



**Growth performance, health and meat quality of broilers
fed diets containing Kalahari melon (*Citrullus lanatus*) seed
cake**

MD Kgaswane



orcid.org/0000-0003-0178-4794

Dissertation submitted in fulfilment of the requirements
for the degree Master of Science in Agriculture (Animal
Science) at the North West University

Supervisor: Prof U Marume

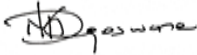
Graduation date: October 2024

DECLARATION

Myself, Mosalakwe Dan Kgaswane hereby certify that this is my original work for the Master of Science in Agriculture: Animal Science program, overseen by Professor Upenyu Marume. No university received this dissertation before. The content source has received full recognition.

Student: Mosalakwe Dan Kgaswane

30-11-2023

Signed:.....


Date:.....

Supervisor: Prof Upenyu Marume



30-11-2023

Signed:.....

Date:.....

DEDICATION

I dedicate this dissertation to the entire Kgaswane family, my parents, my wife, my kids and my siblings.

GENERAL ABSTRACT

The poultry industry is facing challenges of inadequate supply of feed protein sources which subsequently leads to high costs. The study was conducted to determine the effects of dietary inclusion of Kalahari melon seed cake (KMSC) in diets on health and performance in broiler chicken. Five dietary treatments were assigned to four hundred (400) mixed-sex Cobb 500 conventional broiler diet (optifeeds) control; conventional broiler diet + 5% KMSC; conventional broiler diet + 10% KMSC; conventional broiler diet + 15% KMSC and conventional broiler diet + 20% KMSC. Each treatments had eight replicate with a pen holding ten broilers as an experiment unit. The experimental diets were fed *ad libitum* along with freshwater for a period of 6 weeks. Broilers were fed starter (0-14 days), grower (14-28 days) and finisher (28-42 days). The results indicated significant effects of diet ($P < 0.05$) on overall average daily feed intake (ADFI), average body weight gain (ABWG), average daily gain (ADG) and feed conversion ratio (FCR) in dietary inclusion of KMSC. Broilers fed a 15% KMSC diet had the highest overall ADFI ($78.38 \text{ g} \pm 0.93$). The highest overall ADG was obtained in the control-fed diet ($43.63 \text{ g/d} \pm 0.51$) whilst the lowest overall ADG was obtained in the 15% ($39.83 \text{ g/d} \pm 0.51$) fed broilers. Nevertheless, broilers fed control (1883.13 ± 22.17) and 5% (1860.50 ± 22.17) diets had the highest final weights. With regards to overall FCR, the birds fed the control and 5% KMSC diet had the lowest values while those fed the 15% KMSC had the highest value. The highest cumulative weight gain (CWG) obtained was observed from control and 5% KMSC whilst the lowest values were from broilers fed 15% KMSC. The results demonstrated that dietary inclusion influenced protein consumption (PC), protein efficiency ratio (PER), specific growth rate (SGR) and growth efficiency (GE). Dietary treatments significantly affected the haematology parameters, however no significant effects were found on red blood cell (RBC). The dietary treatments showed effect on serum parameters. At 42 days, broilers were slaughtered in order to

determine the carcass traits and meat quality parameters. Four broilers in each replication were chosen randomly to be used for evaluation. The findings from the study indicated that all carcass traits were influenced by diet ($P < 0.05$), apart from dressing % and thigh ratio. Control and 5% KMSC fed broilers had the highest hot carcass weight ($1431.62 \text{ g} \pm 26.55$), ($1426.28 \text{ g} \pm 26.55$), cold carcass weight of ($1373.56 \text{ g} \pm 26.40$), (1369.21 ± 26.40) respectively. The internal organ sizes of broiler were influenced by dietary inclusion apart from caeca and colon. The dietary treatments significantly influenced meat quality parameters. Broilers fed 15% KMSC had lighter meat color (L^*) in both 0 h and 24 h post slaughter. Moreover, broilers fed 15% KMSC had higher cooking loss, whilst those fed 5% KMSC had higher shear force. Also measured, were the tibia parameters, bone breaking strength, bone ash and macro mineral content. Diet influence ($P < 0.05$) tibia biomechanics parameters. Inclusion of KMSC in broiler diets increased the tibia linear parameters. Diet also influenced macro minerals content. The broilers fed 20% KMSC recorded the highest values of total ash, moisture and phosphorus content while broilers fed the control diet had the lowest. The broiler chickens fed 20% KMSC recorded the highest values on all the macromineral content. Deformation was observed in the proximal metaphyseal tibiotarsus region of broiler tibiae fed 5%, 10%, and 20% KMSC. This deformation is linked to the gross lesion analysis. Therefore, this led to the conclusion that since KMSC promoted comparable growth performance, nutrient utilization, blood parameters, carcass traits, meat quality, internal organs and bone development parameters, it might eventually replace soybean meal.

Keywords: Bone development, carcass traits, efficacy, health, nutrient utilization.

ACKNOWLEDGEMENTS

To God be the glory for giving me ability to complete my studies. My profound appreciation is extended to Prof. Upenyu Marume, for his motivating, supervision and support during my study term. His efforts and constructive criticism are greatly appreciated. I want to thank the staff at the North-West University Molelwane Farm: Poultry Department for helping me with my research project. I want to express my gratitude to my wife for all of her support throughout my academic journey. To my two daughters, I would like to show my gratitude to them. And for all this, I want to thank my parents for everything. My Grandmother “I am because of you”. All the glory and honour goes to my God.

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ABBREVIATIONS

a^*		Redness
b^*		Yellowness
L^*		Lightness
ADFI		Average Daily Feed Intake
ADG		Average Daily Gain
CCW		Cold Carcass Weight
HCW		Hot Carcass Weight
KM		Kalahari Melon
KMSC		Kalahari Melon seed cake
N		Newton
NWU		North-West University
PC		Protein Consumed
RBC		Red blood cell
SAS		Statistical Analysis System
SBM		Soybean meal
SGR		Specific growth rate
SAS		Statistical Analysis System
SGR		Specific growth rate
TBS		Tibia Breaking Strength
TDPE		Tibia Diameter Proximal End
TWD		Tibia Width Diameter
TDDE		Tibia Diameter Distal end
TLD		Tibia Length Diameter
WHC		Water Holding Capacity

CHAPTER 1: INTRODUCTION

1.1 Background

The broiler industry dominates the agricultural sector in South Africa (SA) as the main supplier of animal protein (DAFF, 2017). Chicken is healthier than other meat sources and is the most affordable protein source for resource-poor communities. Oluwatayo *et al.* (2016) reported that the production of broilers has the potential to play a vital role in South Africa's smallholder communities as it is easier to establish and run compared to other livestock enterprises. Nevertheless, the broiler industry in SA and the world over still faces major challenges. An inadequate supply of protein for broiler diets has been the major challenges the industry is facing as this is subsequently leading to high feed costs (Lee *et al.*, 2016). The main source of protein used in broiler diets is soybean meal (SBM), which is also included in the food matrix for humans. Soybean production in SA and across the world is currently being affected by climate change, recurring droughts and floods in some cases (Henchion *et al.*, 2017). Moreover, the war among the major soybean-producing nations has affected the production and access to SBM. Consequently, efforts are being made to explore alternative protein sources for broiler diets to reduce the over-dependence on SBM.

Alternative, novel protein sources including sorrel seed meal, moringa leaf meal, bambara nut meal, velvet beans and faba beans have been suggested as potential substitutes for SBM in broiler diets (Ozung *et al.*, 2018). In SA, the growing essential oils industry has seen an increase in the accessibility of some agro by-products with the potential to be included in animal feed sources. For example, Kalahari melon (*Citrullus lanatus*) is currently being grown commercially for purposes of essential oils extraction for use in the cosmetic industry

(African Origin Oil, 2018). The seed cake has a variable nutritional composition with the potential to be incorporated in animal diets as a protein source (Carr, 2007). Kalahari melon may contain as much as 35% protein, and 5% dietary fibre (Shazali *et al.*, 2013). According to Ajilola *et al.* (1990), the Kalahari melon seed contained about 50 per cent of oil, 37.4% protein, 2.6% fiber, 3.6 ash and 6.4 moisture content. The fruit is regarded as a good source of vitamin C, glucose, fibre, lycopene and β -carotene (Gupta & Pandey, 2019). The melon seeds are also excellent sources of essential fatty acids such as linoleic (62%-71%) and oleic acid 12%-20% (PhytoTrade, 2008). The health benefits of linoleic acids include being immune stimulatory, antidiabetic, anticarcinogenic, antiatherogenic and anthelmintic effects (Liu *et al.*, 2017). The remaining cake after oil extraction has the potential to be integrated with animal diets as a source of protein (Carr, 2007). However, its inclusion levels in broiler diets needs to be investigated.

1.2 Problem statement

The most preferred protein source in animal diets is SBM (Azman & Yilmaz, 2005; Iji *et al.*, 2017). It's Crude Protein (CP) is reported to be between 40-48% (Beski *et al.*, 2015). Climate change, droughts, floods are some of the major global concerns influencing soybean output (Ciurescu *et al.*, 2019). Conventional protein sources, such soybean meal, have been shown to raise feed costs, making them unfeasible and unsustainable for commercial farmers and the feed industry. The scarcity of soybean has led to intense research efforts to find alternate sources of protein for SBM in animal diets. Kalahari melon seed cake (KMSC) has been proven to have a nutrient content similar to that of groundnut, soybean and cotton seed cakes making it a good source of protein for livestock (Mustafa and Alamin, 2012). Despite its nutrient content, there is no information pertaining to its's inclusion levels in broiler diets. It is therefore essential to explore KMSC as alternative protein source that will allow for the

least cost diet formulations that ensure sustainable broiler meat production. The study will therefore provide critical information on the use of KMSC as an substitute protein source in the broiler diets. Information on its utilization, optimum inclusion levels and effects on various performance parameters in broilers may enable its integrations in least-cost diet formulation for broilers. Moreover, this may allow for dietary manipulation that results in the production of meat products that can have health benefits for consumers.

1.3 Justification of the study

The KMSC is a potential replacement for conventional protein sources. The crop grows throughout the world in warmer climates and is found in every province in South Africa (Komane *et al.*, 2017). The remaining cake after extraction may be viable alternative to SBM in broiler feeding systems. Shazali *et al.* (2013) demonstrated that the protein content of KMSC is approximately 25%-30% and low fibre of 5%. The seeds contains a high (50%) proportion of oil (Ajilola *et al.*1990), with some significant amounts of polyunsaturated fatty acids including palmitic (8-13%), stearic (5-11%), oleic (10-29%) as well as (55-70%) linoleic acid (African Origin Oil, 2018). These fatty acids profiles may have a positive influence on meat quality. In addition, the cake contains a variety of secondary plant compounds, particularly flavonoids and phenols (Kumar *et al.*, 2014) that may stimulate good general health, improved meat quality and fatty acid profiles. Some secondary plant compounds may prevent it from being used in animal diets. However, despite the potential of KMSC as a alternative source of protein, no exploratory studies have been shown to evaluate its suitability in broiler diets. Therefore, the purpose of the study is to determine the efficacy of adding various levels of KMSC in broiler diets as a protein source and its influences on growth performance, health, meat quality traits and bone development.

1.4 Objectives of the study

Main objectives

The aim of the study was to establish the effects of inclusion of Kalahari melon seed cake as in place of soybean meal on growth performance, health, meat quality characteristics and bone development of broilers.

Specific objectives

To determine the effects of 0%; 5%; 10% ; 15%; 20% KMSC inclusion levels on:

- i. Growth parameters; weight gain, feed intake and protein utilization efficiency of broilers.
- ii. Slaughter performance, meat quality and carcass components
- iii. Nutritional and health status; internal organ development, haematology and serum blood parameters, bone development parameters

1.5 Hypothesis of the study

H_0 : The inclusion levels of Kalahari melon seed cake in diets have no effect on growth parameters: weight gain, feed intake and protein utilization efficiency of broilers.

H_0 : Different inclusions levels of Kalahari melon seed cake in diet have no effect on slaughter performance, meat quality and carcass components of broilers

H_0 : Kalahari melon seed cake inclusions levels in diets do not affect nutritional and health status; internal organ development; haematology and serum blood parameters, bone development parameters in broilers

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CHAPTER 2: LITERATURE REVIEW

2.1 Background

Poultry industrial sector provides SA customers with the most affordable animal protein source making a huge contribution to food security (Davids, 2013). The amount of poultry products consumed for the year 2023 was 2.725 million tonnes (SAPA, 2023). Nevertheless, local poultry production is failing to meet growing consumption levels (BFAP, 2014). Broiler production in SA has also been under pressure from cheaper imports, while feed prices have increased sharply resulting in uncertainty in the profitability of broiler enterprises (Davids 2013; Joubert, 2017). Moreover, the scarcity of conventional protein sources and high prices are proving to be major challenges for broiler production. The cost of feed normally takes up to 65 to 70% of the total cost of broiler production (Babiker, 2012; Srivastava *et al.*, 2013; Swain *et al.*, 2014). Two major leading ingredient sources used in poultry feed are maize and SBM. Oluwatayo *et al.* (2016), observed that the broiler industry is the biggest consumer of SBM. The high prices and the huge demand for SBM make the feed cost a significant challenge for broiler producers (Lee *et al.*, 2016). Alternative sources of protein that can replace SBM in poultry diet should therefore be explored. It is observed that SBM production in SA is lower than demand and the country will remain a net importer for many years (BFAP, 2014).

2.2 Conventional protein sources for broiler diets

Poultry feeds are formulated to meet the bird's nutritional requirements. The feed industry is highly dependent on the use of conventional protein sources, especially seed oilcakes or meals from soybean, cottonseed, canola, sunflower and groundnut (Ramachandran *et al.*, 2007). Several factors such as climate change (Henchion *et al.*, 2017), conflict among

humans and livestock competing for the same feeding stuffs are currently making it more difficult for utilization of conventional sources in animal diets (Beshir & Babiker, 2009). Soybean meal is widely utilised as a source of protein of plant origin the world over (Park *et al.*, 2001; Beski, 2015; Joubert, 2017; Cromwell 2017; Abraham *et al.*, 2019) while Canola meal (CM) ranked second in the world (Khajali & Slominski, 2012). The largest consumer of SBM is represented by the poultry industry, especially the broiler industry (DAFF, 2010; Oluwatayo *et al.*, 2016) and is regarded as the benchmark by which different feed protein sources are evaluated (Cromwell, 2017). A well-balanced profile of essential amino acids can be found in soybean (Ravindran, 2013). Soybean meal accounts for 69%, Canola Meal (CM) 13%, Cottonseed Meal (CSM) 6%, Sunflower Meal (SFM) 5% and Fish Meal (FM) 2% of all sources of protein used in feeds (Cromwell, 2017).

2.3 Alternative protein sources.

Exploration of alternative protein sources of plant origin which do not fall into the human food basket and that can be efficiently incorporated into poultry diets are currently ongoing across the world. According to Iji *et al.* (2017), in order for a product to be considered a candidate/substitute for alternative protein, it should be abundant, easily accessible, reasonably priced and should have sufficient protein and key nutrients. Potential replacements for soybean in broiler diets include novel protein sources like marama bean meal, bambara nut meal, watermelon seed meal, velvet beans, and faba beans (Ozung *et al.*, 2018). They are reported to contain high protein content which are comparable with SBM. They have been reported to be successfully incorporated into broilers' diets. Kalahari melon (KM) is one of the protein sources of plant origin with promising nutritional potential. It is currently attracting a lot of research interest as potential replacement of protein in broiler

diets. Nevertheless, there's a need to explore the nutritive value of seed cakes and the possibility for manipulating them as sources of dietary protein in feedstuffs.

