5.95	5.85	5.98	5.90	0.040	0.438
L^* (lightness)	51.52	52.68	51.91	49.04	49.90	1.250	0.667
a^* (redness)	-1.69	-1.79	-1.52	-1.70	-1.64	0.410	0.521
b^* (yellowness)	4.23	6.21	4.50	4.03	4.15	0.770	0.588
WHC (%)	13.30	12.90	9.20	15.50	12.70	2.340	0.488
Drip loss (%)	3.50	4.59	3.12	4.08	5.01	0.790	0.235
Cooking loss (%)	32.18	35.23	34.06	28.22	33.37	4.310	0.315
Shear force (N)	1.99	1.93	1.87	1.92	1.90	0.050	0.337
Moisture (%)	74.31^a	75.92^a	72.95^b	72.13^b	72.33^b	0.200	0.005
Crude Protein (%)	22.75^b	19.71^c	24.51^a	24.48^a	24.54^a	0.210	0.005
Crude fat (%)	0.90^b	2.09^a	0.65^c	0.66^c	0.65^c	0.060	0.005
Ash (%)	0.91	0.76	0.93	1.07	0.96	0.100	0.687

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; $MLSE_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; $MLSE_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; $MLSE_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water. [2]Parameters: [2]pH_{45min}: pH at 45mins post-mortem; pH_{24hr}: pH at 24hr post-mortem; WHC: water holding capacity. [3]SEM: standard error of mean.

5.4.6 Intestinal morphology

The various levels of MLSE supplementation showed a difference ($p < 0.05$) in the duodenum, jejunum, and ileum length, as shown in Table 4.11. It was observed that birds offered $MLSE_{90}$ and $MLSE_{120}$ had the longest ($p < 0.05$) duodenum, jejunum, and ileum length, while those from the NC were the shortest in these three segments of the small intestine. In the duodenum segment of the small intestine, the villus height was highest ($p < 0.05$) when $MLSE_{90}$ and $MLSE_{120}$ were supplemented, while NC had the lowest ($p < 0.05$) mean value. Furthermore, villus width was increased ($p < 0.05$) with oral administration of $MLSE_{90}$ and $MLSE_{120}$, when compared to other treatment groups. Also, at this duodenum, the samples from the PC group had the lowest ($p < 0.05$) villus height/crypt depth ratio when compared to those from the $MLSE_{90}$ and $MLSE_{120}$ administered groups. For the jejunum segment, the villus height of birds offered NC was lower ($p < 0.05$) when compared to those from the $MLSE_{90}$ and $MLSE_{120}$ groups. The villus width was lowest for birds offered NC, PC, and $MLSE_{60}$, while an increased

($p < 0.05$) villus width was recorded for those that received $MLSE_{90}$ and $MLSE_{120}$. Furthermore, the crypt depth was lower ($p < 0.05$) for birds offered NC compared to those from $MLSE_{120}$, while the villus height/crypt depth ratio was highest ($p < 0.05$) at $MLSE_{90}$ supplementation. At the ileum section of the gut, the villus height was increased ($p < 0.05$) for samples from the MLSE supplemented groups, while those from the NC and PC groups were lower. Also, the oral administration of $MLSE_{90}$ and $MLSE_{120}$ had the highest ($p < 0.05$) villus width and crypt depth when compared to other treatment groups. The villus from the NC group had the lowest muscularis mucosa thickness when compared to those in other groups. For the villus height/crypt depth ratio, the samples from PC were the lowest ($p < 0.05$) compared to those in other treatment groups.

Table 5. 11. Intestinal morphometric parameters of Cobb 500 broilers (n = 125) orally administered moringa leaf + seed extract (MLSE) through drinking water.

	[1]Treatments						
[2]Parameters	NC	PC	$MLSE_{60}$	$MLSE_{90}$	$MLSE_{120}$	[3]SEM	*p* value
Duodenum length (cm)	25.19^{d}	25.97^{c}	26.80^{b}	27.75^{a}	27.31^{ab}	0.190	0.005
Jejunum length (cm)	64.94^{d}	65.84^{c}	66.82^{b}	67.91^{a}	67.22^{ab}	0.250	0.005
Ileum length (cm)	65.44^{d}	66.58^{c}	67.48^{b}	68.41^{a}	67.75^{ab}	0.260	0.011
Duodenum							
Villus height (µm)	1713^{c}	1741^{bc}	1780^{b}	1862^{a}	1862^{a}	15.94	0.005
Villus width (µm)	147.3^{b}	140.8^{b}	153.2^{b}	172.8^{a}	170.8^{a}	4.450	0.005
Crypt depth (µm)	188.6	190.8	191.2	191.4	192.0	3.010	0.668
Lamina propria thickness (µm)	173.4	174.2	190.0	218.6	214.2	19.18	0.748
Muscularis mucosa thickness (µm)	44.80	44.80	0.045	47.40	46.80	0.940	0.551
Muscularis externa thickness (µm)	242.6	242.0	245.4	248.0	247.0	1.910	0.625
Villus height/Crypt depth ratio	9.240^{ab}	9.000^{b}	9.310^{ab}	9.740^{a}	9.700^{a}	0.170	0.003
Jejunum							
Villus height (µm)	1375^{b}	1413^{ab}	1433^{ab}	1479^{a}	1473^{a}	24.90	0.008
Villus width (µm)	138.1^{b}	135.9^{b}	145.1^{b}	163.3^{a}	160.0^{a}	3.340	0.009
Crypt depth (µm)	167.8^{b}	168.8^{ab}	170.4^{ab}	170.6^{ab}	172.0^{a}	1.150	0.011
Lamina propria thickness (µm)	179.2	179.6	205.2	216.2	212.8	15.93	0.258
Muscularis mucosa thickness (µm)	37.80	39.80	41.60	45.00	44.00	2.250	0.357
Muscularis externa thickness (µm)	237.4	236.6	236.2	244.0	242.0	2.960	0.159
Villus height/Crypt depth ratio	8.190^{b}	8.380^{ab}	8.410^{ab}	8.670^{a}	8.560^{ab}	0.130	0.025
Ileum							
Villus height (µm)	1063^{b}	1025^{b}	1100^{ab}	1173^{a}	1173^{a}	31.90	0.014
Villus width (µm)	141.4^{b}	142.8^{b}	145.2^{b}	159.7^{a}	157.4^{a}	1.900	0.035
Crypt depth (µm)	135.0^{d}	136.0^{c}	137.6^{b}	138.2^{ab}	138.8^{a}	0.330	0.015
Lamina propria thickness (µm)	167.8	170.6	206.6	213.6	210.4	17.49	0.094
Muscularis mucosa thickness (µm)	37.40^{b}	38.40^{ab}	38.80^{ab}	41.00^{a}	40.20^{ab}	1.000	0.026
Muscularis externa thickness (µm)	226.0	223.6	230.6	240.8	239.6	5.300	0.251

