Inclusion of enzymes in Kalahari melon-based broiler diets: Effects on growth performance and meat quality

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DECLARATION

I declare that the dissertation, which I hereby submit for the degree MSc (Agric) Animal Science at the North-West University, is my own work and has not previously been submitted by me for a degree at any other tertiary institution.

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GENERAL ABSTRACT

The poultry industry is one of the fastest growing within the agricultural sector. Feed ingredient such as soybean meal have been used widely used in broiler diets but are becoming expensive mainly due to the competition between humans and animals for the same protein sources. It is therefore imperative to explore unconventional protein sources specifically indigenous legumes with potential to supply good quality protein in broiler diet. Therefore, this study was conducted to evaluate the inclusion of enzymes in Kalahari melon based diet on growth performance, protein utilization efficiency, serum biochemistry, meat quality and bone development of broiler chickens. Five isoenergetic and isonitrogenous diets were formulated with the inclusion of Kalahari melon (KM) cake at 10% in place of soybean meal and various enzymes combinations to the grower and finisher diets. The following diets were formulated: Control (commercial diet), KM (commercial diet + 10% KM), KMP (commercial diet + 10% KM + protease), KMXB (commercial diet + 10% KM + xylanase and *β-glucanase*) and KMXAP (commercial diet + 10% KM + xylanase. amylase and protease). Four hundred unsexed day-old broiler chicks were random distributed to the five dietary treatments, replicated 8 times. The chicks were offered commercial starter diet from 1 to 14 days. The experimental diets were offered during the grower and finisher phase.

From the results, Broilers fed control diet had the highest average daily feed intake (ADFI) in the grower phases, whilst birds fed the KMP diet had the lowest. Similarly, the birds fed the KMP diet had the lowest average daily gain (ADG) compared with the other treatments. With regards to the feed conversion ratio (FCR), the birds fed the KMP diet had the highest value while those fed the KMXAP had the lowest value. In the finisher phase, the birds fed the control diet had the highest ADFI and ADG respectively compared with the other treatments. The birds fed the control diet also had the lowest FCR. Like the grower phase, the birds fed the KMP had the lowest values for ADFI and ADG respectively. In grower phase, protein consumed was higher in birds fed KM followed by control. Protein efficiency ratio (PER) and specific growth rate (SGR) was however, highest in birds fed the KMXAP diets. In all instances, the birds fed the KMP diets had the lowest values for PC, PER, SGR and growth efficiency (GE) in the grower phase. In the finisher phase, the birds fed the control diet had the highest PC although not significantly different from all other treatments apart from the KMP diet. Similarly, the birds fed the control diet had the highest value for PER followed by those fed KMXB diet. SGR was however highest in birds fed the KMXB diet followed by those fed the KMXAP diet. As with the grower phase the birds fed the KMP diet consistently had the lowest values for all parameters. Birds fed control had the highest ($P < 0.05$) symmetric dimethylarginine , whilst birds fed KMP had the lowest. Birds fed control had the highest amount of SDMA followed by the KMXAP diet, whilst KMP had the lowest. Birds fed KM had the highest amount of GLOB whilst KMXAP had the lowest. Birds the fed KMXB had the highest amount of ALB/GLOB followed by KM. However, birds fed KMP had highest amount of alkaline phosphate followed by the KMXAP.

Broilers fed KMXB had the highest final weight, whilst KMP had lowest. Broilers fed control had the highest breast weight, whilst KMP had lowest. No significant effects were found between the dietary treatment and hot carcass weight (HCW), cold carcass weigh (CCW), carcass yield, breast index, thigh index and drum index. Broilers fed KMXAP had lighteR meat color (L), whilst control had darker meat color in both 0 h and 24 h P slaughter. Moreover, broilers fed KM had higher cooking loss, whilst those fed KMXAP had higher shear force. Additional, diet did not affect meat pH ,meat colors and water holding capacity . Broilers fed KMXB had highest tibia weight and tibia diameter proximal compared to other treatments. Broiler fed KM and KMP had highest tibia width diameter, whilst control had lowest. With regards to tibia length and tibia diameter distal end, diet did not affect the tibia parameters. In conclusion, enzymes inclusion in diets containing Kalahari melon improved growth performance, meat quality and health and welfare of broiler chickens. Inclusion of enzymes in Kalahari melon-based diets is therefore, recommended for improved broiler production and decreased raw material costs in feed production.

KEY WORDS

Anti-nutritional factors, bone development, exogenous enzymes, fatty acids, phytogenic compounds.

DEDICATION

I dedicate this dissertation to my mother, Pretty Samaria Mcobokazi and my little sister Nandi Phephile Mashela for nursing me with love and affections and their dedicated partnership for success in my life.