2.4 Characteristics of Kalahari Melon (*Citrullus lanatus*).



Plate 2.1: Kalahari melon seeds, oil and fruit (Source: Bionatconsult, 2018).

2.4.1 Description, origin and distribution of Kalahari Melon.

The *Citrullus lanatus* (Thunb.) known as Kalahari melon belong to the family of Cucurbitaceae that is spread in the warmer parts of all continents (Maggs, 1998; Haraja *et al.*, 2020; Cheikhoussef *et al.*, 2017). Kalahari melon has many names which originated from where it grows such as Karkoer, Mankataan, Oatango, White and Wild Watermelon

(Highland Essential Oils). Kalahari melon fruit is round, has a green or pale yellow colour, and can have stripes. The flesh has a bitter taste (Plate 2.1). It is regarded as an underutilized fruit crop (Highland Essential Oils, Vermaak, 2011). Variations exist in the seed characteristics such as thickness, size, texture and thickness of the seed edges (Ahmed, 2004). The crop is extremely resilient to drought and erratic weather (African Origin Oil, 2018). The crop grows well in sandy soil at a soil pH of 5.6 to 7 (Maggs, 1998), it requires a temperature between 25-27 °C for all growth stages and requires 400 to 650 mm of rain a year (Erickson *et al.*, 2005; DAFF, 2013). No necessity for the application of fertilisers or pesticides to the crop (Carr & Mallet, 2008). Nevertheless, fertilizer application can improve yields (Schipper, 2000).

The Kalahari melon is found across Kalahari desert, spanning across Botswana, Namibia and South Africa and also thrives in dry lakes and river banks (African Origin Oil, 2018). It grows throughout the world in warmer climates, including the Mediterranean region and is found in every province in South Africa (Komane *et al.*, 2017). The fruit contains up to 92 % water and in the Kalahari region, the fruit is used as an alternative of water consumption for both man and livestock (Kaura, 2009; Vermaak, 2011). According to Kaura (2009), Kalahari melon seeds are mostly used in the cosmetic and homeopathic industries. Maggs (2003) reported that the Kalahari melon seeds can be consumed as food by humans. Carr (2007) observed that the seed cake is fed to animals as a source of protein and to treat human malnutrition.



Plate2.2: Distribution of Kalahari Melon across Southern Africa (Komane *et al.*, 2017).

African Origin Oil (2018) reported that the seed oil is extremely high in Vitamin E and rich in fats and protein. The rich yellow oil from the seed helps to grow hair, shields the skin from the sun and is a ingredients component of soap (Vermaak, 2011).



Plate 2.3: Kalahari melon seed cake (Source: M.D. Kgaswane, 2021)

2.4.2 Nutrient composition of Kalahari melon seed.

Mustafa and Alamin (2012) noted that *C. lanatus* has a nutrient content similar to that of groundnut, soybean, rape and cotton seed cakes making it a good source of protein for livestock. Composition of oil cakes and meal differs depending on the environmental factors, seed quality and extraction techniques (Iji *et al.*, 2017). Touré *et al.* (2016) noticed that the CP content of *C. lanatus* seed cake is 27.0% whereas Ojieh *et al.* (2007); observed crude protein for “Egusi” type was 29.42%. Muhammad *et al.* (2020); Beshir and Babiker (2009) observed that the crude protein content was 23.62% and 23.4%, respectively. Ajilola *et al.* (1990) observed that Kalahari melon seed comprises 37.4% of protein content. The findings by Bolu *et al.* (2011) revealed that the content of crude protein for sun-dried, roasted and defatted melon was 26.74%, 32.38%, and 40.03%. The seeds of the Kalahari melon are rich in nutrients and may be a good source of unsaturated fat, vitamins, antioxidants, minerals and proteins (Mogotlane, 2015). According to Berry (2015), seed oil has several health benefits

and contains essential fats like isomers of linoleic, palmitic, stearic and oleic acids. Cheikhyyoussef *et al.* (2017), concluded that *C. lanatus* seed oil contains fatty acids including linoleic acid, palmitic stearic, oleic acid (Marume *et al.*, 2020). These results were approximately comparable to those obtained from PhytoTrade (2008) whereby the oil content was observed to consist of linoleic 62%-71% and oleic 12%-20%.

Thiamine and vitamins A, B, C are also thought to be present in good amounts in Kalahari melon seeds. The seed cake contains considerable amounts of aspartic acid, glutamic acid, leucine and arginine but contains low concentrations of lysine (Ojieh *et al.*, 2007; Egbuonu, 2015; Folade *et al.*, 2020). In addition, the seeds contain large amounts of minerals that contribute to growth and development, which include calcium, iron, manganese, phosphorus, potassium, sodium, zinc, copper as well as magnesium (Mogotlane, 2015). The *C. lanatus* seed contains 5% fibre (Shazali *et al.*, 2013). The findings differ with Ajilola *et al.* (1990) who observed that *C. lanatus* seed comprises about 2.6% fibre. The higher levels of fibre content may probably limit the use of *C. lanatus* seed meal in broiler rations (Ahmed, 2004). Fibre has important physiological effects in the gastrointestinal tract whereby it expands inside the walls of the colon making passage of waste products easy consequently relieving constipation (Haraja *et al.*, 2020). The addition of excessive fiber more than broilers can digest will have a detrimental effect on their performance and ability to absorb nutrients (Bustamante *et al.*, 2013). Beshir and Babiker (2009) observed that CF was 29.9% for *C. lanatus* seed cake, almost similar to findings by (Mustafa and Alamin, 2012). The ash content of *C. lanatus* ranges between 1.82-5.2% (Ajilola *et al.*, 1990; Al Khalifa, 1996).

2.4.3 Secondary plant metabolites.

Despite its potential as a nutrient source, Kalahari melon seed contains diversity of secondary plant metabolites namely flavonoids, phenols, alkaloids and tannins and (El Zawawy, 2015). In smaller amounts, these secondary metabolites may stimulate immunocompetence and improve fatty acid profiles of meat (Kumar *et al.*, 2014). However, the secondary metabolites presence in Kalahari melon may outweigh any potential nutritional advantages of utilisation depending on their amounts in diets. They may have both favorable and unfavorable impacts on the use of feed by broilers. Because of their antioxidant properties, phenols found in fruits have garnered attention over time (Tabiri *et al.*, 2016). Phenols are thought to enhance human well-being by reducing the danger of various illnesses, including cancer and heart problem (Mogotlane, 2015).

2.5 Potential inclusion of Kalahari Melon in broiler diets

2.5.1 Effects on growth performance on broilers

Literature search did not provide documentation on the use of dietary inclusion of KMSC in broiler diets. However, the study conducted by Mukhtar *et al.* (2016) as well as Touré *et al.* (2016) revealed that *C. lanatus* - Watermelon Seed Cake (WMSC) can be incorporated in diets of broilers up to 20-26.6% without affecting daily feed consumption and subsequently improving broilers growth. The conclusions are contrary to the findings from previous research which showed that broiler diets containing up to 8% WMSC won't have an adverse effect on the growth performance of the birds (Khlil, 2011). Protein efficiency ratio, feed conversion efficiency and weight gain of broilers were considerably improved by addition levels of up to 10% (Ahmed, 1998; Bolu *et al.*, 2011; Ahmed, 2004; Shazil *et al.*, 2013). However, Bolu *et al.* (2011) stated that broilers fed 10% consumed less feed and gained more weight. Moreover, the feed intake decreases if seed cake is added in large quantities. This result is inconsistent with findings by Ahmed (1998) as the author observed the increase in

feed intake as a large quantity of WMSC is added to broilers diets. Tiamiyu *et al.* (2014) observed that raw WMSC can be fed *Cyprinus carpio* at the inclusion level of 10% without adverse effects on growth and nutrient utilization. The author also reported that the protein efficiency ratio, protein utilization and specific growth varied significantly. The addition of WMSC in diets was observed to improve nutrient utilization (Bolu *et al.*, 2011). According to Ahmed (2004), adding WMSC to broiler diets can enhance nutrient absorption and digestion. Watermelon seed cake is however different in terms of nutritional composition from KMSC and hence it is not known what effects it may have on the performance of broiler.

2.5.2 Effects on carcass characteristics and meat quality.

The most significant influence for determining the quality of meat in poultry is diet, age, gender and management system (Kuźniacka *et al.*, 2020). No evidence exists on the inclusion of KMSC in broiler diets. Khlil (2011) reported no depressive influence of the inclusion of WMSC on the broiler carcass traits. Similarly, no effect observed on dietary inclusion of WMSC on carcass weights, dressing percentages and organ weights (Bolu *et al.*, 2011, Mukhar *et al.*, 2016). In contrast, Shazali *et al.* (2013) concluded that dressing percentage was significantly higher in the broilers given the graded levels of watermelon full fat (5%, 10%, and 15%). One of the essential characteristics that has a significant impact on acceptance and consumer perception of freshness in meat is its colour (Muchenje *et al.*, 2009). Kuźniacka *et al.* (2020) on the effect of Faba bean-based diets substituted with SBM on meat quality observed no effects on meat quality. Water holding capacity, dripping loss and meat color coordinates for breast muscles (L^* , a^* , and b^*) were similar in both groups. In broiler chickens, the thymus and bursae of Fabricius are major lymphoid organs that have a role to play in immune responses. According to Al-Saganet *et al.* (2020), adding 20 and 30% of blue lupine did not significantly alter the percentages of the spleen, thymus and bursa, at

21 and 35 days. This suggests that diets containing blue lupine provide sufficient nutrients for the improvement of immune organs. According to Marume *et al.* (2020), layer hens fed diets with Kalahari melon oil seeds showed the largest intestines and heaviest weight in the spleen. Nevertheless, gaps still exist regarding the impact of KMSC on the immune organs of broilers. Moreover, no information is currently available on how KMSC affects meat quality in broilers and hence needs to be investigated.

2.5.3 Effects on haematology and serum biochemistry.

Serum biochemical and haematological indices are important as they give an insight into the health status and production performance potentials of animals (Ojo *et al.*, 2019). In order to detect stress caused by environmental, nutritional and pathological factors in broilers, haematological parameters can be used (Li *et al.*, 2015). The impact of *C. lanatus* on animal diets has not been thoroughly studied. Significantly higher values of white blood cell (WBC), red blood cell (RBC), Hb, PCV, neutrophils and lymphocytes were recorded in dietary inclusion of WMSC (Bulo *et al.*, 2011). The high values for RBC and Hb were related to high-quality dietary protein levels of WMSC. Ozung *et al.* (2018) concluded that haematological parameters of broilers fed toasted sorrel seed meal were not affected. Research conducted by Algabe (2018) on the haematological parameters of weaner rabbits fed diets added with watermelon peels showed no significant differences in the amount of peels included in the diet. Marume *et al.* (2020) noted that inclusion of the *C. lanatus* oil in layer diets affected WBC, RBC, neutrophils, monocytes, and eosinophils of broilers. With regards to serum biochemistry, WMSC supplementation in broiler diets positively influenced total glucose, uric acid, serum protein and albumin (Bulo *et al.*, 2011). Moreover, higher levels of WMSC increase the serum content above the normal ranges reducing blood flow and damaging the liver and kidney. Serum blood parameters of rabbits fed diets integrated

with watermelon peels did not affect the liver of the rabbits (Algabe, 2018). Haraja *et al.* (2020) observed no effect on aspartate transaminase (AST), alkaline phosphate (ALP) and alanine transaminase (ALT) in the serum of supplemented broilers with different levels of WMSC. According to Muhammad *et al.* (2020), adding WMSC powder in broiler diet demonstrated not to affect the ALT and AST.

2.5.4 Effects on bone development and related diseases.

A crucial factor in broiler production that can eventually enhance growth performance and bone development is proper nutrition. In poultry production practices emphasis should be focused more on provisioning of diets containing balanced calcium (Ca), phosphorus (P) and vitamins. The major macrominerals is Ca and P which are essential for bone formation and maintenance of the skeleton of broilers. Deficiencies of such nutrients can cause abnormality in poor growth and bone development. Although nutritional approaches have improved the situation in poultry industry, leg disorders have been a significant challenge for the industry at large (Fleming, 2008). Strong bone formation and development is necessary for broiler chickens to carry their bodies without experiencing any variety of effects on bone disorders, such as rickets, dyschondroplasia, tibia lameness and bone deformities (Disetlhe, 2017). Xu *et al.* (2021) observed that nutritional imbalances may result in rickets.

Reduced daily feed intake which automatically affects the weight gain can also occur from bone disorders that impair walking ability (Świątkiewicz & Arczewska-Wlosek, 2012). Fleming (2008) observed that bone disorders may also lead to mortality from starvation and dehydration. The bone health of broiler chickens is of major concern to the poultry industry and can cause economic loss to the producers. Determinants of mineral deficiencies and bone development in broiler chickens include bone-breaking strength, tibia linear measurement

and bone ash (Swiątkiewicz *et al.*, 2014). Including KMSC may help the gut absorb nutrients more effectively and promote active bone growth development by releasing vitamins and macrominerals (Ca and P). Broiler might show poor growth development if it receives excessive Ca and P. However, more investigation is required to determine whether KMSC adequate dietary protein could not increase bone formation of broilers.

2.6 Potential of Kalahari melon integration in diets of broilers.

The protein content of *C. lanatus* seed is reported to vary from 25-30% (Shazali *et al.*, 2013). The proximate analysis has shown that seed cake contains nutrients which are required in broilers diets (Rajab, 2002; Ahmed, 2004; Ahmed, 2007; Ojieh *et al.*, 2007). Except for lysine and sulfur-containing amino acids, the seed cake is abundant in all important amino acids (Ojieh *et al.*, 2007; Mustafa & Alamin, 2011). It is crucial to balance the diets of broilers to ensure that the required essential amino acids are met during formulation of diets. The *C. lanatus* seed meal contains 5% fibre (Shazali *et al.*, 2013). High fibre content is the most important limitation of utilization of diets in broilers. *Citrullus lanatus* seed cake contains a variety of secondary plant compounds particularly flavonoids and phenols that may stimulate good general health. Numerous researches have shown that WMSC (sweet melon) can replace SBM partially in broiler diets. Incorporation of WMSC (sweet melon) up to 8-10% in broilers' diets was observed to significantly improve weight gain, feed conversion and protein efficiencies of broilers (Ahmed, 1998; Ahmed, 2004; Bolu *et al.*, 2011; Shazil *et al.*, 2013), however, some authors' reported that inclusion level may be up to 20-26% without affecting daily feed intake and improve performance of broilers (Mukhtar *et al.*, 2016; Touré *et al.*, 2016). A general recommendation is to limit the use of WMSC to 10% in broiler diets. However, higher inclusion rates of WMSC were observed to reduce feed intake (Bolu *et al.*, 2011).