Villus height/Crypt depth ratio	7.87^{ab}	7.54^{b}	7.99^{ab}	8.49^{a}	8.45^{a}	0.230	0.018

[a,b,c,d] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; $MLSE_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; $MLSE_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; $MLSE_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water. [3]SEM: standard error of mean.

Results presented in Table 5.12 showed that jejunal and ileal segments of the small intestine from birds offered $MLSE_{90}$ and $MLSE_{120}$ had the highest ($p < 0.05$) acidic goblet cell counts when compared to the other groups. The mixed goblet cell count at the jejunum segment of the small intestine was lowest for birds offered PC treatment compared to those in other treatments. Also, the total goblet cell count was highest ($p < 0.05$) for villus samples from $MLSE_{90}$ and $MLSE_{120}$ when compared with the other treatments. The oral administration of $MLSE_{90}$ and $MLSE_{120}$ had comparable higher ($p < 0.05$) mean values for mixed goblet cell count in the ileum segment of the small intestine, while those from NC, PC, and $MLSE_{60}$ had similar values. Furthermore, the PC group had the lowest total goblet cell count ($p < 0.05$), whereas $MLSE_{90}$ and $MLSE_{120}$ administration yielded comparable higher ($p < 0.05$) goblet cell counts values.

Table 5. 12. Goblet cell count (per 100 µm villus height) of Cobb 500 broilers (n = 125) orally administered moringa leaf + seed extract (MLSE) through drinking water.

		[1]Treatment						
[2]Intestinal segment	**[3]Goblet cells**	**NC**	**PC**	**$MLSE_{60}$**	**$MLSE_{90}$**	**$MLSE_{120}$**	**[4]SEM**	***p* value**
Duodenum	Acidic	52.94	51.55	54.27	54.98	54.68	1.090	0.753
	Mixed	39.18	39.75	38.66	41.45	39.92	2.620	0.951
	Total	92.12	91.30	92.93	96.43	94.60	1.670	0.852
Jejunum	Acidic	57.17^{b}	55.55^{b}	59.66^{b}	68.19^{a}	66.75^{a}	1.470	0.005
	Mixed	43.56^{ab}	42.69^{b}	45.41^{ab}	52.07^{a}	51.80^{a}	2.850	0.008
	Total	100.7^{b}	98.20^{b}	105.1^{b}	120.3^{a}	118.6^{a}	3.310	0.005
Ileum	Acidic	57.78^{b}	51.29^{c}	60.96^{b}	70.84^{a}	68.68^{a}	2.130	0.011
	Mixed	47.28^{c}	47.51^{c}	50.44^{bc}	60.82^{a}	57.44^{ab}	2.930	0.041
	Total	105.1^{bc}	98.80^{c}	111.4^{b}	131.7^{a}	126.1^{a}	3.170	0.005

[a,b,c] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; $MLSE_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; $MLSE_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; $MLSE_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water. [4]SEM: standard error of mean.

5.4.7 Bone parameters

The result from Table 5.13 showed was no difference ($p > 0.05$) for diaphysis diameter, FPHT, MPHT, and robusticity index of the tibia that were measured. The tibia weight was significantly ($p < 0.05$) influenced by varying oral administration of MLSE. Tibia weight was lowest ($p < 0.05$) for birds offered NC administration while $MLSE_{90}$ supplementation promoted the highest

($p < 0.05$) weight of 14.75g, which was comparable to those offered $MLSE_{120}$. The various levels of MLSE affected ($p < 0.05$) the tibia length, with NC being the longest (103.67mm) while those on $MLSE_{90}$ were the shortest (95.12mm). The BBS was affected ($p < 0.05$) by the MLSE offered, with MLSE supplemented groups being the strongest when compared with the NC and PC. Observations also indicated that the TW/TL index was highest ($p < 0.05$) in the tibia from MLSE groups while those from the NC and PC had the lowest values. The highest ($p < 0.05$) mean values for TMW and TLW were obtained when $MLSE_{120}$ and $MLSE_{90}$ were administered, whereas the lowest ($p < 0.05$) comparable mean values were observed in the tibia of the NC, which were comparable to the PC. However, the MCD of the tibia of the birds offered the different levels of MLSE supplementation was significantly ($p < 0.05$) reduced when compared with NC and PC. The highest ($p < 0.05$) tibiotarsal index values were obtained in tibia of birds offered MLSE compared to the control groups.

Table 5. 13. Tibia morphometric parameters of Cobb 500 broilers (n = 225) orally administered moringa leaf + seed extract (MLSE) through drinking water.