2.7 Summary

The feed industry depends entirely on the use of SBM because of protein content and amino acid profile. Protein content of Kalahari melon can replace the insufficient supply of SBM and thus reduce feed costs. Kalahari melon is found in all provinces of SA. The main justification for using KMSC in broiler diet formulation is their high protein content, which ranges from 25% to 30% and their high fatty acid content which includes linoleic (62%–71%) and oleic (12%–20%). The essential amino acids present in Kalahari melon seed cakes are high with the exception of lysine and sulfur amino acids. The melon contains a variety of secondary plant compounds, particularly flavonoids and phenols that may stimulate good general health, improved meat quality and fatty acid profiles. Kalahari melon seed cake in the diets could enhance feed efficiency and improve broilers growth performance and meat quality without affect bone growth development. Therefore, it is imperative to investigate the impact of various inclusion levels of KMSC in broiler diets.

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CHAPTER 3

GROWTH PERFORMANCE , HEALTH AND PROTEIN UTILIZATION IN BROILERS FED KALAHARI MELON BASED DIETS

Abstract

This study was performed to investigate the effects of inclusion of KMSC in broiler diets on growth performance, protein utilisation efficiency and blood parameters. Five diet were assigned randomly to four hundred (400) mixed sexed Cobb 500 day old chicks in pens stocked at ten chicks per pen. Birds were fed control (conventional diet); (conventional broiler diet + 5% KMSC, conventional broiler diet + 10% KMSC, commercial broiler diet + 15% KMSC, commercial diet + 20% KMSC. Each treatment was repeated eight times. Broilers were fed a starter (0-14 days), grower (14-28 days) and finisher (28-42 days). Results of the study showed a significant effect ($P < 0.05$) of diet on overall growth performance measurements. Broilers given 15% KMSC diet exhibited the greatest ADFI ($78.38 \text{ g} \pm 0.93$). The ADG was highest in the control ($43.63 \text{ g/d} \pm 0.51$), while the lowest ADG was obtained in the 15% ($39.83 \text{ g/d} \pm 0.51$). Broilers fed the control (1883.13 ± 22.17) and 5% (1860.50 ± 22.17) diets had the highest final weights. During the finisher feeding phase, an increase in CWG was observed in birds fed both the control and 5% diets. Diet also influenced overall PC, PER, SGR and GE. Broilers fed a 20% diet having the highest overall PC (16.53 ± 0.18). Broilers fed 10% KMSC had highest haematocrit, MCV values (11.96 ± 0.75) and (71.66 ± 3.95) respectively while 15% KMSC birds fed had highest MCH values. Broilers fed 20% KMSC had highest values for WBC differential counts (25.42 ± 2.63) while the lowest values were birds fed control diet (15.40 ± 2.81). The diet significantly influenced serum parameters. Broilers fed 15% & 20% KMSC had highest values for most amylase, lipase and ALT values. The results of the study suggested that inclusion of KMSC on diet positively influenced performance and health of broilers.

Keywords: Protein utilization, serum biochemistry, haematology, fibre, physiological

3.1 Introduction

Broilers (*Gallus gallus domesticus*) are reared intensively with faster growth rates and high-quality meat products enhanced by superior genetics, high-quality diets and the use of some growth promoters (Pellattiero *et al.*, 2020). They are fed high-performance and balanced diets with the inclusion of high-quality protein sources. Globally, soybean meal is the most preferred protein source plant origin in broiler diets (Park *et al.*, 2001; Azman & Yilmaz, 2005). There has been increased concern regarding the availability and accessibility of SBM over the years due to caused by recurring drought, climate change and wars among grain-producing countries. Consequently, the purchasing costs are becoming unsustainable (Brand, 2007). It is critical to explore alternative protein sources for least-cost dietary formulations for broilers.

Currently many alternative protein sources are being explored for utilisation in broiler feeding systems. These include, among others, sorrel seed meal, lupins, bambara nut meal, velvet beans, faba beans and marama. From literature, the protein proportion of marama bean seed ranges between 30-39% (Chingwaru *et al.*, 2011) whilst velvet bean ranges between 20–25% (Carew & Gernat, 2006). However, the protein content are comparable with SBM. Nevertheless, the utilization of many of these alternative sources is being limited either by high fibre content or by high amounts of some adverse secondary plant metabolites (Ojieh *et al.*, 2007). Strategies are being developed to process such protein sources to reduce the effects of the high fibre content and some undesirable bioactive compounds while increasing their utility in animal diets (Ahmed, 2004; Tihamiyu *et al.*, 2014; Alagbe, 2018).

In South Africa and other Sub-Saharan countries, the Kalahari melon is prevalent and has been grown for consumption mainly by animals during the dry season. It has also been regarded as a medicinal plant and herb commonly used by societies (Liu *et al.*, 2009). Currently, Kalahari melon is being grown commercially for essential oil extraction for use in cosmetic products in South Africa (Vermaak *et al.*, 2011). The cake that remains after oil extraction may be utilized to make animal feed. The cake contains considerable amounts of protein (up to 37%) albeit high fibre content when compared to SBM. The efficacy of its inclusion in broiler diets as a protein source has not yet been assessed in exploratory studies. The study therefore evaluated the effects of inclusion levels of KMSC. It is hypothesized that substituting SBM with KMSC can positively affect growth performance, nutrient utilization, haematology and serum biochemical indices.

3.2 Experimental procedures

3.2.1 Study site

The feed trial was done at NWU Research Farm, which is at Mahikeng of Ngaka Modiri Molema (NMM) district in the North West Province of South Africa. Farm is situated between (25°40.459'S, 26°10.563'E) and is located 1400 meters above sea level.

3.2.2 Kalahari melon seed cake and ingredients.

Highland Essential Oils S.A (PTY) Ltd provided KMSC that was used for the study. The KMSC is derived from the oil extraction process. The GMP Laboratories (Labworld) analyzed the chemical composition of the KMSC. The seed cake was first ground using a Hammer Mill (TRF 400) to pass through a 2mm sieve. The other ingredients were obtained from Nutroteq (PTY) Ltd and Chicken Ranch Animal Feeds.

3.2.3 Treatments and Experimental design

Five different treatments were established with inclusion levels of KMSC as follows: control conventional (optifeeds) broiler diet, conventional broiler diet + 5% KMSC, conventional broiler diet + 10% KMSC, conventional broiler diet + 15% KMSC, conventional broiler diet + 20% KMSC. Table 3.1 and Table 3.2 present the ingredients and proximate compositions of the diets. A total of 400 unsexed day-old chicks (Cobb 500) purchased from Chicken Ranch Animal Feeds & Agricultural Supplies (PTY) Ltd and were placed in 2.5 x 1 x 2 m pens. A complete random design (CRD) was used to conduct the study.

Table 3.1: Dietary treatments ingredients (g/kg as fed basis).

Ingredients	Dietary treatment ¹														
	Starter					Grower					Finisher				
	Control	5% KMSC	10% KMSC	15% KMSC	20% KMSC	Control	5% KMSC	10% KMSC	15% KMSC	20% KMSC	Control	5% KMSC	10% KMSC	15% KMSC	20% KMSC
Yellow Maize-Fine	63.2	63.2	63.2	63.2	56.88	69.9	69.8	69.8	69.8	69.8	76.1	76.1	76.1	76.1	76.1
Prime Gluten 60	1.17	1.20	1.20	1.20	1.08	1.8	1.80	1.80	1.80	1.80	1.27	1.27	1.27	1.27	1.27
Fullfat Soya	0.00	0.00	0.00	0.00	0.00	5.1	5.07	5.07	5.07	5.07	1.53	1.53	1.53	1.53	1.53
Soybean Meal	31.67	30.0	28.50	26.95	24.25	19.7	18.72	17.73	16.75	15.76	18.00	17.1	16.20	15.30	14.40
Kalahari Melon	0.00	1.59	3.17	4.76	6.34	0.0	0.99	1.97	2.96	3.94	0.00	0.90	1.80	2.70	3.60
Limestone Powder-Fine	1.62	1.62	1.62	1.62	1.46	1.45	1.45	1.45	1.45	1.45	1.30	1.30	1.30	1.30	1.30
MCP/Mono Cal KK	0.90	0.90	0.90	0.90	0.81	0.72	0.72	0.72	0.72	0.72	0.50	0.50	0.50	0.50	0.50
Salt-Fine	0.34	0.34	0.34	0.34	0.30	0.32	0.32	0.32	0.32	0.32	0.33	0.33	0.33	0.33	0.33
Koeksoda	0.18	0.18	0.18	0.18	0.16	0.17	0.17	0.17	0.17	0.17	0.13	0.13	0.13	0.13	0.13
Choline Powder	0.08	0.08	0.08	0.08	0.07	0.075	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Lysine	0.25	0.25	0.25	0.25	0.23	0.279	0.28	0.28	0.28	0.28	0.26	0.26	0.26	0.26	0.26
L-Threonine	0.05	0.05	0.05	0.05	0.04	0.041	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03
Methionine	0.23	0.23	0.23	0.23	0.21	0.187	0.19	0.19	0.19	0.19	0.16	0.16	0.16	0.16	0.16
PX P1 Br Str with Phytase	0.17	0.17	0.17	0.17	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Coxistac	0.05	0.05	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Olaquinox	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Str=Starter, Gr= Grower, Fin = Finisher, Br = Broiler.

¹Dietary treatment Control (Conventional broiler diet), Conventional broiler diet + 5% KMSC, Conventional broiler diet + 10% KMSC, Conventional broiler diet + 15% KMSC, Conventional broiler diet+ 20% KMSC

Table 3. 2: Proximate compositions of experiemental diets (g/kg, as fed basis)

Parameters ²	Dietary treatment ¹											
	Starter				Grower				Finisher			
	5% KMSC	10% KMSC	15% KMSC	20% KMSC	5% KMSC	10% KMSC	15% KMSC	20% KMSC	5% KMSC	10% KMSC	15% KMSC	20% KMSC
Dry matter	88.104	88.367	88.679	88.991	87.947	88.234	88.536	88.848	87.902	88.171	88.471	88.783
Moisture	11.896	11.633	11.321	11.009	12.053	11.766	11.464	11.152	12.098	11.829	11.529	11.217
Protein	22.000	22.318	23.028	23.739	20.000	20.538	21.243	21.953	18.500	18.781	19.483	20.193
Ash	4.593	4.677	4.869	5.061	4.079	4.219	4.396	4.589	4.027	4.119	4.294	4.486
Fat EE	2.852	2.939	2.998	3.057	2.971	3.043	3.105	3.164	3.037	3.124	3.187	3.246
Fibre	6.662	9.074	11.924	14.774	6.319	8.928	11.650	14.500	6.328	8.844	11.554	14.404
ME (MJ/kg)	11.500	11.500	11.500	11.500	12.000	12.000	12.000	12.000	12.500	12.500	12.500	12.500
Calcium	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Total P	0.645	0.626	0.625	0.625	0.560	0.549	0.545	0.544	0.584	0.568	0.563	0.562
DP PTRY	0.450	0.447	0.450	0.450	0.450	0.458	0.450	0.450	0.450	0.461	0.450	0.450
Potassium	1.053	1.034	1.031	1.027	0.960	0.950	0.949	0.945	0.887	0.866	0.864	0.860
Sodium	0.237	0.160	0.160	0.160	0.179	0.180	0.180	0.181	0.160	0.160	0.160	0.160
Chlorine	0.237	0.235	0.230	0.228	0.235	0.230	0.228	0.226	0.234	0.232	0.228	0.226
LYS TOT	1.279	1.274	1.272	1.270	1.166	1.163	1.160	1.158	1.056	1.05	1.049	1.047
LYS APTRY	1.100	1.100	1.100	1.100	1.000	1.000	1.000	1.000	0.90	0.90	0.90	0.90

¹Dietary treatment =Control (Conventional broiler diet), Conventional broiler diet + 5% KMSC, Conventional broiler diet + 10% KMSC, Conventional broiler diet + 15% KMSC, Conventional broiler diet+ 20% KMSC.

²Parameters=ME-metabolizable energy.

3.2.4 Proximate composition of the KMSC experimental diets

The formulated experimental diets were chemically analyzed (Table 3.2) for CP, CF, NDF, ADF and EE. Metabolisable energy (ME) and amino acids contents were predicted using near-infrared reflectance spectroscopy (SpectaStar XL, Unity Scientific, Emu Heights, Australia). Atomic ICP spectrophotometer (AAS–Buck) was used to determine macrominerals following procedures by the AgriLASA (1998).

3.2.5 Animals and Housing Management

Before the chicks were placed drinkers, feeders and poultry house were cleaned and thereafter be disinfected in preparation for their arrival. Sunflower hulls were used for bedding. The brooding temperature was kept between 33 °C and 32 °C for three days and gradually lowered periodically to 27 °C when chicks were two weeks of age. The infrared lights were used for the provision of heat. Thermometer was used to control temperature. Chicks were provided stress pack in the first 3 days. Broilers were fed in starter, grower and finisher according to a feeding program. For biosecurity purposes a footbath was positioned at the front of the house and drinking water was provided *ad-lib*. The study protocols were evaluated and certified by the North-West University REC of and the following ethics number was granted: NWU-00819-21-A5- (Appendix 7.1)

3.2.6 Daily feed intake

Broiler feed was given and daily records were made. Refusal was gathered prior to feeding and weighed every morning. Before feeding, feed was weighed as well. Adam equipment or TSW weighing scales was used to weigh the feed. Average feed intake was computed as follows:

$$ADFI = \frac{\text{Feed offered}-\text{Feed refusal}}{14 \text{ Days}} \dots\dots\dots (1)$$

3.2.7 Growth performance of broilers.

An electronic/digital weighing scale (CNP 300 Max 300) was used for weighing broilers . Upon the arrival of chicks, initial weight (IW) was determined by weighing each replicate as an experimental unit. Thereafter broilers were weighed each week i.e. Day 7, 14, 21, 28, 35, 42 respectively. Prior to feeding time, the weighing took place in the morning. Growth performance of broilers was computed as ADG and FCR:

$$ADG = \frac{\text{Finish weight}-\text{Start weight}}{\text{Age (Days)}} \dots\dots\dots (2)$$

$$FCR = \frac{\text{Feed Intake (g)}}{\text{Weight Gain (g)}} \dots\dots\dots (3)$$

3.2.8 Protein Utilization Efficiency.

Crude protein concentration (CPd) was multiplied to determine the protein intake (PI g/bird). The following formulas were used to calculate PER,GE and SGR, which is expressed as a percentage of growth per feeding phase:

$$PI = CPd \times FI \dots\dots\dots (4)$$

$$PER = \frac{BWG}{PI} \dots\dots\dots (5)$$

$$GE = \frac{BWG}{\text{Initial Weight}} \dots\dots\dots (6)$$

$$SGR = \frac{\text{In FW}-IW}{14 \text{ days}} \times 100 \dots\dots\dots (7)$$

3.2.9 Determination of serum biochemical and haematological parameters

At the end of the trial, two broilers was chosen randomly for blood collection from each pen. Blood sample (ml) was withdrawn into purple tubes containing anticoagulant for heamatological analysis and into the red tubes for serum biochemistry from the brachial vein. Haematology was conducted using the Idexx Lasercyte. The haematological indices examined included the full blood count including the haemoglobin (HGB), white blood cells (WBC), red blood cells (RBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), haematocrit (HCT), mean corpuscular volume (MCV) and red cell distribution width (RDW). For serum biochemistry, blood samples were allowed to coagualter and subsequently centrifuged for 10 min at 4000 rpm for serum separation at room temperature. The 1.5 ml Eppendorf tubes were filled with pure serum samples using a pipette. Serum biochemical parameters such as alanine aminotransferase (ALT), alkaline phosphate (ALP), aspartate aminotransferase (AST), bilirubin, total proteins, albumin, creatinine, potassium, sodium, chloride and urea were subsequently determined.

3.2.10 Statistical Analysis

The infleunces of KM inclusion in diets on body weight changes, feed inatke, protein utilization efficiency and blood parameters were analysed by using the Proc Glm procedures of SAS (2010) taking into account the effect of diet only (model 1), while the mixed models procedure for repeated measures that considers the effects of diet and time (model 2) was used for cumulative weight gain measurements. The significance level was set at ($P < 0.05$). The following models were used:

$$Y_{ijk} = \mu + T_i + E_{ijk}$$

Y_{ij} = observations (growth parameters, protein utilization efficiency , blood parameters)

μ = constant mean

T_i = dietary effect ($i=0\%$, 5% , 10% , 15% , 20% of KMSC levels),

E_{ij} =random error

For cumulative weight gain, the following model was used

$$Y_{ijk} = \mu + D_i + W_j + (D*T)_{ij} + E_{ijk}$$

Where:

Y_{ij} = observation (CWG, BWT)

μ = overall mean observation

T_i = dietary effect ($i=0\%$, 5% , 10% , 15% , 20% of KMSC levels)

W_j = effect of week of measurement

$W*T_{ijk}$ = diet and week of measurement interaction

E_{ij} =random error

3.3 Results

3.3.1 Growth performance and feed intake

Table 3.3 presents the influence of KMSC in diet on overall growth performance of broilers. The broilers all had the same initial weight at the start of the feeding trial. However, broilers in the control treatment obtained the highest overall final BWT, followed by broilers given the 5% KMSC diet. The lowest final BWT was obtained in broilers fed the 15% KMSC diet. It appeared that the final weight decreased with increasing levels of KMSC. In the grower and finisher phases, ADFI was influenced by the addition of KMSC to diets. In the grower phase,

ADFI inclined as the levels of KMSC in diets increased. The birds fed diets containing 5% KMSC and control obtained the greatest ($P < 0.05$) ADG. In all feeding phases, the birds fed the 5% KMSC diet had similar values for ADG as the birds fed the control diet. Notably, ADG in all feeding phases declined with increasing levels of KMSC in diets. Although diet did affect the overall FCR, it appeared that the control and the 5% KMSC diets had numerically lower values compared to the treatments with higher levels of KMSC in the diets. In the different feeding phases, the FCR increased with increasing levels of KMSC in diets. The CWG values of all dietary treatments were similar from starter until grower feeding phases (Figure 3.1). From week 4 of age (finisher feeding phases), the broilers fed the control and 5% dietary treatment groups accrued more body weight gain than all other treatments.

Table 3.3 Overall growth performance of broilers of broilers fed KM based diets

Parameters ²	Dietary treatments ¹					SEM ³	<i>P-Values</i>
	Control	5% KMSC	10% KMSC	15% KMSC	20% KMSC		
Initial BWT (g/bird)	50.62 ^{ab}	53.25 ^a	51.62 ^{ab}	51.75 ^{ab}	50.14 ^b	0.90	0.001
Final BWG (g/bird)	1883.13 ^a	1860.50 ^a	1737.75 ^c	1725.00 ^c	1771.14 ^b	22.17	0.001
Overall ADFI (g/d)	76.49 ^c	77.70 ^b	77.60 ^b	78.38 ^a	77.95 ^b	0.93	0.001
Overall ADG (g/d)	43.63 ^a	43.02 ^a	40.14 ^b	39.83 ^c	40.75 ^b	0.51	0.001
Overall FCR	1.65 ^c	1.72 ^b	1.82 ^a	1.85 ^a	1.80 ^a	0.03	0.001

^{a, b} Significant differences ($P < 0.05$) exist between the means in the same rows with different superscripts. Control (Conventional broiler diet),

¹Dietary treatments=Conventional broiler diet + 5% KMSC, Conventional broiler diet +10% KMSC, Conventional broiler diet + 15% KMSC, Conventional broiler diet+ 20% KMSC.

²Parameters=BWG :body weight gain, ADFI :average daily feed intake, ADG: average daily gain, FCR:feed conversion ratio

³SEM = standard error of the mean.

Table 3. 4: Growth performance of broilers fed kalahari melon based diets in different feeding phases

	Dietary treatments ¹						
Parameters ²	Control	5% KMSC	10% KMSC	15% KMSC	20% KMSC	SEM ³	<i>P-Values</i>
<i>Starter phase</i>							
ADFI (g)	25.98 ^c	26.77 ^{bc}	28.17 ^a	27.09 ^b	26.97 ^{bc}	0.39	0.004
ADG (g/d)	19.11 ^{ab}	17.99 ^b	20.12 ^a	18.73 ^{ab}	18.85 ^{ab}	0.88	0.111
FCR	1.36	1.50	1.40	1.45	1.43	0.05	0.360
<i>Grower phase</i>							
ADFI (g)	78.99 ^a	79.29 ^a	81.10 ^a	80.91 ^a	80.45 ^a	0.93	0.820
ADG (g/d)	46.91 ^a	46.15 ^{ab}	43.38 ^{cd}	42.64 ^d	44.59 ^c	0.51	0.003
FCR	1.68 ^a	1.72 ^a	1.87 ^c	1.90 ^c	1.81 ^b	0.03	0.012
<i>Finisher phase</i>							
ADFI (g)	124.49	127.05	123.53	127.14	126.45	1.67	0.763
ADG (g/d)	64.86 ^b	64.94 ^b	56.92 ^a	58.14 ^a	58.82 ^a	1.00	0.001
FCR	1.92 ^c	1.96 ^{bc}	2.18 ^a	2.20 ^a	2.15 ^{ab}	0.03	0.022

^{a, b} Significant differences (P <0.05) exist between the means in the same rows with different superscripts. Control (Conventional broiler diet),

¹Dietary treatments=Conventional broiler diet + 5% KMSC, Conventional broiler diet +10% KMSC, Conventional broiler diet + 15% KMSC, Conventional broiler diet+ 20% KMSC.

²Parameters:ADFI-Average daily feed intake:ADG-Average daily intake: FCR-Feed conversion ratio

³SEM :standard error of the mean.

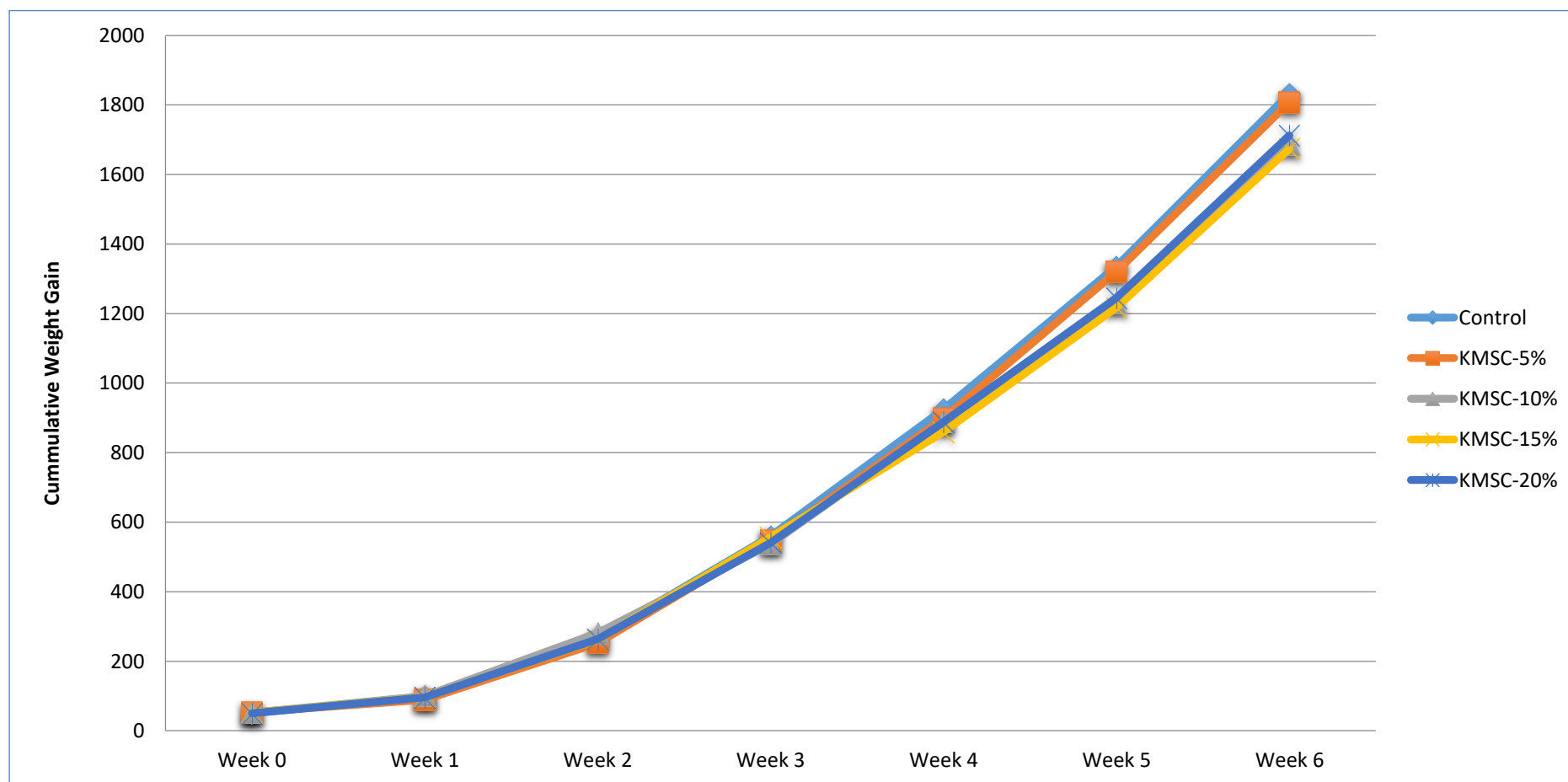


Figure 3. 1: Influence of feeding broilers Kalahari melon seed cakes on cumulative weight gain.

3.3.2 Protein utilization and growth efficiency

Diet affected all protein utilization parameters (Table 3.5). Broilers fed a 20% KMSC dietary inclusion had the highest overall PC (16.53 ± 0.18) whilst the control diet had the lowest overall PC value (13.11 ± 0.18). The PC appeared to incline with increasing KMSC inclusion levels in diets. Nevertheless, the highest PER values were observed in control diets, whilst diets containing 15% and 20% KMSC had the lowest values. The PER also declined with rising levels of the dietary KMSC. The SGR and GE were influenced by diet. In different feeding phases, the PC inclined with inclining levels of KMSC in diets. Nevertheless, the PER and SGR declined with increasing levels of KMSC in diets although the birds fed the Control and 5%KMSC diet had statistically similar values.

3.3.3 Haematological parameters

Table 3.7 presents the results of haematological parameters. Diet significantly affected the haematological parameters apart from red blood cell count (RBC). Broilers fed the control and 10% KMSC diets showed the highest haematocrit values while the broilers given the 5% KMSC diet obtained the least value. Moreover, the control fed birds had the greatest ($P < 0.05$) MCV values (72.60 ± 3.42) as related to other treatments. MCH concentrations were highest in birds fed the 15% KMSC (59.11 ± 4.32). The birds offered the 20% KMSC diet obtained the highest WBC values, while the control diet obtained the least. The birds offered the KMSC dietary inclusion had higher values for the WBC compared with the control. The broilers fed 20% KMSC consistently had the higher values for lymphocytes, monocytes, eosinophils and basophils. Moreover, the control diet had the lowest values on neutrophils, lymphocytes, monocytes, eosophils.

Table 3. 5: Overall protein utilization efficiency of broilers fed KM based diets

Parameters ²	Dietary treatments ¹					SEM ³	<i>P-Values</i>
	Control	5% KMSC	10% KMSC	15% KMSC	20% KMSC		
PC (g/bird)	13.11 ^b	15.11 ^{ab}	15.23 ^{ab}	16.06 ^a	16.53 ^a	0.18	0.001
PER (g/bird)	47.85 ^a	40.58 ^{ab}	39.05 ^b	36.60 ^b	36.31 ^b	0.68	0.001
SGR (% day ⁻¹)	8.61 ^a	8.46 ^a	8.37 ^b	8.34 ^b	8.45 ^{ab}	0.08	0.001
GE	2.76 ^a	2.61 ^b	2.71 ^a	2.62 ^b	2.69 ^{ab}	0.05	0.001

^{a, b} Significant differences (P <0.05) exist between the means in the same rows with different superscripts

¹Dietary treatments=Conventional broiler diet + 5% KMSC, Conventional broiler diet +10% KMSC, Conventional broiler diet + 15% KMSC, Conventional broiler diet+ 20% KMSC.

²Parameters=PC:protein consumption,PER:protein efficiency ratio,SGR:specific growth rate,GE:growth efficiency

³SEM = standard error of the mean.

Table 3. 6: Protein utilization efficiency of broilers fed kalahari melon based diets

	Dietary treatments ¹						
Parameter ²	Control	5% KMSC	10% KMSC	15% KMSC	20% KMSC	SEM	<i>P</i> - <i>Values</i>
<i>Starter phase</i>							
PC (g)	5.19 ^c	5.97 ^b	6.28 ^a	6.23 ^a	6.40 ^a	0.06	0.001
PER	51.67 ^a	42.15 ^a	44.96 ^a	42.05 ^a	41.20 ^a	0.15	0.001
SGR (% day-1)	13.12 ^a	12.46 ^b	13.32 ^a	12.87 ^{ab}	13.04 ^a	0.23	0.026
GE	5.28 ^a	4.75 ^b	5.46 ^a	5.07 ^{ab}	5.22 ^a	0.01	0.027
<i>Grower phase</i>							
PC (g)	14.22 ^c	15.86 ^b	16.22 ^b	17.19 ^a	17.66 ^a	0.18	0.001
PER	46.23 ^a	40.84 ^b	37.62 ^c	34.82 ^c	35.44 ^c	0.68	0.001
SGR (% day-1)	8.00 ^a	8.13 ^a	7.41 ^b	7.61 ^b	7.82 ^{ab}	0.08	0.005
GE	2.07 ^{ab}	2.12 ^a	1.82 ^c	1.91 ^{bc}	1.99 ^{bc}	0.05	0.006
<i>Finisher phase</i>							
PC (g)	19.93 ^d	23.50 ^{bc}	23.19 ^c	24.76 ^{ab}	25.53 ^a	0.31	0.001
PER	45.63 ^a	38.76 ^b	34.59 ^c	32.93 ^c	32.31 ^c	1.18	0.001
SGR (% day-1)	4.70 ^{ab}	4.79 ^a	4.38 ^c	4.55 ^{abc}	4.50 ^{cb}	0.09	0.008
GE	0.93 ^{ab}	0.95 ^a	0.61 ^c	0.89 ^c	0.88 ^{abc}	0.14	0.009

^{a, b, c, d} Significant differences ($P < 0.05$) exist between the means in the same rows with different superscripts.

²Parameters=PC:protein consumption,PER:protein efficiency ratio,SGR:specific growth rate,GE:growth efficiency

Table 3. 7 Haematology parameters of broilers fed kalahari melon based diets.