[2]Parameters	[1]Treatments					[3]SEM	*p* value
	NC	**PC**	**$MLSE_{60}$**	**$MLSE_{90}$**	**$MLSE_{120}$**		
Tibia weight (g)	10.75^{d}	12.00^{c}	13.75^{b}	14.75^{a}	14.25^{ab}	0.210	0.005
Tibia length (mm)	103.7^{a}	101.9^{b}	97.66^{c}	95.12^{d}	96.40^{c}	0.440	0.033
Diaphysis diameter (mm)	8.58	8.90	9.05	8.48	8.95	0.210	0.349
FPHT (mm)	26.27	26.12	25.57	25.48	25.59	0.460	0.854
MPHT (mm)	19.52	19.96	18.74	19.32	18.79	0.410	0.772
BBS (N)	253.4^{b}	254.1^{b}	261.5^{ab}	276.3^{a}	278.1^{a}	6.230	0.024
TW/TL (g/mm)	0.09^{b}	0.12^{b}	0.14^{a}	0.16^{a}	0.15^{a}	0.010	0.017
TMW (mm)	1.260^{c}	1.33^{c}	1.56^{b}	1.63^{ab}	1.69^{a}	0.030	0.005
TLW (mm)	2.25^{c}	2.30^{c}	2.55^{b}	2.64^{ab}	2.72^{a}	0.040	0.003
MCD (mm)	5.06^{a}	5.27^{a}	4.95^{ab}	4.21^{c}	4.54^{bc}	0.170	0.041
Tibiotarsal index	40.98^{b}	40.93^{b}	45.36^{a}	50.48^{a}	49.37^{a}	0.720	0.026
Robusticity index	31.84	21.82	21.21	20.84	21.02	4.370	0.427

^{a,b,c,d} Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; $MLSE_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; $MLSE_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; $MLSE_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water. [2]Parameters: FPHT: Femoral side proximal head thickness; MPHT: Metatarsal side proximal head thickness; BBS: bone breaking strength; TW/TL: tibia weight/tibia length index; TMW: thickness of the medial wall; TLW: thickness of the lateral wall; MCD: Medullary canal diameter. [3]SEM: standard error of mean.

Table 5.14 presented the tibia ash and mineral composition of the bones measured in broilers administered MLSE. All parameters considered were significantly ($p < 0.05$) influenced by the supplementation of different levels of MLSE except for the iron content of the bone. The highest ($p < 0.05$) mean value for ash and calcium concentration was obtained when $MLSE_{120}$

and $MLSE_{90}$ were supplemented, whereas the lowest ($p < 0.05$) comparable mean values were observed in the NC and PC groups. The phosphorus concentration of the tibia observed in $MLSE_{120}$ was the highest ($p < 0.05$), which was comparable to the NC and $MLSE_{90}$ groups, while those from PC and $MLSE_{60}$ gave the lowest ($p < 0.05$) mean values. Similarly, treatment effects were observed for the Ca:P, with the oral administration of various levels of MLSE recording the highest ($p < 0.05$) and the lowest ($p < 0.05$) observed with samples from the PC and NC groups. Finally, the administration of the NC presented the lowest ($p < 0.05$) mean values for magnesium and zinc concentrations in the tibia, while the highest ($p < 0.05$) mean values were recorded with the PC and the MLSE supplemented groups.

Table 5. 14. Tibia ash and mineral composition of Cobb 500 broilers (n = 225) orally administered moringa leaf + seed extract (MLSE) through drinking water.

	[1]Treatments						
[2]Parameters	NC	PC	$MLSE_{60}$	$MLSE_{90}$	$MLSE_{120}$	SEM	*p* value
Ash (g)	43.82^{d}	45.65^{c}	47.88^{b}	50.15^{a}	51.18^{a}	0.490	0.005
Calcium (mg/g)	219.7^{c}	226.0^{bc}	234.3^{b}	245.3^{a}	251.7^{a}	3.360	0.001
Phosphorus (mg/g)	119.4^{a}	111.5^{b}	112.2^{b}	117.2^{ab}	119.0^{a}	1.890	0.005
Ca: P (mg/mg)	1.97^{b}	1.90^{b}	2.09^{a}	2.09^{a}	2.12^{a}	0.030	0.009
Magnesium (mg/g)	5.10^{b}	6.70^{ab}	7.12^{ab}	8.02^{a}	6.45^{ab}	0.090	0.028
Iron (µg/g)	39.74	53.93	62.18	62.59	60.39	9.640	0.627
Zinc (µg/g)	1.25^{b}	1.73^{a}	1.75^{a}	1.73^{a}	1.57^{ab}	0.150	0.019

[a,b,c,d] Values within the same row with different subscripts are significantly differed ($p < 0.05$). [1]Treatments: NC = negative control; PC = positive control; $MLSE_{60}$: 30 mL moringa leaf extract + 30 mL moringa seed extract/L of drinking water; $MLSE_{90}$: 45 mL moringa leaf extract + 45 mL moringa leaf extract/L of drinking water; $MLSE_{120}$: 60 mL moringa leaf extract + 60 mL moringa seed extract/L of drinking water. [3]SEM: standard error of mean.

5.5 Discussion

5.5.1 Growth performance

The increments in the final weight observed at the starter, finisher, and culmination of the experiment may be attributed to the minerals, vitamins, and bioactive compounds in MLSE (Tables 5.1 and 5.2) and the permeability of the soluble nutrients inherent in MLSE. This finding is supported by the report of Sigolo et al. (2021) who observed an increased FBW, BWG, and ADWG of broilers when they investigated the effect of a blend of nettle, dill, thyme, and coriander extracts. The process of action of phytogenic plant additives has not been very clearly explained yet, although previous studies have noted that administering some plant extracts increased growth performance in broilers (Carboni et al., 2020; Paul et al., 2020; Mogire et al., 2021).