Parameters ²	Dietary treatments ¹					<i>P-Values</i>
	Control	5% KMSC	10% KMSC	15% KMSC	20% KMSC	
Haematocrit (g/dl)	11.46±0.64 ^b	10.14±0.58 ^a	11.96±0.75 ^b	10.27±0.61 ^a	10.48±0.55 ^a	0.239
MCV (fl)	72.60±3.42 ^b	70.63±3.06 ^b	71.66±3.95 ^b	70.53±3.22 ^b	64.90±2.91 ^a	0.443
MCH (pg)	47.10±4.10 ^a	58.48±4.10 ^b	55.00±5.29 ^{ab}	59.11±4.32 ^b	53.35±3.91 ^{ab}	0.266
Haemoglobin (g/dl)	8.15±0.36	8.01±0.34	8.64±0.35	8.83±0.34	8.60±0.34	NS
RBC (x 10 ¹² /l)	1.00±0.16	1.11±0.15	1.08±0.16	1.01±0.15	1.14±0.15	NS
WBC (x 10 ⁹ /l)	15.40±2.81 ^a	19.63±2.63 ^b	19.75±2.72 ^b	20.26±2.63 ^{bc}	25.42±2.63 ^c	0.153
Neutro (x 10 ⁹ /l)	1.76±0.81 ^a	2.97±0.76 ^b	4.54±0.78 ^c	3.65±0.76 ^{bc}	3.79±0.76 ^{bc}	0.154
Lym (x 10 ⁹ /l)	12.76±2.64 ^a	15.14±2.47 ^{ac}	15.03±2.55 ^{bc}	16.92±2.47 ^b	20.88±2.47 ^b	0.231
Monocytes (x 10 ⁹ /l)	0.49±0.29	0.60±0.27	0.93±0.28	0.77±0.27	1.30±0.27 ^b	0.286
Eosinophils (x 10 ⁹ /l)	0.37±0.34 ^a	0.72±0.29 ^a	0.77±0.30 ^a	0.51±0.29 ^a	1.26±0.29 ^b	0.323
Basophils (x 10 ⁹ /l)	0.26±0.30 ^b	0.01±0.28 ^a	0.02±0.30 ^b	0.02±0.28 ^a	0.63±0.28 ^a	0.503
Retic (K/uL)	1.33±1.68 ^{ab}	2.65±1.57 ^b	4.27±1.68 ^c	0.68±1.57 ^a	4.65±1.57 ^c	0.327
RDW %	20.79±1.64 ^a	25.82±1.64 ^c	24.22±1.96 ^{ab}	25.90±1.73 ^c	24.00±1.57 ^{ab}	0.205

^{a,b,c,d} Significant differences (P <0.05) exist between the means in the same rows with different superscripts.

¹Dietary treatment :Control (Conventional broiler diet), Conventional broiler diet + 5% KMSC, Conventional broiler diet + 10% KMSC, Conventional broiler diet + 15% KMSC, Conventional broiler diet+ 20% KMSC.

²Parameters: MCV= mean corpuscular volume, Neutro = Neutrophils, WBC= White blood Cell, MCH = mean cell haemoglobin, RBC=Red Blood Cell, Lym = Lymphocytes.

3.3.4 Serum biochemical parameters

Diet significantly affected ($P < 0.05$) serum biochemical parameters (Table 3.8). Broilers offered the control diet had the highest levels of ALT (44.31 ± 3.53), followed by those fed 20% KMSC (37.81 ± 3.53). However, the 20% KMSC-fed broilers had the lowest ALT levels (238.56 ± 21.46). With regards to digestive enzymes, diet significantly affected both the amylase and lipase. The amylase levels were highest in the birds fed the 15% KMSC whereas those fed the 20% KMSC got the lowest levels. The highest levels of lipase were obtained in birds fed a 20% KMSC diet. The least levels of lipase were found in broilers fed 5% KMSC diet. With an increase in KMSC inclusion on a broiler diet, the total bilirubin concentration dropped. Glucose levels also declined with the increased level of KMSC. The control diet had the highest glucose levels while the 20% KMSC diet had the lowest. Diet did not affect cholesterol, serum protein parameters and serum mineral concentrations.

Table 3. 8: Serum biochemistry of broilers fed kalahari melon based diets

Parameters ²	Dietary treatments ¹					SEM	<i>P-Values</i>
	Control	5% KMSC	10% KMSC	15% KMSC	20% KMSC		
ALT (IU/L)	44.31 ^c	33.99 ^a	34.74 ^a	33.56 ^a	37.81 ^b	3.53	0.184
ALKP (IU/L)	254.87	273.31	270.68	291.93	238.56	21.46	0.479
Amylase (IU/L)	406.56 ^b	411.43 ^b	396.43 ^{ab}	444.31 ^c	371.43 ^a	27.43	0.454
Lipase (IU/L)	143.12 ^b	114.81 ^a	119.62 ^a	143.62 ^b	148.18 ^c	19.35	0.639
Total bilirubin	5.17 ^c	4.12 ^b	4.06 ^b	3.37 ^a	3.31 ^a	0.84	0.534
Cholesterol(mmol/L)	3.66 ^b	3.73 ^c	3.47 ^a	3.42 ^a	3.59 ^{ab}	0.13	0.485
Glucose (mmol/L)	7.01 ^c	6.42 ^b	6.65 ^b	5.81 ^a	5.47 ^a	0.52	0.231
Globulin (g/L)	21.00 ^{ab}	22.81 ^c	21.00 ^{ab}	20.68 ^a	21.93 ^b	1.04	0.598
Albumin (g/L)	14.06 ^{ab}	13.81 ^a	13.87 ^a	14.18 ^b	14.18 ^b	0.31	0.867
ALB/GLOB	0.73 ^b	0.63 ^{ab}	0.68 ^{ab}	0.82 ^c	0.64 ^a	0.08	0.490
Total protein (g/L)	38.31 ^c	36.56 ^b	35.00 ^a	35.00 ^a	35.12 ^{ab}	1.39	0.371
Urea (mmol/L)	0.90 ^c	0.88 ^{ab}	0.86 ^a	0.89 ^b	0.90 ^c	0.01	0.225
Phosphorous	3.14 ^a	3.19 ^b	3.19 ^b	3.36 ^c	3.65 ^d	0.15	0.142
Calcium (mmol/L)	2.34 ^c	2.30 ^{ab}	2.29 ^a	2.32 ^b	2.29 ^a	0.02	0.406

^{a, b} Significant differences ($P < 0.05$) exist between the means in the same rows with different superscripts.

¹Dietary treatment :Control (Conventional broiler diet), Conventional broiler diet + 5% KMSC, Conventional broiler diet + 10% KMSC, Conventional broiler diet + 15% KMSC, Conventional broiler diet+ 20% KMSC.

²Parameters:ALKP =Alkaline phosphatase =, ALT Alanine aminotransferase .

3.4 Discussions

3.4.1 Growth performance

The current study assessed the effects of dietary KMSC inclusion as a substitute protein source on broiler for growth performance. During the finisher stage broilers fed the control and 5 % KMSC had similar values for BWG, ADG, and lower FCR. Based on the comparable values, the lower levels KMSC has the likelihood of improve gut health, increase appetite and improve digestibility and general performance of broilers. Interestingly, the inclusion in the diet at 15% KMSC levels gave higher overall ADFI values. This concurs with the observations by Shazali *et al.*, (2013), who reported that the ADFI of broilers fed WMSC increased by 15% and 20%. The finding is inconsistent with the studies by (Ahmed 2004; Khilil, 2011; Bolu *et al.*, 2011) who stated that WMSC can be included in broiler diet up to 10% without any negative effect on their growth performance. *Citrullus lanatus* contains fibre content (Shazali *et al.*, 2013). However, the higher levels of fibre content have been associated with reduced nutrient utilization of seed meal in broiler rations (Ahmed, 2004). Bustamante *et al.* (2013) reported that excessive fibre in broiler diets might cause a reduction in broilers performances.

3.4.2 Growth and protein utilisation efficiency

The results showed that when KMSC was included to the diet of broilers, protein utilization and GE declined statistically. This reflects a negative effect of the high inclusion levels of KMSC on digestibility and nutrient utilisation. It was apparent that the incline in PC with increasing levels of the KMSC in diets was in close association to the observed increase in ADFI as the calculation of PC is based on ADFI. The observed reduction in the protein efficiency ratio (PER) is reflective of a reduction in protein value of a protein source. According to the study's findings, when the PC level increases, PER decreases which may

indicate reduced digestibility and lower release and availability of essential amino acids that ultimately affects chickens' growth. The typical PER values for soybean concentrates ranges from 2.0-2.2. The PER is a commonly used method in the evaluation of the quality of different protein sources (Brody, 1999). Rat's studies demonstrated that, growth rate of weanling rats is a valid indicator of dietary protein value hence, PER may be predicted as a ratio of gain in weight over the protein consumed. This may also apply to broilers. The apparent decline in PER with increasing levels of KMSC in diets can therefore point to the effects of some factors in the KMSC such as high fibre or some secondary plant metabolites that can affect protein quality (Bressani, 2003; Kurpad, 2013). Thus, treatment of the KMSC, including prior to its inclusion in diets, may enhance the effective release of essential amino acids for optimum animal growth (Bressani, 2003).

3.4.3 Haematology parameters

Haematological parameters are considered a vital measurement tool for evaluating the health status of broilers and other animals (Disetlhe *et al.*, 2018). The haematological parameters further provide critical information regarding the condition of animals and aid in distinguishing between healthy and unhealthy ones. In the present study, inclusion levels of KMSC affected several haematological blood parameters. Nevertheless, no dietary effects observed on the RBC and Hb. These results are inconsistent with findings from previous studies involving graded levels of WMSC on the performance, carcass assessment and blood parameters of broilers (Bolu *et al.*, 2011). The WBC of the broilers' blood in this study demonstrated that KMSC positively impacted the formation of protective cells of the body. The haematological indices also revealed that the lymphocytes, monocytes, neutrophils and eosinophils values generally tended to increase with incremental levels of KMSC. The elevation of these protector cells could have been stimulated by the bioactive compounds such

as flavonoids contained in the KMSC which normally promotes immunocompetence in body systems as reported elsewhere (Marume *et al.*, 2020)

3.4.4 Serum biochemical indices

Serum biochemical indices are used to track an animal's nutritional and physiological status as well as identifying some subclinical illnesses (Tijani *et al.*, 2015). Animal health status may be indicated by serum biochemical parameters and any changes resulting from internal or external factors such as dietary modifications (Toghyani *et al.*, 2010). In the current investigation, the blood serum metabolites were measured to evaluate the nutritional status and health of broiler. The inclusion of KMSC in diets affected serum blood parameters. Liver enzymes such as alkaline phosphatase and alanine transferase indicate changes in liver functions caused by some dietary factors. Elevation of liver enzymes may indicate toxicity or strain on the liver caused by dietary alteration (Toghyani *et al.*, 2010). In general, the increasing inclusion of KMSC in broiler chickens appeared to depress the ALT levels. The decrease in ALT levels resulted in the reduction of the liver damages. The ALT is an enzyme found inside the liver cells. With regards to digestive enzymes, diet significantly affected both the amylase and lipase. The increasing levels of dietary KMSC tended to increase the lipase content indicating the increasing dietary levels of KMSC may predispose to liver and kidney damage (Tijani *et al.*, 2015). Nevertheless, there were no high incidents of death throughout the experimental period.

3.5 Conclusion

Findings from the study showed that KMSC can replace SBM in broiler diets at lower levels (5%) without affecting their growth performance, nutrient utilisation and maintain their general health. The study showed that the inclusion of KMSC to 5% significantly induced

better growth and feed efficiency. There was a decline in key growth parameters as KMSC increases. The possible inclusion of KMSC as a source of protein in broiler diets was reflected by the reduced FCR and overall higher ADG as well as the improved overall protein utilization efficiency and nutritional status of broilers. Higher inclusion levels beyond 20% KMSC may reduce performance of broiler probably due to the increased fibre content that may reduce digestion and nutrient utilisation by the birds.

3.6 References

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CHAPTER 4

CARCASS AND MEAT QUALITY CHARACTERISTICS, AND INTERNAL ORGAN MORPHOMETRY IN BROILERS FED KALAHARI MELON BASED DIETS

Abstract

The study assessed the carcass traits, meat quality and internal organ development in broilers fed KM based diets. At 42 days, broilers were slaughtered in order to determine internal organs morphometry, carcass traits and measurements related to meat quality. Four broilers was randomly selected from each pen for analysis. Broilers fed control and 5% KMSC diets had the highest hot carcass weight (HCW) ($1431.62 \text{ g} \pm 26.55$, $1426.28 \text{ g} \pm 26.55$) and cold carcass weight (CCW) of ($1373.56 \text{ g} \pm 26.40$, 1369.21 ± 26.40), respectively. No effect of diet was observed on caeca and colon. Similarly, diet significantly ($P < 0.05$) affected meat quality parameters. It can be concluded that broilers carcass traits, internal organs sizes and meat quality were adversely affected by KMSC inclusion levels in broiler diets.

Keywords: carcass traits, chicken, interna organs, meat colour, slaughter

4.1 Introduction

A broiler chicken requires a balanced diet containing protein sources to enable it to grow well to be able to reach the target market weight. A broiler chicken should weigh between 1,90 kg and 2 kg at six weeks of age (Grain SA, 2021). Broilers are well known to be reared within a short time and specifically for meat production for human consumption. Soybean meal is the ideal source of protein that can be included in livestock diets. Efforts are being directed towards the identification and evaluation of alternative sources of dietary protein to reduce the over-reliance on SBM, which is currently becoming scarce and expensive due to a variety of

factors. Nevertheless, the alternative protein source should be able to sufficiently provide for the protein requirements of broilers while enhancing growth performance, meat quality and welfare. Quality of meat is becoming a critical aspect of purchasing decisions among the health-conscious consumers. Dietary manipulation can influence growth, healthiness and quality of meat (Zhai *et al.*, 2018).

Kalahari melon meal has the potential to be used as a possible substitute for broiler conventional protein sources. Apart from the high protein content, Kalahari melon is reported to contain a variety of secondary plant compounds, particularly flavonoids and phenols (Kumar *et al.*, 2014) that may stimulate good general health, improved meat quality and fatty acid profiles. High concentrations of n6 and n9 essential FAs, such as oleic, stearic, palmitic and some isomers of linoleic acid are also obtainable in the Kalahari melon oil cake (Berry, 2015) that may have a desirable influence on quality of meat and fatty acid profiles. Furthermore, KMSC may contain certain vitamins, minerals and antioxidants (lycopene and carotenoids) that may help affect vital aspects of meat quality like color, pH and tenderness (Das *et al.*, 2012; Mogotlane, 2015; Tabiri *et al.*, 2016). Currently, there have not been any attempts to evaluate the potential influences of KMSC based diets on internal organ development, carcass measurements and meat quality characteristics in broilers. The current study, therefore, explored the possible effect of KMSC based diets on carcass and meat quality characteristics in broilers.

4.2 Methodology

4.2.1 Diet, study design and study site

The research site, diets and design of the study are described in Sections 3.2.1-3.2.3, while Section 3.2.4 provides an explanation of the dietary treatments, experimental design and animal management.