Park et al. (2014) also noted an increased FBW and ADWG in broilers when a blend of *Saposhnikovia divaricata*, *Lonicera japonica*, and *Chelidonium majus* extracts were administered for 35 days. According to Vidanarachchi et al. (2010) a combination of the cabbage tree, acacia, and Undaria seaweed supplemented in diets did not increase broiler BWG. However, Hernandez et al. (2004) and Garcia et al. (2007) reported no treatment effects on BWG when a blend of sage, thyme, and rosemary extracts was supplemented in broiler diets for 42 and 49 days, respectively. In this study, $MLSE_{120}$ significantly increased the overall ADWG of broilers. This finding agrees with the reports of Farahat et al. (2021), who administered a blend of Curcuma roots, chamomile flowers, licorice roots, and olive leaf extracts to broilers for 42 days. The variation in results may possibly be ascribed to the synergistic effect among different bioactive compounds in the respective blend, the route of administering the extracts, and the dosage of the extracts administered.

Furthermore, $MLSE_{120}$ decreased feed intake (FI) and average daily feed intake (ADFI) at the finisher and overall phases while feed conversion ratio (FCR) was at the starter, finisher, and overall phases which is not consistent with the report of Farahat et al. (2021). They noted no effect on FI and ADFI, but FCR was better. Hernandez et al. (2004) observed no effect on FI and FCR, while Garcia et al. (2007) observed better FCR. Also, Park et al. (2014) reported no effect on FI and FCR. In their study with neem, garlic, and plantain extracts, Camy et al. (2019) found no increase in FI but no effect on the FCR of broilers after a 28-day trial. In contrast to this current study was the report of Attia et al. (2017), who found no effect on FI, ADFI, and FCR when an aqueous extract blend of oregano, fenugreek, chamomile, and fennel was administered in broiler diets for 42 days. In this present study, it can be inferred that oral administration of MLSE increased the efficiency of the diets consumed since it reduced FI with an increased BWG. Additionally, it was noted in this study that the apparent nutrient digestibility was comparatively increased in MLSE-administered groups. It may seem that the reduced FCR in birds from MLSE groups is due to the elevated digestibility indices observed among them.

5.5.2 Nutrient digestibility

The current study showed that oral administration of MLSE had a positive effect by increasing ether extract, ash, calcium, and phosphorus digestibility. Earlier studies have reported that phytogenic extracts can increase nutrient digestibility by stimulating bile secretion, elevating pancreatic and intestinal enzyme secretion, and/or decreasing harmful bacteria colonization in the intestine (Murugesan et al., 2015; Akuru et al., 2021). Rezvani et al. (2018) reported an elevated ether extract, decreased dry matter, and crude protein digestibility with pomegranate seed extract supplementation in the diets of broilers for 39 days. Also, bioactive compounds (flavonoids and phenols) in MLSE have been noted to be beneficial in increasing nutrient utilization due to their digestive enzyme stimulatory attribute, appetizing effect (due to their saponin content), and stabilization of the digestive tract ecosystem (Karthivashan et al., 2015). Attia et al. (2017) reported an increased dry matter, crude protein, and ether extract, but no effect on ash, calcium, and phosphorus digestibility when a blend of oregano, fenugreek, chamomile, and fennel was administered to broilers. Contrarily, Reisinger et al. (2012) reported no significant increase in the nutrient digestibility of broilers due to the inclusion of a 125-ppm blend of oregano, anise, and limonene extracts.

5.5.3 Blood parameters

Conceivably, the haematological parameters of broilers are a useful indicator for evaluating the safety of phytogenic plant extracts on their health status. The elevated haematocrit percentage and reticulocyte count as influenced by the MLSE is not supported by Kamboh et al. (2018). The authors administered a 5mg blend of genistein and hesperidin extracts to broilers for 21 days and noted no statistical difference in haematocrit percentage or reticulocyte count. Saha et al. (2010) administered an organic soluble water additive to broilers and observed significantly elevated values for RBC and no difference for haematocrit percentage. Park et al. (2014) found no difference in the haematocrit percentage and reticulocyte count of broilers when a blend of *Saposhnikovia divaricata*, *Lonicera japonica*, and *Chelidonium majus* extracts were supplemented in their diets. The increased reticulocyte count and haematocrit percentage possibly suggesting increased oxygen-carrying capacity of birds administered MLSE, which implies that MLSE administration increased haematopoiesis.

However, Zanu et al. (2012) reported no significant effect on haematocrit percentage and reticulocyte count when 15% *M. oleifera* leaf meal was supplemented in broiler diets. Tijani et al. (2015) reported reduced MCH, PVC, Hb, WBC, and neutrophil concentrations in birds fed 20% moringa leaf meal. These observations are not consistent with the current study. Perhaps the enhanced haematological parameters in this study may be a combined effect of the blend of moringa leaf and seed extracts, the extraction process, and the route of administration. Also, increasing supplementation levels of moringa leaf meal in the diets of rats decreased the haematological parameters (Odetola et al., 2012). Investigations showed a positive correlation between tannin intake and reticulocyte count in broiler chickens administered a phytogenic additive (Akanji et al., 2016).

Perhaps the elevation of haematocrit and reticulocyte in this study is due to the bioavailability of iron in MLSE, made possible by water extraction. Elbashier and Ahmed (2016) opined that iron is essential for the formation of haemoglobin and myoglobin. According to Olugbemi et al. (2010), reticulocytes play a role in the transportation of oxygen and carbon dioxide in the blood as well as the manufacture of haemoglobin. Hence, elevated values among MLSE groups suggested an optimum performance of these functions and an improved state of health. A significant increase in the number of haematocrit and reticulocyte values may be attributed to the rich bioavailable nutrients in MLSE (Elbashier and Ahmed, 2016). Similarly, Jiwuba et al. (2016) observed that *M. oleifera* leaf meal inclusion did not compromise the haematological parameters of broiler chickens, which may be due to the higher quality of the protein in the leaves, an opinion shared by Alabi et al. (2021). According to Elbashier and Ahmed (2016), *M. oleifera* has a blood-boosting effect because of the significant quantities of most essential amino acids.

Haemoglobin, MCH, MCHC, and MCV are essential haematological parameters whose concentrations are used to detect the appearance and severity of anaemia (Aikpitanyi and Egweh, 2020). A decrease in their concentration levels may indicate that the birds are vulnerable and do not cope well with stressors (Vicuña et al., 2015). In the current study, no significant differences were noted among the treatments in haemoglobin, MCH, MCHC, and MCV values. These results possibly imply that the oral administration of MLSE generally did not compromise the bird's ability to withstand stress. It has also been reported that a low mean corpuscular haemoglobin concentration (MCHC) value of less than 29.0% can be accredited to iron and other trace element deficiency (Essien and Udoh, 2021). In the present study, all the measured haematological parameters were within the optimum reference range for clinically healthy birds (Talebi et al., 2005; Oyeagu et al., 2019; Iyaode et al., 2020; Matshogo et al., 2020; Matshogo et al., 2021). Based on the determined and available literature on the micronutrient composition of the test ingredients, it can be inferred that the phytogenic additives increased the availability and utilization of essential nutrients.

Serum biochemistry generally describes the metabolism of nutrients and physiological status in the body concerning growth and development (Egbu et al., 2022). In the current study, serum biochemistry parameters suggested that oral administration of MLSE did not decrease the metabolism of proteins, lipids, and minerals, which supports the current growth performance of birds in the MLSE groups. This result is not consistent with previous studies that reported similar mean values for serum calcium concentration when rapeseed meal (Fu et al., 2021) and seaweed (Matshogo et al., 2021) were included in the chickens' diet. Chowdhurya et al. (2018) reported that serum total protein concentration increased in broiler chickens consuming a diet containing a blend of cinnamon seed oil, clove seed oil, and ajwain seed oil. In the present study, the supplementation of MLSE did not compromise blood serum parameters by elevating the concentrations of total protein, albumin, globulin, and urea (Sigolo et al., 2021). Serum protein levels are vital for maintaining the immune system and can increase under disease and stress conditions such as toxicity (Tekce and Gül, 2017). In birds, serum total protein consists mainly of albumin and globulin (Sigolo et al., 2017). Thus, low total protein levels are synonymous with low serum concentrations of albumin and globulin and vice versa (Sigolo et al., 2019). Urea is a protein metabolite and is a useful indicator of nitrogen utilization. In birds, an increased serum urea concentration is akin to a decreased amino acid incorporation into tissue muscle proteins (Sahebi-Ala et al., 2021).

It was expected that MLSE administration would have a serum cholesterol-lowering impact because numerous researchers have documented a reduction in blood total cholesterol levels in previous investigations (Alimohamadi et al., 2013; Farahat et al., 2017; Waheed et al., 2017; Ashour et al., 2020). However, no such difference was seen in this study. Mohammadi et al. (2014) found that supplementing clove seed oil at varying levels (0.1, 0.3, and 0.5 g/kg) did not significantly affect serum cholesterol concentrations in broiler chickens. These findings are comparable to the results of the present study, which showed that the bioactive contents of MLSE interfered with the activity of 3-hydroxy-3-methyl glutaryl-CoA reductase, a vital enzyme in cholesterol synthesis (Adeyemi et al., 2021a). The reduction in the activity of 3-hydroxy-3-methyl glutaryl-CoA reductase decreases the low-density lipoprotein receptor activity and, consequently, low-density lipoprotein concentrations and increases apolipoprotein A activity bound to high-density lipoproteins (Adriani et al., 2018). This may be responsible for why the serum cholesterol was not increased following the oral administration of MLSE in broilers (Al-Ramamneh, 2018). In conformity with these results, the favorable role of phytogenic additives on serum cholesterol concentration and the distribution of cholesterol lipoproteins, from low-density lipoproteins to high-density lipoproteins, has been noted (ABD EL-HACK et al., 2020).

The liver is the epicenter of several digestive, metabolic, and productive activities and, as such, is vulnerable to changes in chemical and biological compromise. Such compromise is made obvious by the serum levels of specific enzymes emanating from the liver. Based on their levels, these enzymes may cause some disturbances to bodily functions, thereby resulting in impaired health and production performance. The activities of alkaline phosphatase (ALKP), alanine transaminase (ALT), and gamma-glutamyl transferase (GGT) in the blood are biological indexes of liver function and damage (Yildirim et al., 2018). Increased levels of these enzymes are associated with liver or muscle damage resulting from the body's response to stress (Annongu et al., 2014). Therefore, the lack of elevation of these enzymes in this study due to MLSE administration may imply that liver function was not compromised. These results are consistent with the report of (Aikpitanyi and Egweh, 2020). The lack of elevation in the concentration of serum AST in the MLSE-supplemented birds indicated the hepatoprotective effect of MLSE phytochemicals. Likewise, the supplementation of quercetin (Kim et al., 2015) and a blend of bitter leaf and *M. oleifera* leaf extracts (Daramola, 2019) reduced serum AST in broilers.

5.5.4 Carcass yield and internal organs weight

The statistical difference observed in the slaughter weight (SLWT), dressing percentage, hot carcass weight (HCW), cold carcass weight (CCW), and thigh weight among treatments possibly reflects the combining effect of both the *M. oleifera* leaf and seed extracts. The beneficial impact of MLSE on carcass yield may be attributed to the activity of the phytogenic components (flavonoids, phenols, and protein) of MLSE. The quantitative phytochemical analysis of MLSE showed that it contained numerically more flavonoids, phenol, and protein than MLE and MSE. These observations were also affirmed by earlier reports on the effect of phytogenic feed additives on broiler chicken carcass yield (Ragaa et al., 2016; Sigolo et al., 2021). However, the supplementation of *Mentha cordifolia* leaf (Abdel-Wareth et al., 2019), herb residues (Lokaewmane et al., 2020), onionskin waste (Adeyemi et al., 2021a), and *Crassocephalum crepidioides* leaf (Adeyemi et al., 2021b) did not affect the dressing percentage and relative weights of carcass cuts of broiler chickens. The variation in results despite similar use of phytogenic feed additives could perhaps be attributed to the combination of the following factors: energy levels of the diets (Attia et al., 2021), broiler breed used (Abouelezz et al., 2019); the extraction process; dosage of the phytogenic feed material (Matshogo et al., 2021b); the higher concentration of phytogenic compounds in the MLSE; and route of administration.