4.2.2. Slaughter procedures

The experiment was terminated on day 42. The birds were placed in crates and taken to a chicken abattoir for slaughter. They were left without feed for 13 hours in order to facilitate crop clearance (Ari *et al.*, 2013), during which they were supplied with drinking water only. When the broilers arrived at the slaughterhouse, they were divided into groups based on the diets they were given (Control; 5% KMSC; 10% KMSC; 15% KMSC & 20% KMSC). Broilers were then held upside down for stunning. Using a sharp knife, the jugular vein of the broilers were cut and they were exsanguinated for up to two minutes followed by de-feathering and eviscerated. After the evisceration were performed, the carcasses were placed separated in plastics and then weighed. The internal organs were also packed separated according to their treatments. The carcasses were placed in a chiller overnight at 4 °C for 24 hours and subsequently transported to to the NWU farm laboratory for the purpose of determining internal organ, carcass and meat quality parameters.

4.2.3 Carcass and internal organs measurements

Carcasses were weighed immediately after slaughter to determine the hot carcass weight (HCW) and chilled overnight. At 24 hours post slaughter, the carcasses were reweighed to obtain the cold carcass weight (CCW). The carcasses were then apportioned into different

carcass cuts. The weights of the breast, thighs and drumstick were subsequently taken. The internal organs including the livers, gizzards, spleens, hearts and intestines were carefully dissected and weighed individually. The intestinal length was also taken using a measuring tape. The dressing percentage was computed follows.

$$\text{Dressing\%} = \frac{\text{Carcass Weight (g)}}{\text{Live Weight (g)}} \times 100$$

The breast yields were also calculated as follows:

$$\text{Breast yield} = \frac{\text{Breast meat weight (g)}}{\text{Live Weight (g)}} \times 100$$

4.2.4 Meat pH and Temperature

Meat pH and temperature measurements were taken immediately after slaughter at 45 minutes, and 24 hr. Breast muscles at a depth of 2 cm, as described by (Maliwan *et al.*, 2017) was used by inserting the electrodes into the meat samples using a contact pH meter system (Barbosa-Filho *et al.*, 2017). Four broilers in each replication were chosen randomly to be used for evaluation of meat pH and temperature.

4.2.5 Meat colour measurement

Meat color was determined using a Minolta color guide (Spectrophotometer CM 2500c, Konika Minolta, Osaka, Japan). After slaughter (45 minutes and 24 hours), meat color measurements were made using the CIELAB system, which evaluate the meat's color in accordance with the following guidelines: L^* = Lightness, a^* = Redness, and b^* =

Yellowness. Four broilers in each replication were chosen randomly to be used for evaluation.

4.2.6 Meat cooking loss

Breast meat sample were obtained to determine cooking loss. Raw breast muscles were weighed to get the initial weight (W1) of raw meat. Meat sample were put into sealable plastic and bags placed in water bath and cooked at 80°C for 40 minutes. Thereafter, the samples were allowed 20 min of cooling and re-weighed to obtained the weight at cooking (W2). Loss due to cooking was determined as follows:

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

4.2.7 Meat drip loss %

Breast muscle strips (3 cm) were obtained from the raw meat and weighted (W1). The strips were hooked onto the sample bottle tops using wire steel in hung inside plastic bottles. The bottles were tightly sealed and placed in a chiller for 72 hours at 4 °C. The meat samples were the retrieved and strips re-weighed to obtain weight after drip (W2). Drip loss was computed as

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

4.2.8 Meat water holding capacity

Filter-paper press method developed by Grau and Hamm (1957), was used to determine the water holding capacity. Thins breast meat slices (5-6 grams) were freshly cut and weighted (W1). The slices were placed inbetween 2 filter papers and held for 5 minutes under weight

(60 kg pressure). The samples was then re-weighed (W1) to determine water holding capacity.

$$\text{WHC \%} = \frac{W1 - W2}{W1} \times 100$$

4.2.9 Meat tenderness

Cooked meat samples were used to measurement of cooking loss for determination of meat tenderness. The Meullenet Owens Razor Shear Blade (A /MORS) was utilized to compute shear force values. It was attached on a texture analyzer (TA XT plus, Stable Micro Systems, Surrey, UK). The average of the peak force measurements for each sample was expressed in Newtons (N).

4.2.10 Statistical analysis

Data on meat quality, carcass characteristics and internal organs and of broiler meat was analysed using GLM procedure of SAS (2010). Diet was considered as the only factor. The level of significance was set at $P < 0.05$ for all tests. The following statistical model was used as follows:

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

Where:

Y_{ij} = Response variables

μ = Overall mean observation,

T_i = effects of diets. ϵ_{ij} = random error

4.3. Results

4.3.1 Carcass characteristics

Dietary effects on carcass characteristics of broilers are shown in Table 4.1. The diet influenced all carcass characteristics apart from dressing percentage and thigh ratios. The control diet had the highest slaughter weights, HCW (1431.62g $\pm$ 26.55), CCW(1373.56g $\pm$ 26.40), average breast weight (282.15g $\pm$ 7.82) and average thigh weight (112.34g $\pm$ 2.94) followed by those fed the 5% KMSC fed broilers. Broilers fed 20% KMSC diet had the least values throughout.

4.3.2 Internal organs

The effect of partial replacement of SBM with KMSC on the size of internal organs in broilers are presented in Table 4.2. Apart from the weight of ceaca and colon, diet had a significant influence on all measured internal organs. The chicken fed 15% KMSC diet had the heaviest gizzard (42.42g $\pm$ 4.10) while the chicken fed with a 10% KMSC diet had the lowest gizzard weight (34.24g $\pm$ 4.17). However, the proventriculus and spleen weights were heaviest ($P < 0.05$) in broilers fed 10% KMSC. The duodenal weight was marginally higher in broilers fed the 15% KMSC diet while those fed the 20% KMSC had the highest jejunum and ileal weight.

Table 4.1: Carcass characteristics of broilers fed diets containing inclusion levels of Kalahari melon seed cake fed broilers.

Parameters	Dietary treatments ¹					<i>P-Values</i>
	0% KMSC	5% KMSC	10% KMSC	15% KMSC	20% KMSC	
Slaughter weight (g)	1883.12±10.57 ^c	1860.50±10.57 ^c	1737.16±10.74 ^a	1725±10.57 ^a	1762.50±10.57 ^b	0.001
Hot carcass weight (g)	1431.62±26.55 ^b	1426.28±26.55 ^b	1370.80±26.97 ^a	1357.03±26.55 ^a	1408.06±26.55 ^b	0.185
Cold carcass weight (g)	1373.56±26.40 ^b	1369.21±26.40 ^b	1334.35±26.83 ^a	1314.50±26.40 ^a	1354.06±26.40 ^b	0.481
Dressing %	76.01±1.61	76.71±1.61	79.23±1.63	78.82±1.61	77.45±1.61	NS
Av Breast weight (g)	282.15±7.82 ^b	278.43±7.82 ^b	244.03±7.95 ^a	242.18±7.82 ^a	257.03±7.82 ^a	0.001
Av Wing weight (g)	82.03±1.64 ^b	86.09±1.64 ^b	79.51±1.66 ^a	76.40±1.64 ^a	79.68±1.64 ^a	0.001
Av Thigh weight (g)	112.34±2.94 ^b	112.18±2.94 ^b	110.00±2.99 ^b	107.65±2.94 ^{ab}	105.62±2.94 ^a	0.421
Av Drumstick weight (g)	96.56±2.44 ^a	102.65±2.44 ^b	95.32±2.48 ^a	97.50±2.44 ^a	99.21±2.44 ^{ab}	0.867
Breast ratio	0.19±0.00 ^c	0.19±0.00 ^c	0.17±0.00 ^a	0.17±0.00 ^a	0.18±0.00 ^b	0.001
Thigh ratio	0.07±0.00	0.07±0.00	0.08±0.00	0.07±0.00	0.07±0.00	NS

^{a, b, c} Significant differences ($P < 0.05$) exist between the means in the same rows with different superscripts.

¹Dietary treatment :Control (Conventional broiler diet), Conventional broiler diet + 5% KMSC, Conventional broiler diet + 10% KMSC, Conventional broiler diet + 15% KMSC, Conventional broiler diet+ 20% KMSC.

²Parameters:Av:Average,g:gram .

Table 4.2: Internal organs sizes of broilers fed inclusion levels of Kalahari melon seed cake.

Parameters	Dietary treatments ¹					<i>P-Values</i>
	0% KMSC	5% KMSC	10% KMSC	15% KMSC	20% KMSC	
Gizzard (g)	36.32 ±4.10 ^a	34.40 ± 4.10 ^a	34.24 ± 4.17 ^a	42.42 ±4.10 ^b	34.67 ±4.10 ^a	0.587
Proventriculus (g)	9.26 ±0.45 ^{ab}	10.67 ± 0.44 ^b	8.78 ± 0.46 ^a	9.74 ± 0.44 ^{ab}	8.39 ± 0.48 ^a	0.005
Spleen (g)	2.01 ±0.69 ^b	3.87 ± 0.69 ^c	1.90 ± 0.70 ^a	2.03 ± 0.69 ^b	1.93 ± 0.70 ^a	0.210
Liver (g)	35.22 ±1.20 ^b	37.63 ±1.20 ^c	35.97 ± 1.22 ^b	34.28 ±1.20 ^a	35.92 ±1.20 ^b	0.387
Duodenum (g)	29.93 ± 1.10 ^a	29.52 ± 1.10 ^a	29.17 ± 1.12 ^a	31.41 ± 1.10 ^b	29.13 ± 1.10 ^a	0.582
Jejunum (g)	69.88 ± 2.16 ^a	69.66 ± 2.16 ^a	66.72 ± 2.20 ^a	70.76 ± 2.16 ^{ab}	72.25 ± 2.16 ^b	0.491
Ileum (g)	61.69 ± 2.62 ^{ab}	57.53 ± 2.62 ^a	61.09 ± 2.66 ^{ab}	58.59 ± 2.62 ^a	65.05 ± 2.62 ^b	0.292
Caeca (g)	18.32 ± 0.43	18.25 ± 0.43	18.31 ± 0.44	18.62 ± 0.43	19.14 ± 0.43	NS
Colon(g)	4.79 ± 0.36	4.90 ± 0.36	4.83 ± 0.36	4.79 ± 0.36	4.92 ± 0.36	NS

^{a, b} Significant differences (P <0.05) exist between the means in the same rows with different superscripts.

¹Dietary treatment :Control (Conventional broiler diet), Conventional broiler diet + 5% KMSC, Conventional broiler diet + 10% KMSC, Conventional broiler diet + 15% KMSC, Conventional broiler diet+ 20% KMSC.

4.3.3 Meat quality

Table 4.3 presents the KMSC inclusion level in broiler diet on meat quality measurements. Diet significantly affected the meat quality coordinates in response to inclusion levels of KMSC. Breast muscle pH and temperature were higher ($P < 0.05$) for carcasses fed control and 15% KMSC post 24-hour chilling. Broilers fed the 15% KMSC diets had the highest L^* values (47.14 ± 0.33), while the control had the highest a^* value (2.88 ± 0.09) and 5% KMSC fed broilers for b^* (3.79 ± 0.17). In terms of WHC% in breast meat, the broilers fed 15% KMSC had the lowest value (13.88 ± 2.55). The highest value observed was in broilers fed 10% KMSC (19.80 ± 2.55). The dripping loss was observed to be lower on the broilers fed 5% KMSC (6.81 ± 0.48). The dripping loss % increased significantly ($P < 0.05$) with incremental levels of KMSC. The highest cooking loss was observed in broilers fed 15% KMSC (32.30 ± 1.11), while the lowest was observed in 5% KMSC (29.14 ± 1.11). The control diet had the strongest shear force texture (6.30 N) ($P < 0.05$). Nonetheless, meat tenderness was lowest in birds fed the 5% KMSC (5.41 N).

Table 4.3:Meat quality measurements of broilers fed inclusion levels of Kalahari melon seed cake.

Parameters ¹	Dietary treatments					<i>P-Values</i>
	0% KMSC	5% KMSC	10% KMSC	15% KMSC	20% KMSC	
Meat pH (0 hr)	5.72±0.01 ^b	5.70±0.01 ^{ab}	5.74±0.01 ^c	5.66±0.01 ^a	5.74±0.01 ^c	0.020
Meat temp (0 hr)	26.19±0.36 ^a	26.30±0.36 ^b	26.59±0.37 ^c	26.33±0.36 ^c	26.41±0.36 ^b	0.955
Ultimate pH _u	5.79±0.01 ^b	5.77±0.01 ^a	5.77±0.01 ^a	5.78±0.01 ^b	5.74±0.01 ^{ab}	0.137
Meat temp (24hrs)	15.71±0.18 ^{bc}	15.62±0.18 ^b	15.11 ± 0.18 ^a	16.12 ± 0.18 ^b	15.74±0.18 ^c	0.007
<i>Meat colour(0 Hrs)</i>						
<i>L*</i>	46.28±0.46 ^a	47.25±0.46 ^b	46.03±0.47 ^a	47.62±0.46 ^c	46.64±0.46 ^a	0.101
<i>a*</i>	2.19±0.13 ^{ab}	1.97±0.13 ^a	2.35±0.13 ^c	2.34±0.13 ^c	2.18±0.13 ^{ab}	0.254
<i>b*</i>	2.12±0.15 ^a	3.11±0.1 ^c	2.17±0.15 ^a	2.73±0.15 ^b	2.77±0.15 ^b	0.001
<i>Meat colour(24 Hrs)</i>						
<i>L*</i>	45.70±0.33 ^a	46.09 ± 0.33 ^b	45.19 ± 0.34 ^a	47.14±0.33 ^c	46.41±0.33 ^b	0.001
<i>a*</i>	2.88 ± 0.09 ^c	2.78±0.09 ^{ab}	2.62 ± 0.10 ^a	2.81±0.09 ^b	2.80±0.09 ^b	0.477
<i>b*</i>	3.00 ± 0.17 ^a	3.79 ± 0.17 ^d	3.40 ± 0.17 ^{ab}	3.48 ± 0.17 ^{ab}	3.62 ± 0.17 ^c	0.020
WHC (%)	18.80 ± 2.55 ^b	18.45±2.55 ^b	19.80±2.55 ^c	13.88 ± 2.55 ^a	17.48 ± 2.55 ^b	0.531
Drip loss %	6.82 ± 0.48 ^a	6.81 ± 0.48 ^a	7.70 ± 0.48 ^b	7.66±0.48 ^b	7.83 ± 0.48 ^b	0.382
Cooking loss (%)	29.45 ± 1.11 ^a	29.14 ± 1.11 ^a	29.34 ± 1.11 ^a	32.30 ± 1.11 ^c	30.74 ± 1.11 ^b	0.225
Shear force (N)	6.30±0.56 ^c	5.41±0.56 ^a	6.15±0.56 ^c	5.78±0.56 ^b	5.88±0.56 ^b	0.830

^{a,b,c,d}Significant differences (P <0.05) exist between the means in the same rows with different superscripts.

¹Parameters:L*=lightness,a*=redness,b*=yellowness;WHC=WaterHoldingcapacity

4.4 Discussion

4.4.1 Carcass characteristics

Diet significantly affected all the measured carcass parameters except the dressing percentage and thigh ratios. The observed high carcass values for the birds fed the control diet were expected, considering that the control diet contained SBM as the protein source. When it comes to broiler diets, soybean meal is commonly used because of its rich amino acid profile (Ravindran, 2013). The most limiting amino acids in KMSC is lysine (Ojeh *et al.*, 2007). The Kalahari melon cake is characterized by balanced CP content and high fibre content which could have affected the utilization of the diets containing the cake. The observation of the current study are in contrast to the findings of Mukhtar *et al.* (2016), who reported that incorporating WMSC up to 20% in diets of broiler chicken showed no significant difference in carcass components and meat/bone ratio. The disparity in observations between the two studies could be due to the fact that Mukhta *et al.* (2016) used the sweet melon based diets while the current study used the bitter Kalahari melon and hence there are some taste variability among the 2 melon types. Unpalatability and unpleasant smell of KMSC might also be contributing factor. Composition of seed cakes differs depending on the environmental factors, seed quality and extraction techniques (Iji *et al.*, 2017).