Earlier observations (Waheed et al., 2017; Camy et al., 2019) on the effect of phytogenic feed additives on the visceral organ weight of broiler chickens have been laden with inconsistencies. The current study showed that MLSE administration did not compromise the normal functioning of the visceral organs. The similar gizzard, heart, and heart weight are substantiated by the findings of Lokaewmane et al. (2020), who noted no effect of herbal residue on the visceral organ development of broiler chickens. Much as Sigolo et al. (2021) noted enhancements in visceral organ weight when phytogenic plant extracts were administered to broiler chickens, Adeyemi et al. (2021b) did not record such enhancements. Contrarily, Waheed et al. (2017) noted that a blend of ajwain, sweet violet, and fenugreek extracts increased liver weight but decreased gizzard and heart weight. Also, Camy et al. (2019) noted that neem leaf extract reduced liver weight, while plantain and garlic leaf extracts elevated it.

A poorly developed gizzard limits the broiler chicken's ability to effectively digest large feed particles (Kheravii et al., 2018). The observed similar gizzard weight is at variance with the report of Matshogo et al. (2021b), who reported heavier gizzards in birds reared on seaweed that was treated with both the fibrolytic and protease enzymes compared to untreated seaweed. The gizzard weights observed imply normal mechanical digestion (Nhlane et al., 2020). Similar liver weight among treatments possibly infers that supplementation of MLSE did not cause toxicities.

The increased weights of the bursa, spleen, and thymus observed in the MLSE administered groups may suggest that the bioactive compounds in MLSE increased lymphocyte enlargement. The findings of the present study corroborated with the earlier reports of M'Sadeq et al. (2018) and Su et al. (2021), which noted a positive correlation between the weights of the bursa, spleen, and thymus and the immune response of birds. The bursa, spleen, and thymus are very important central immunity organs, which are indicators of better health status and good physiological response to the body's immune system. Mohamed et al. (2021) reported increased bursa, spleen, and thymus weight when *Boswellia serrata* was supplemented in the diets of broiler chickens. Waheed et al. (2017) reported increased bursa and thymus weight but reduced spleen weight. Liu et al. (2020) observed that chestnut wood extract had no effect on spleen and thymus weights but increased bursa weight.

5.5.5 Meat quality

Surprisingly, no significant differences were observed for meat pH_{45min}, pH_{24hr}, lightness (*L**), redness (*a**), yellowness (*b**), WHC, drip loss, cooking loss, and shear force among treatments. Similar results were reported by Adeyemi et al. (2021a), who administered onionskin waste to broilers. In contrast to this study, Suliman et al. (2021) reported reduced broiler meat pH_{24hr}, WHC, CL, shear force, lightness (*L**), and yellowness (*b**) with increasing inclusion levels of *Syzygium aromaticum* extract in diets. The similar mean values for pH_{45min} and pH_{24hr} among treatments in this study may suggest that oral administration of MLSE did not decrease the bird's glycogen content at slaughter and the glycolysis rate post-mortem (Salwani et al., 2015). Furthermore, Yang et al. (2017) proposed that pale meat color is consistently associated with lower pH. The meat pH_{24hr} (5.85–5.98) from this study falls within the optimal range (5.5–6.5) for chicken meat as posited by Glamoclija et al. (2015). Also, as clarified by Uhlířová et al. (2018) the similarity in pH values may be explained by the similarity in preslaughter responses to stress, storage time, and temperature. This observation could possibly illustrate why there was a similarity in mean values for meat drip loss, cooking loss, and WHC (Nhlane et al., 2020). Jang et al. (2008) observed higher meat pH_{24hrs} but no variation in broiler WHC after administering a combination of mulberry leaf, Japanese honeysuckle, and goldthread extract. Meat with a low pH has a lower WHC in general, which was noted in this study.

Consumers' acceptance of meat is predicated upon the meat's colour (Adeyemi et al., 2021a). Comparable to this study, Matshogo et al. (2021a) noted that enzyme treatment of seaweed had no effect on colour indicators. An et al. (2015) administered onion extracts to white mini broilers noted no variations among treatments for meat colour. Apart from the effect of nutrition, there are separate factors influencing meat colour such as total haemoglobin and myoglobin content, muscle pH, age, breed, and sex of birds (Wideman et al., 2016).

The noted increase in meat's crude protein content with decreasing moisture and crude fat content contradicts the findings of Camy et al. (2019), who found no difference in meat moisture, crude protein, or ash but a difference in crude fat content when plantain, garlic, and neem extracts were administered to broilers. Also, when untreated moringa leaf and fermented moringa leaf were supplemented in broiler diets, Nkukwana et al. (2015) and Sugiharto et al. (2020) detected no influence on the moisture content of broiler meat. The discrepancy in outcomes might be attributed to the treatment adopted and the route of administration. Previous research treated phytogenic additions before use, such as fermented moringa leaf (Sugiharto et al., 2017; Sugiharto et al., 2020), fermented cottonseed meal (Nie et al., 2015), and fermented bioproducts (Marcinčák et al., 2018), supported the increased muscle crude protein content. Perhaps the processing of these materials increased the bioavailability of nutrients for absorption, which then elevated the intramuscular protein anabolism of broiler chickens (Hossain et al., 2012).

The reduced muscle crude fat observed might imply that MLSE subdued hepatic lipogenesis as observed in the lack of treatment effect on blood serum cholesterol concentration in the birds noted in this study. Comparably, the inclusion of *Mentha cordifolia* leaf (Abdel-Wareth et al., 2019) and onion and garlic mix (Al-Ramamneh, 2018) decreased fat in broilers. Likewise, Wang et al. (2014) and Nie et al. (2015) noted a significant decrease in the crude fat content of breast meat from broiler chickens. Oral administration of MLSE possibly increased the degrading of tissue triglycerides and β-oxidation of fatty acids, resulting in reduced fat deposition in the muscles of broilers (Sugiharto and Ranjitkar, 2019).

5.5.6 Intestinal morphology

The gastrointestinal tract's primary functions include digestion and nutrient absorption, as well as immune system maintenance (Song et al., 2018). The increased length of the duodenum, jejunum, and ileum in the MLSE administered groups may indicate the increased surface area for nutrient absorption, which is supported by the noted performance. The increased length of the duodenum, jejunum, and ileum in $MLSE_{90}$ and $MLSE_{120}$ groups contradicts the findings of Brenes et al. (2010), who found a substantial reduction in the relative length of the jejunum and ileum in birds given grape seed extract (0.6, 1.8, and 3.6 g/kg diet) at 21 days of age. Also, Thomas et al. (2007) reported a decrease in intestine length in birds fed 0.5% green tea diets, which contain higher polyphenolic flavonoids, primarily catechins. Sehm et al. (2007) found that feeding red wine pomace high in flavan-3-ol and proanthocyanidins decreased jejunum length in pigs. After 42 days of administering grape seed extract to hens, Hajati et al. (2015) found no influence on their duodenal, jejunal, or ileal lengths. Rezvani et al. (2018) found that feeding a 2% pomegranate seed extract to broilers for 39 days resulted in a shorter intestine length. According to Bolacali et al. (2021), administering date palm extract to Japanese quail for 42 days increased their intestine length. Humer et al. (2015) also observed increased intestine length in broilers when phytogenic supplements were provided. The differences in results seen can be attributed to the age of the birds, the route of administration of the phytogenic additive, the extraction procedure, and the dosage provided.

Research has indicated that villus height, villus width, crypt depth, and the villus height/crypt depth ratio are all significantly correlated to nutrient absorption and health in monogastric animals (Qureshi et al., 2016; Li et al., 2020). Similarly, decreasing crypt depth might be interpreted as a sign of decreased development of immature enterocytes, resulting in a lower tissue turnover rate and fewer maintenance requirements for the formation of new enterocytes (Attia et al., 2017). Therefore, the increase in villus height or a higher villus height/crypt depth ratio for the supplemented groups can be linked to greater nutritional absorption as well as the observed performance. These findings disagree with that of Attia et al. (2017), who administered a blend of oregano, fenugreek, chamomile, and fennel extract to broilers and noted no increase in villus height, crypt depth, or villus height/crypt depth ratio across the duodenal, jejunal, and ileal segments of the small intestine.

The results obtained in this study are supported by Hazrati et al. (2019), who noted that the inclusion of plants with bioactive compounds increased villus height and villus height/crypt depth in the small intestine. Likewise, Abdaljaleel et al. (2018) reported increased villus height and a villus height/ crypt depth of the duodenum and jejunum in birds supplemented with phytogenic products. The height of the jejunal villus increased in broilers supplemented with *Zingiber officinale* and a propolis extract blend (Tekeli et al., 2010). Increased villus height and villus height/crypt depth are associated with increased intestinal turnover (Bolacali et al., 2021), and longer villi are associated with cell mitosis initiation (Attia et al., 2021). The observed improvements in intestinal morphology suggest that MLSE has the potential to be a proponent of improving gastrointestinal morphology in broiler chickens. This may possibly be responsible for the increased growth performance.

When using marigold and grape seed extracts in broilers, negative effects on villus height and width, as well as a shortened intestinal tract, have been reported (Brenes et al., 2010, Gurbuz et al., 2010). Other studies have shown that fermented ginkgo leaves, which have been used in many chicken experiments, increase intestinal absorption function (villus height and villus height/crypt depth ratio) (Zhang et al., 2013; Zhang et al., 2015; Yu et al., 2015). Basit et al. (2020) reported that plant extract blends administered in their study increased villi height and villi height/crypt depth ratio in three segments of the small intestine and a significant decrease in the crypt depth of the duodenum. Polyphenols in MLSE may have reduced the intestinal microbial population, lowering the presence of toxins at this level, which is linked to changes in intestinal structure, such as increased villi height and villi height/crypt ratio. More research is needed to determine the effects of various polyphenol-rich supplements on nutrient digestion.

The mucus in the goblet cells serves as a lubricant, a source of nutrition for normal commensals, and a barrier against pathogens in the gut (Majidi-Mosleh et al., 2017). This study found increased acidic and mixed-nature goblet cells in the jejunum and ileum segments of the small intestines in the MLSE administered group, which was supported by Sikandar et al. (2017). It is possible that MLSE maintains goblet cell activity by regulating its mucin gene and thus positively contributes to the gut's protective mechanisms. The underlying mechanism that increased acidic and mixed-nature goblet cell populations in the jejunum and ileum is not clear after 42 days of trial. However, it may be in response to the higher microbial population in those segments compared to the duodenum. This finding is consistent with the report of (Pascual et al., 2020), who noted that supplementation of yeast cell wall extracts induces goblet cell wall proliferation as a defense mechanism in broiler chickens.

The increase in the number of total goblet cells observed in the MLSE groups can be viewed positively considering the protective effect of mucus production by goblet cells under challenging conditions (Reisinger et al., 2012). Alizadeh et al. (2016) reported an increase in the number of acidic, mixed, and total goblet cells in broilers fed diets supplemented with phytogenic plant extracts. MLSE administration to birds had a positive effect on animal health by modulating the mucosal morphology of the small intestine. This microarchitecture modulation is linked to increased immunity, implying that MLSE could be a viable alternative to antibiotics in broiler production.