It is worth noting that the higher the breast muscle weight the more meat deposited in the carcass of broilers. In the current study, the breast muscle weight tended to decrease with the incremental levels of KMSC. This is consistent with the observed decline in carcass weight with incremental levels of KMSC in the diets. These results are also consistent with of Al-Sagan *et al.*, (2020) who observed a decrease on breast muscle weight when the inclusion levels of blue lupine (*Lupinus angustifolius*) meal increases at the incremental rate up to 30% in broiler diets. The decrease in breast muscle may be due to the low essential amino acid

concentrations on the broiler diet that is essential for muscle protein deposition (Al-Sagan *et al.*, 2020). The KMSC is reported to contain high levels of essential amino acids except lysine and sulphur containing amino acids (Ojeh *et al.*, 2007; Mustafa and Alamin, 2011). The lack of effect of KMSC inclusion levels in diet on dressing percentage is consistent with findings from (Bolu *et al.*, 2011; Mukhar *et al.*, 2016) when WMSC was included in diets of broilers.

4.4.2 Internal organs

The internal organs characteristics and gut microbiome are indicative of alterations in diets through inclusion of new ingredients ultimately affecting animal productivity (Kogut *et al.*, 2017). Changes in internal organs morphometry may be reflective of the influences of diet and its components on the internal organ development and function (Disetlhe, 2017). The results of the current study showed variability among internal organs that may be ascribed to the presence of KMSC in the diets with the birds fed KMSC with the birds fed the 20%KMSC having the highest values. The results are contrary to the findings by (Mukhar *et al.*, 2016) who did not find any significant effect of WMSC inclusion in diet of broilers in internal organ weights. This could be attributed to the fact that KMSC with high fiber could have increased the retention time of the feed to enable the optimization of digestive enzyme activity to deal with the increase fiber and other compounds in the diets. The diet effect on the liver weight suggest that the KMSC diet had high levels of bioactive compounds that could affect the liver functions.

4.4.3 Meat quality

Diet, age, sex of birds and management system are the most contributing factors determining the quality of broiler (Kuźniacka *et al.*, 2020), while meat pH may influence on other meat

quality parameters (Hofmann, 1994). In the current study, breast muscle pH and temperature and meat color were not affected by the incremental levels of KMSC amongst the treatment groups. The meat pH range (5.74-5.79) found in this study falls within the range that is appropriate for monogastric animals (pH 5.5-6.5) (Aoe *et al.*, 2008; Ribarski & Genchev, 2013). Meat colour is one of the major determinants of purchasing decision by consumers. While diet had no influence on meat colour, generally there was an observed increase in (L^* and b^*) and decrease in a^* meat colours coordinates with incremental levels of KMSC. This could be ascribed to the antioxidants lycopene and carotenoids present in Kalahari melon which may influence meat quality (Das *et al.*, 2012; Tabiri *et al.*, 2016). Various studies have reported that kalahari melon is a good source of glucose, lycopene, vitamin C, β -carotene and fiber (Mogotlane, 2015; Gupta & Pandey, 2019).

Water holding capacity in breast meat appeared to be lower at higher inclusion of KMSC in the diets. However, when compared to other treatments the broilers fed 10% KMSC demonstrated a higher ability of the breast meat to retain water. The broilers fed higher inclusions of KMSC obtained the higher drip and cooking loss implying that the KM inclusion at levels higher than 10% may negatively affect the meat quality and storage of the meat. The drip loss % increased with an incremental levels of KMSC. The breast meat tenderness was significant different among the treatments with birds fed 5% KMSC showing high meat tenderness in comparison with the other treatment.

4.5 Conclusion

From the results, the observed lower carcass weight in broilers fed the diets containing the KMSC based diet compared to the control could be attributed to the lower protein value and

high fibre in the KMSC. The results of the current study also showed variability among internal organs that may be ascribed to the KMSC inclusion in the diets with the birds fed KMSC with the birds fed the 20%KMSC having the highest values. Meat pH and colour were influenced by diet. Nevertheless, water holding capacity was lower at highest KMSC inclusion levels while the drip loss increased as levels of the KMSC in diets increased. In general, this study showed inclusion levels of KMSC up to 20% had effects on carcass and meat quality traits as there was a decline of parameters as inclusion level increased.

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CHAPTER 5

EFFECT OF DIETARY INCLUSION LEVELS OF KALAHARI MELON SEED CAKE ON TIBIA BONE DEVELOPMENT AND INCIDENCE OF RICKETS OF BROILERS

Abstract

The current study assessed bone growth and development and incidents of rickets in broilers fed KM based diets. Five dietary treatments were assigned to four hundred(400) unsexed Cobb 500: conventional diet control; conventional broiler diet + 5% KMSC; conventional broiler diet + 10% KMSC; conventional broiler diet + 15% KMSC; and conventional broiler diet + 20% KMSC. Each of the treatments were repeated eight times with each pen holding ten broilers as an experiment unit. Ten right tibia from each treatment as experiemental unit were surgically removed, defleshed and cleaned during the 48 day necropsy. Diet influenced ($P < 0.05$) tibia linear measures. Inclusion of KMSC in diets increased the tibia linear parameters. The total bone ash and moisture were influenced by the dietary treatments ($P < 0.05$). Diet also influenced minerals and phosphorus ($P < 0.05$) content. The broilers fed 20% KMSC recorded the highest values of total ash, moisture and phosphorus content. The broiler chickens fed 20% KMSC recorded the highest values on all the macromineral content. The tibiae from broilers fed 5%, 10% and 20% KMSC showed deformation in the region of the proximal metaphyseal tibiotarsus which is associated with the gross lesion analysis. It was concluded that the inclusion of KMSC improved bone strength and agility in broiler chickens.

Keywords: calcium, mineral, welfare, phosphorous, tibia.

5.1 Introduction

Chickens are reared for dual purpose which includes production of meat and eggs for human consumption. Nonetheless, the bone health hampers chicken productivity particularly in broiler production causing economic losses to the producers. In the broiler industry, bone health conditions that result in leg weakness have been identified as a serious issue which raises concerns about the welfare of the chickens (Nari & Ghasemi 2020). Some bone health indicators include bone linear parameters, mineral content particularly calcium (Ca) and phosphorous (P) and bone breaking strength (Evaris *et al.*, 2021). Calcium and P deficiency has serious implication on bone development, growth performance and welfare in broiler chickens (Nari & Ghasemi 2020).

Leg complications such as tibial dyschondroplasia and lameness appear in broilers as a result from a deficiency of nutrients (Faustin-Evaris *et al.*, 2022). The chicken with bone disorders can display characteristics of rickets and laminitis which ultimately will results in decrease in feed intake and body weight gain (Świątkiewicz & Arczewska-Włosek, 2012; Nari & Ghasemi 2020). The bone disorders may also lead to mortality from starvation and dehydration (Fleming, 2008). Therefore, dietary inclusions of minerals should always ensure sufficient provision of important minerals such as Ca and P to ensure normal bone growth and development. Dietary minerals are often provided in diets by some major ingredients such as monocalcium phosphate. Some protein and energy ingredients may also be sources of minerals while other ingredients may influence mineral assimilation during digestion.

While containing some important minerals, KMSC also contains some secondary plant metabolites which can influence minerals release and assimilation in the digestive system. Therefore the inclusion of KMSC may induce effective release of minerals and other

nutrients in the gut, while elevating the nutrient utilization and assimilation resulting in effeiceint bone growth and development that ensures healthy feet and welfare. However, information on the possible effects of KMSC inclusion in broiler diets on bone development is largely unavailable hence requires a comprehensive investigation.

5.2 Experiemntal procedures

5.2.1 Diets, study design and animals

The diets, animals and experiment design were explained in Section 3.2.1-3.2.3, while Section 3.2.4 provides an explanation of the animal management.

5.2.2 Slaughter proceduers

The outline of the slaughter technique has been provided in Section 4.2.2.

5.2.3 Tibia gross lesion analysis

In order to evaluate the incidence of rickets in broilers on a variety of dietary treatments groups, tibia gross anatomy were performed. Carefully removing the muscles from around the tibia, the proximal section of the bode were dissected uing sharp knife to expose the epiphyse plate. Visual examination of gross lesions was performed on growth plates from the tibia.

5.2.4 Tibia linear measurement and bone strength

After slaughter, 10 right thighs from each treatments as an experiment unit were sampled, surgically removed from the flesh, and all tissue, including cartilage caps, was cleaned.

Tibiae were weighed to get the Tibia weight (TW). The tibia linear measurements were determined using a (150mm Electronic Digital, Vernier Caliper Micrometer). The measurements were recorded in millimetres. Following bone breaking strength, every tibia was positioned on a 3-point bend/snap fixture that could be adjusted. These fixtures were then fitted on a heavy-duty TA-XT plus platform from Stable Microsystems in Surrey, UK. The peak load prior to the break was recorded to be the bone-breaking strength (BBS) in newtons.

5.2.5 Bone ash content

Following the recording of the bone-breaking strength measurement, tibia fragments were gathered and sent to the Nutri Feeds Laboratory (Viljoenskroon) for analysis. In order to fit into the muffle furnace cubes and facilitate defatting and ashing, dry tibia samples were broken into small pieces. To calculate the percentage of bone ash, bone fragments were shed at 600 °C in a muffle furnace for over an entire day. They were then allowed to cool in a desiccator and weighed.

$$\text{Tibia bone ash \%} = \frac{(\text{Tibia ash weight})}{(\text{Tibia dry weight})} \times 100$$

5.2.6 Bone mineralisation

The bone mineral concentraions were determined at the Nutri Feeds Laboratory (Viljoenskroon) following the AgriLASA recommendations. Brielfy, after ashing, the contents digested overnight in 55% nitric acid. The contents were subsequently decanted into McCartney bottles while ensuring no mixing of the contents. An ICP Spectrometer was used

to measure the concentrations of moisture, calcium, magnesium, sodium, ” or “ phosphorus. The macromineral content was shown as %.

5.2.7 Data analysis

Dietary effects on on tibia parameters, mineral content and bone breaking strength was assessed using the GLM procedure of SAS (2010). The following model was used

$$Y_{ijk} = \mu + D_i + \varepsilon_{ijk}$$

Where: Y_{ijk} = observations (tibia linear measurements, BBS, mineral content)

μ = constant, D_i = dietary effect

ε_{ijk} = error term.

5.3 Results

5.3.1 Gross lesion assessment

Incidence of rickets and gross lesion analysis are shown in Plate 5.1 up until 5.5. Control group showed normal bone. However, the white articular cartilage or growth plate of the tibia bone from broilers fed diet containing 5%, 15% and 20% KMSC were little bit enlarged. The tibiae from broilers fed 5%, 10% and 20% KMSC showed deformation in the region of the proximal metaphyseal tibiotarsus compared to other treatments groups. These were a clear signs of bone abnormalities associated with leg weakness connected with various metabolic disorders which cause fracture of the bones in broiler chickens.

5.3.2 Tibia Linear Parameters and BBS

Table 5.1 shows tibia biomechanics in broilers. Diet however, significantly affected ($P < 0.05$) tibia biochemical parameters. The broilers provided with the 15% KMSC diet had the biggest TW, while the broilers fed the 10% KMSC diet obtained the smallest TW. The birds in the 15% KMSC treatment obtained the greatest TL and TWidth. The broilers fed 5% KMSC had the highest ($P < 0.05$) values for bone-breaking strength (208.82 ± 0.59). The lowest observed were broilers fed 15% KMSC (177.61 ± 0.59). It appeared that the increasing levels of KMSC in diets caused a decline in the BBS. The addition of KMSC to the diet was found to increase tibia measurements. Generally, the 15% KMSC-fed broiler chickens consistently had the highest values on tibia biomechanical parameters except for BBS.

5.3.3 Tibia bone mineralization

Table 5.2 shows tibia macromineral content of the broilers. Total ash, moisture and all minerals content were influenced by diet. Generally, the broilers fed 20% KMSC recorded the highest values of total ash, moisture and phosphorus content. The broilers fed diets containing KMSC appeared to have marginally higher levels of Ca and potassium.



Plate 5.1: Normal bone from broilers fed control diet.



Plate 5.2: Proximal metaphyseal tibiotarsus in broilers fed 5% KMSC demonstrating signs of rickets, including widening and minor distortion.



Plate 5.3: Deformation in broilers fed 10% KMSC in the proximal metaphyseal tibiotarsus, confirming rickets



Plate 5.4: Expansion in the proximal metaphyseal tibiotarsus area, confirming rickets in broilers given 15% KMSC

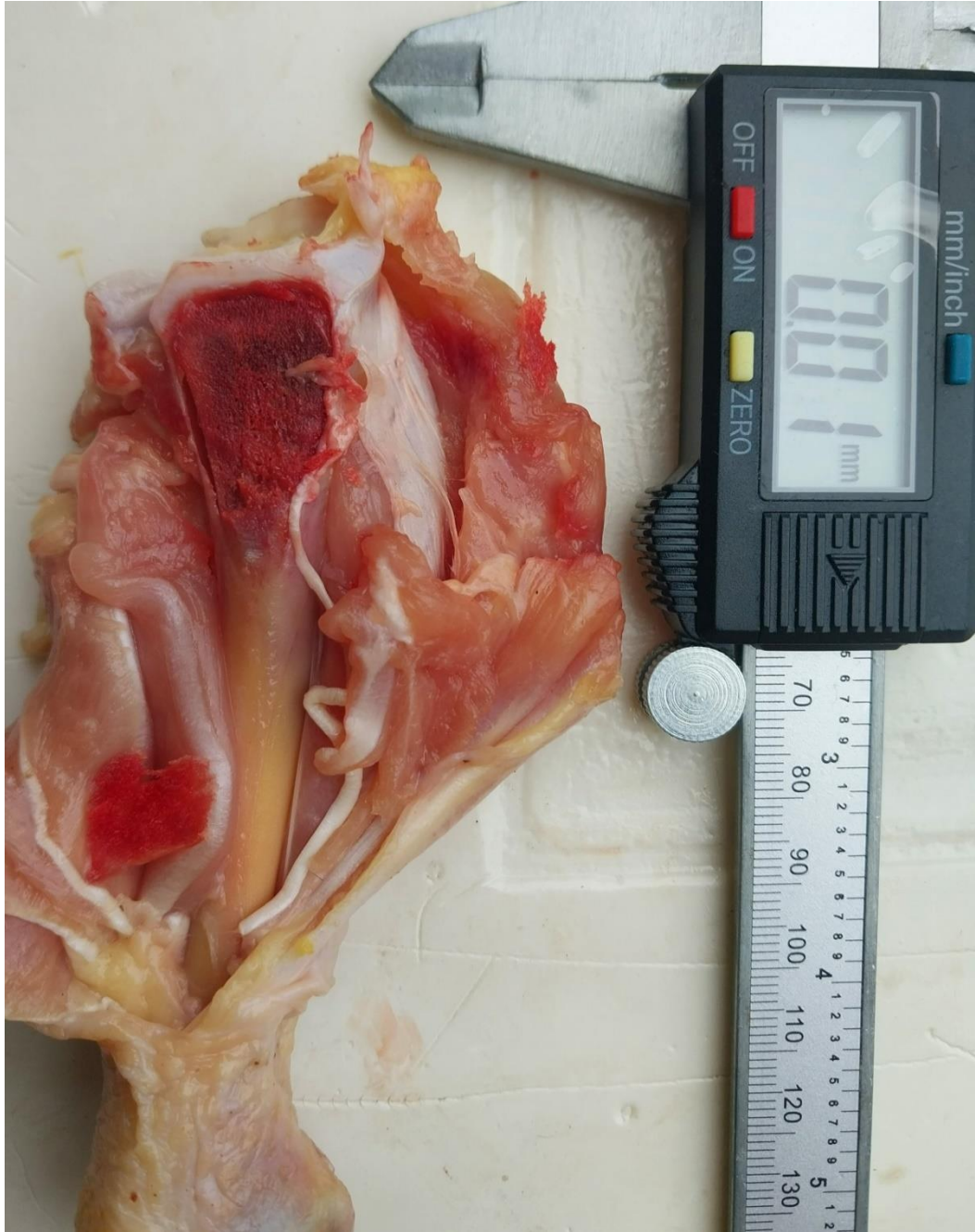


Plate 5.5 Minor distortion and enlargement in the area of the proximal metaphyseal tibiotarsus, which confirmed rickets

Table 5. 1: Tibia biomechanics of the broilers fed Kalahari melon based diets.