5.5.7 Bone parameters

Mirakzehi et al. (2018) discovered that supplementing diets with *Withania somnifera* root and *Withania coagulans* fruit extract had no effect on broiler chicken tibia weight, length, or bone breaking strength (BBS). In another similar study in swine, Njoku et al. (2021) reported a reduction in BBS, the thickness of the medial wall (TMW), and the thickness of the lateral wall (TLW) when a blend of moringa and basil leaves or neem and basil leaves was supplemented. Mohasesi et al. (2021) reported no significant effect on tibia weight, length, or BBS when dill extracts were supplemented in broiler diets. The results of the current study are consistent with the findings of Hosseini et al. (2016), who found that supplementing hydroalcoholic extract of *Withania coagulans* enhanced the tibia morphometric parameters of birds. Furthermore, Santos et al. (2019) found variation in observed tibia weight, length, and BBS when vitamin C, an antioxidant, was included in broiler diets.

Additionally, Sgavioli et al. (2017) found a significant effect on wall thickness, medullary canal diameter, and tibiotarsal index of broiler chickens, corroborating the conclusions of this study. MLSE, which is composed of calcium, phosphorus, and vitamin D, has been shown to improve tibia morphometric characteristics and strength. They enhance bone health through a variety of processes, including preventing bone loss. They promote osteoblast genesis, repress osteoclast genesis, and boost osteoimmunological activity via antioxidative and anti-inflammatory properties (Njoku et al., 2021).

The study of Mirakzehi et al. (2018) demonstrated that bone mechanical strength is affected by the amount of bone density and mineralization as well as the architectural spatial arrangement of the bone. According to Skřivan et al. (2020), tibial ash content may be used as an adequate predictor of bone mineral composition. The most important elements for bone development are calcium and phosphorus (Broch et al., 2021). Oral delivery of MLSE increased tibia ash and mineral composition in this research, except for iron concentration, which was unaffected. This study contradicts the findings of Hossain et al. (2013), who found no variations in bone macro mineral concentrations of broiler chickens across treatments. The reported increased calcium in the present study supports the earlier increased calcium concentration in the blood, which can aid deposition in the bone as well as contribute to bone weight.

5.6 Conclusion

The results showed that water extraction of *Moringa oleifera* leaf and seed powder with the subsequent administration through drinking water improved growth performance, nutrient digestibility, blood parameters, carcass yield, meat quality, intestinal morphology, and bone parameters in Cobb 500 broilers.

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CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The purpose of this study was to determine the effect of administering *Moringa oleifera* extracts through drinking water on broiler chicken growth performance, nutrient digestibility, blood parameters, carcass yield, internal organs weight, meat quality, intestinal morphology, and bone parameters. The MLE, MSE, and MLSE were chemically analyzed and administered respectively in Chapters 3, 4, and 5 to determine their effects on the physiological and meat quality responses of Cobb 500 broiler chickens.

Chapter 3 of this study hypothesized that administration of MLE improves growth performance, nutrient digestibility, blood parameters, carcass yield, internal organs weight, meat quality, intestinal morphology, and bone parameters of Cobb 500 broiler chickens. The study did not reject the hypothesis and indicates that MLE administration increased weight growth, better FCR, enhanced nutrient digestibility, carcass yield, some internal organs weight, meat quality, intestinal morphology, and bone parameters in Cobb 500 broiler chickens while not compromising blood parameters.

In Chapter 4, the tested hypothesis was accepted, and the results indicated that MSE administered orally has the potential to be used as an alternative to AGP in Cobb 500 broilers. MSE in drinking water enhanced FCR, calcium and phosphorus digestibility, certain blood parameters, carcass yield, internal organs weight, meat quality, intestinal morphology, and bone parameters in Cobb 500 broilers.

In Chapter 5, the study aimed to determine the effect of the combination of MLE and MSE on the physiological and meat quality of Cobb 500 broilers. Water extraction of *Moringa oleifera* leaf and seed powder, followed by administration via drinking water, improved growth performance, nutrient digestibility, blood parameters, carcass yield, internal organs weight, meat quality, intestinal morphology, and bone parameters in Cobb 500 broilers.

6.2 Recommendations

Cobb 500 broiler chickens that were administered with moringa leaf extract, moringa seed extract, and moringa leaf + seed extract had the highest physiological performance and meat quality with regards to growth performance, nutrient digestibility, blood parameters, carcass yield, internal organ weight, meat quality, intestinal morphology, and bone parameters. These effects need to be validated with further studies.

- Although observations from this study showed that MLE, MSE, and MLSE have the potential to be administered as an alternative to AGP in broiler production, further investigations at higher dosages in drinking water are required, hence some of the findings in this study were not quite clear. Thus, there is need to conduct further studies using different extraction solvents and methods to ascertain the results.
- Further studies should be conducted on the application of other extraction solvents and methods of extraction on *M. oleifera* leaf and seed powder. The studies should be conducted in communal areas in conjunction with farmers; in that way, they will gain more knowledge about the benefits of *M. oleifera* leaves and seeds and their properties.
- Fatty acids, especially polyunsaturated fatty acids, such as linoleic and arachidonic acids found in chickens, undergo oxidative damage during storage. This leads to oxidative rancidity and nutritional losses when polyunsaturated fatty acids are oxidized, which break down into potentially carcinogenic and mutagenic products. Hence, it is important to determine the fatty acid profiles of broiler chicken, MLE, MSE, MLSE, and experimental diets.
- It is important to determine the actual phytate content in *M. oleifera* leaves and seed powder before and after extraction and its influence on overall nutrient digestibility, growth performance, blood parameters, carcass yield, meat quality, intestinal morphology, and bone parameters.
- Bone parameter analyses at all stages of growth should be conducted.