Parameters ²	Dietary treatments ¹					SEM ³	<i>P-Values</i>
	0% KMSC	5% KMSC	10% KMSC	15% KMSC	20% KMSC		
Tibia Weight (g)	7.37 ^a	7.63 ^a	7.00 ^a	8.16 ^b	7.32 ^a	0.36	0.248
Tibia diameter proximal end (cm)	2.36 ^b	2.32 ^{ab}	2.28 ^a	2.44 ^a	2.31 ^{ab}	0.04	0.131
Tibia width (cm)	0.72 ^a	0.81 ^c	0.76 ^b	0.80 ^c	0.78 ^b	0.02	0.079
Tibia length (cm)	10.15 ^b	10.01 ^{ab}	9.89 ^a	10.36 ^c	10.05 ^{ab}	0.15	0.341
Tibia diameter distal end (cm)	1.78 ^a	1.85 ^{ab}	1.83 ^{ab}	1.94 ^b	1.84 ^{ab}	0.04	0.101
Bone breaking strength (N)	194.61 ^b	208.82 ^c	193.26 ^b	177.61 ^a	187.33 ^{ab}	0.59	0.760

^{a, b, c} Significant differences ($P < 0.05$) exist between the means in the same rows with different superscripts

¹Dietary treatment :Control (Conventional broiler diet), Conventional broiler diet + 5% KMSC, Conventional broiler diet + 10% KMSC, Conventional broiler diet + 15% KMSC, Conventional broiler diet+ 20% KMSC.

²Parameters:g-gram, cm-centimeter, N-Newton

³SEM = standard error of the mean.

Table 5. 2: Mineral content of the broilers fed Kalahari melon based diets.

Parameters ²	Dietary treatments ¹					SEM ³	<i>P-Values</i>
	0% KMSC	5% KMSC	10% KMSC	15% KMSC	20% KMSC		
Tibia ash (%)	33.80 ^a	36.36 ^b	33.92 ^a	36.13 ^b	37.43 ^b	3.53	0.932
Moisture (%)	8.14 ^b	8.18 ^b	8.19 ^b	7.83 ^a	7.90 ^a	0.28	0.836
Calcium (%)	16.03 ^a	16.79 ^b	16.38 ^{ab}	16.15 ^a	17.11 ^c	1.24	0.969
Potassium (%)	0.26 ^b	0.22 ^a	0.25 ^{ab}	0.30 ^c	0.29 ^c	0.01	0.022
Magnesium (%)	0.23 ^c	0.19 ^{ab}	0.18 ^a	0.18 ^a	0.20 ^b	0.02	0.758
Sodium (%)	0.40 ^c	0.36 ^a	0.37 ^b	0.35 ^a	0.37 ^b	0.03	0.909
Phosphorus (%)	7.90 ^a	8.22 ^{ab}	7.90 ^a	8.01 ^{ab}	8.61 ^b	0.59	0.902

^{a,b,c} Significant differences ($P < 0.05$) exist between the means in the same rows with different superscripts

¹Dietary treatment :Control (Conventional broiler diet), Conventional broiler diet + 5% KMSC, Conventional broiler diet + 10% KMSC, Conventional broiler diet + 15% KMSC, Conventional broiler diet+ 20% KMSC.

²Parameters:%-percentage

³SEM = standard error of the mean.

5.4 Discussion

5.4.1 Gross lesion assessment

From the results, the addition of KMSC in diets significantly affected tibia gross lesions. Broiler bone health has been observed to be negatively influenced by mineral imbalances and deficiency in diets (Li *et al.*, 2020) ultimately affecting their welfare. The results from a recent study showed that broilers were susceptible to incidences of bone deformities and rickets often attributed to the high growth rates and body mass in broilers. Dinev (2011) described these bone deformities and rickets as metabolic disorders in growing broilers which is characterized by thickening and poor mineralization of the growth plates. The widening of the growth plate can therefore be an indicator of the effects of heavy weight that the bones are not able to carry (Diseth *et al.*, 2018). As observed in this study, there was thickening or irregularity of growth plate and leg deformities in broilers fed diet containing 5%, 15% and 20% KMSC treatment groups. Generally, this could be attributed to mineral imbalance or the inability of broilers to utilize Ca, P or Vitamin D (Gröne *et al.*, 1995; Dinev, 2011). The balance of calcium and phosphorus in broiler chickens is kept in a homeostatic state by vitamin D (Proszkowiec-Weglarz & Angel, 2013). Another explanation might be that the diets were not sufficiently feed-mixed to ensure uniformity as observed elsewhere (Riddell, 1992). Nevertheless, the results from the study did not show the negative effects of the presence of the KMSC in diets on bone development.

5.4.2 Tibia linear parameters and bone breaking strength

The outcomes from our recent study showed that diet influenced all tibia linear measurements apart from the TW and BBS. Incorporating KMSC into diets was found to generally increase tibia measurements, even though treatments. The presence of more minerals (P and Ca) in the

broiler diets as a result of KMSC, could be a factor of the improvement in biomechanical properties. Unsufficient Ca and P in broiler diets may result in poor bone development (Nari & Ghasemi 2020). High bone-breaking strength is an indication of strong bones associated with adequate mineral provision in broiler diets. The higher BBS observed in the broilers fed the 5%KMSC diet might be indicative of the mineral adequacy in broilers' diets enabled by the KMSC in diets. An insufficient amount of calcium in the blood can cause hypocalcaemia, which can impair bone growth and therefore lower bone mineral density poor and hence reduced BBS (Talaty *et al.*, 2009),.

5.4.3 Tibia bone mineralization

Broiler chickens reared intensively are becoming more prone to bone growth problems and defects as a result of inadequate supply of Calcium/Phosphorus. From the study, birds offered KMSC diets obtained higher bone ash amounts resulting in high concentrations of Ca and P content. This confirms that the the KMSC inclusion in the diet may positively influence mineral absorption in the gut. Like other ingredients of a similar nature, KMSC is composed of certain saponins and flavonoids that can enhance the bioavailability and absorption of minerals into the gut, thereby increasing effective mineral utilisation during bone growth and development.

5.5 Conclusions

The study showed that the presence of KMSC in diets improved bone mineralisation and development. Although the growth plates in broilers-fed diets containing KMSC were enlarged, this did not necessarily signify incidences of rickets in the broilers. The graded levels of KMSC in diets generally improved the tibia weight and bone-breaking strength. The

high TW and BBS were also associated with the high tibia ash and mineral concentrations in KM fed broilers. In conclusion, the inclusion of KMSC in broiler diets improved mineral assimilation and ultimately bone development. In conclusion, inclusion in diets containing KMSC improved bone mineralization, development and general welfare in broiler chickens.

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CHAPTER 6

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATION

6.1 Discussion

Broiler production contributes massively towards the agricultural sector in SA. The total production of broiler meat for 2017 was 1.658 million tonnes (SAPA, 2017) and consumption amounted to 2.9 million tonnes (DAFF, 2017). Local broiler production is failing to meet the growing demand and consumption level of South Africans (BFAP, 2014). However, broiler meat remains the most affordable and readily available protein source on the market as compared to beef, pork and mutton (DAFF, 2017). Nevertheless, the high feed cost and the inaccessibility of protein ingredient sources for broiler production remain a challenge in SA. Two major leading ingredient sources used for poultry feed are mainly maize (energy source) and soybean meal (protein source) and prices of these two commodities are likely to be expensive.

According to BFAP (2014) projections, SA will continue to import SBM for the next coming years. Nonetheless, this situation can be addressed by exploring unconventional protein sources, especially such as sorrel seed meal, moringa leaf meal, Bambara nut meal, velvet beans and faba beans that could ensure sustainability in intensive poultry production (Ozung *et al.*, 2018). The main objective of the study was to explore the efficacy of KMSC as an alternative protein for broiler chickens.

The effects of dietary inclusion levels of KMSC to the broiler diet on growth performance and health were evaluated in Chapter 3. The chapter revealed that dietary inclusion levels of KMSC on broiler diets influence growth performance traits of broiler chickens. Nevertheless, the inclusion of the KMSC in diets caused a decline in the performance of the broilers. Moreover, the high fibre of the KMSC diet could have reduced the utility of the diet. Strategies to reduce dietary fibre, includes enzyme inclusion in KMSC based diets (Mukhtar

et al., 2016). This strategy might therefore improve fibre digestibility and reduce its effects on the utilization of the diets. The dietary inclusion of KMSC in broiler diet influenced blood parameters. Overall, KMSC can partially replace SBM without affecting the growth performance and health of the broilers.

The study was carried out to assess the possible effects of KMSC dietary inclusion levels in broiler diets on carcass traits, meat quality and internal organ development in Chapter 4. Broilers fed the control and 5%KMSC diets had the highest slaughter weights, HCW, CCW, breast and thigh weights. The pH, temperature and colour were significantly affected by the inclusion levels of KMSC amongst the treatment groups. However, the range of meat pH values (5.74-5.79) were within the acceptable meat pH range (pH 5.5-6.5) (Aoe *et al.*, 2008; Ribarski & Genchev, 2013). However, the dietary inclusion of KMSC in broilers diet appeared to slightly influence meat colour attributes such as lightness and yellowness indicating that the pigmentation of meat was impacted by the carotene that was deposited in the muscles. In addition, it was interesting to observe that broilers fed 15% KMSC showed the capability of the breast meat to retain water compared to other treatments. Moreover, broilers fed 10% KMSC showed the inability to retain water. The breast meat tenderness was observed from the broilers fed 5% KMSC.

In Chapter 5, the dietary inclusion levels of KMSC in broiler diets on bone development and occurrences of rickets were assessed. Nevertheless, the inclusion of KMSC in diets appeared to improve the tibia biomechanics. It is reflected by the slightly higher tibia parameters and bone-breaking strength. This could be attributed to the increased availability and assimilation of some important minerals such as Ca, P or Vitamin D aided by some desirable secondary plant metabolites such as flavonoids in KMSC diets as observed previously (Gröne *et al.*, 1995; Dinev, 2011). A recent study has shown that including KMSC

in the diet is linked to improvements in bone parameters. Overall, KMSC has great potential to be used as an substitute protein source in diets because it does not compromise bone development and the well-being of the broilers.

6.2 Conclusion

The study demonstrated that the growth performance, protein utilization, health status, internal organs development, carcass characteristics, meat quality traits and bone development in broiler chickens were not adversely affected when SBM is replaced. KM meal can be added to broiler diets by up to 5% without negative influences on broilers performance. Inclusions of KMSC at lower levels might result in the same performance as the SBM especially with regards to growth performance. Therefore, KMSC is a potential protein source in place of SBM in broiler diets.

6.3 Recommendation

The study justified the following recommendations

- i. Feed companies or commercial farmers can partially replace SBM with KMSC in broilers diets. However, the inclusion level of KMSC beyond 20% needs further investigations, given the adverse effects which were observed on bone development.
- ii. Future research can look at the possibilities of utilizing enzymes in KMSC diets to lessen the negative impacts of fiber content, which may have an adverse influence on animal growth and health.
- iii. Future research should focus on determining cost-effectiveness.

6.4 References

- Bureau for Food & Agricultural Policy (BFAP BASELINE) .2014. Agricultural Outlook: 2014 – 2023.
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- Mukhtar, M. A. and Ibrahim, I. S E. 2016. Effect of watermelon seed cake in broiler chicken performance. *World Journal of Pharmacy and Pharmaceutical Sciences* 5:360-365
- Ozung, P., Ubua, J. A., Aboluja, B. A. (2018). Growth performance, carcass and haematological characteristics of broiler fed toasted sorrel meal as replacement for soybean meal. *IARCJ*.8:90-109
- South African Poultry Association (2017) Broiler industry stats summary for 2017

7 Lists of appendice

7.1 Ethics certificate of the study



Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Fax: 018 299-4910
Web: <http://www.nwu.ac.za>

Research Ethics Regulatory Committee
Tel: 018 299-4849
Email: nkosinathi.machine@nwu.ac.za

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the **North-West University Animal Production Sciences Research Ethics Committee (NWU-AnimProdREC)** on 02/12/2021, the NWU Animal Production Sciences Research Ethics Committee hereby **approves** your study as indicated below. This implies that the North-West University Senate Committee for Research Ethics (NWU-SCRE) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Growth performance and meat quality of broilers fed diets containing Kalahari Melon oil cake

Study Leader/Supervisor (Principal Investigator)/Researcher: Prof U Marume

Student: Kgaswane MD

Ethics number:

N W U - 0 0 8 1 9 - 2 1 - A 5

Institution Study Number Year Status
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single Study

Commencement date: 2022/01/01

Risk Category:

2

Expiry date: 2022/12/31

Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special in process conditions of the research for approval (if applicable):

- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the NWU-AnimProdREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the NWU-AnimProdREC:
 - annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and
 - without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.
- The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the NWU-AnimProdREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.
- Annually a number of studies may be randomly selected for an external audit.
- The date of approval indicates the first date that the study may be started.
- In the interest of ethical responsibility, the NWU-SCRE and NWU-AnimProdREC reserves the right to:



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
MAFIKENG CAMPUS

Tel: +27 18 3892328

Fax: +27 86 6068714

E-mail: Dave.Kawadza@nwu.ac.za

Internet: <http://www.nwu.ac.za>

27th November 2023

Language Editing Certificate

This serves to confirm that I have read and edited the dissertation of Mr M D KGASWANE

(Student number: 29231922) entitled:

**Effects of Kalahari Melon (*Citrallus lanatus*) seed cake as a protein source for
broilers on growth, physiology and meat quality parameters**

The candidate corrected the language errors identified.

The document presentation is of an acceptable academic and linguistic standard.

Thank you

A handwritten signature in black ink, appearing to read 'DKawadza'.

Dr T.D. Kawadza

Language Editor