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LIST OF ABBREVIATION

AAs	Amino acids
ADFI	Average daily feed intake
ADG	Average daily gain
ANF	Antinutritional factors
BWG	Birth weight gain
C. Lanatus	*Citrullus lanatus*
CPd	Crude protein diet
DM	Dry matter
FAs	Fatty acids
FI	Feed intake
FCR	Feed conversion ratio
GE	Growth efficiency
KM	Kalahari melon
LSM	Linseed meal
LTL	Latency to lie
MUFAs	Monounsaturated fatty acids
NSP	Non-starch polysaccharides
PC	Protein consumed
PER	Protein efficiency ratio
PKC	Palm kernel cake
PNM	Peanut meal
PUFAs	Polyunsaturated fatty acids
SBM	Soybean meal
SGR	Specific growth rate
SFAs	Saturated fatty acids
SI	Small intestine
SPM	Secondary plant metabolites
TP	Total protein
TDDE	Tibia diamter distal end
TDPE	Tibia diameter proximal end
TLD	Tibia length diameter
TW	Tibia weight
XB	Xylanase, *B-glucanase*
XP	Xylanase Proteases
XAP	Xylanase, Amylase and Protease

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Poultry production is one of the fastest growing industries in the agricultural sector, with large research and developmental interests focusing on animal nutrition, improving health, disease resistance and improved productivity (Mohammed *et al*., 2018). Poultry production plays a key role in alleviation of poverty, particularly in resource poor areas, as well as ensuring food national security. Compared to other domestic animal and plant food sources for humans, poultry has the greater capacity to provide high quality and cheap protein and other critical micronutrients through meat and egg consumption. Globally, per capita consumption of poultry meat and meat products, broiler chicken, has been increasing steadily leading to the need to increase broiler meat production to meet the rising demand (Bahadori *et al*., 2017). The growth in poultry production is therefore having a profound effect on the demand for feed and other raw materials for broiler production. The price of corn and soybean like broiler feed ingredients, protein sources in particularly, keeps fluctuating with seasons and production (Singh *et al*., 2018). Moreover, the competition between humans and animals for the same protein sources have also exacerbated the problem. However, there has been an increase on the global market due to the feed-fuel demand of soybean (Klir *et al.,* 2019; Tona, 2018). Thus, making production less profitable when soybean is included in livestock diets (Tona, 2018). This has stimulated many efforts focusing on exploration of alternative locally available protein sources for broiler diets particularly those that do not fall within the food matrix for humans.

A variety of unconventional protein sources for broiler diets are being explored world-wide. Many of these unconventional protein sources appear to have comparable nutritional composition to that of soya bean meal (Ayssiwede *et al*., 2011). Thus, to contribute to the sustainable poultry production and to the food security, it is necessary to evaluate the efficacy of inclusion of such unconventional and local protein sources in broiler diets and increase their productivity by improving strategies of feeding through. There are many unconventional feed sources such as sunflower oilcake, pumpkin, palm oil and cowpea leaves that can be used as animal feed. Studies are also being conducted to assess the efficacy of other unconventional feed sources such as palm kernel cake (PKC), which is a residue and main by-product after palm oil extraction (Alshelmani *et al*., 2014; MPOB 2019 & 2020). The crude protein content of PKC reaches 14–18% (Sathitkowitchai *et al*., 2018), which is higher than that in corn and bran. Azizi *et al*. (2021) reported that because of moderate crude protein content, PKC has been accepted as one of the common feeds to partially substitute soybean meal and yellow corn in the breeding industry. Among some of these unconventional protein sources is Kalahali melon (KM) oil cake (*Citrullus lanatus*), it is the biological ancestor of the watermelon, which is now found all over the world, but which originated in the Kalahari region of Southern Africa. *C. lanatus* (Thunb.) Matsum & Nakai commonly known as Kalahari Tsamma melon, is one of the species of the Cucurbitaceae family (Schippers, 2000). According to these authors (Shayo *et al*., 1997; Loukou *et al*., 2007; Ojieh *et al*., 2008; Ayssiwede *et al*., 2011; Ogbe & George, 2012; Shazali *et al.,* 2013; Ngoran *et al*., 2015), the protein contents of *C. lanatus* seeds varies from 25 to 30%.

Nevertheless, some of the unconventional protein sources also contain high fiber content that may offset the nutritional benefits that may be realized from their use. Moreover, some of them contains high levels of secondary plant metabolites that may have both positive and negative effects on feed utilisation by chickens depending on their levels in diets. Therefore, apart from exploring the efficacy of their basic inclusion in broilers diets, efforts should also be made to assess strategies to offset the effects of high fibre and secondary plant metabolites including the use of enzymes and other products that may improve utilisation of diets containing such protein sources.

1.2 Problem statement

Kalahari melon (*C.lanatus*) oil cake is one of the potential alternative unconventional protein sources. In the Kalahari regions of Southern Africa, the fresh Kalahari fruits are used as a stock feed in times of drought (Van Wyk & Gericke, 2000). In South Africa, a number of companies are involved in Kalahari Melon essential oil production. The remaining cake as a by-product may cause ecological problems related to the proliferation of insects and rodents; therefore, ways in which the by-products may be efficiently used still need to be researched (Hussein *et al*., 2001). Nevertheless, the dry press cakes contains between 34.0 and 39 % protein depending on the region and environmental condition and 17.4% crude fiber (Danlami *et al*., 2015; Petkova & Antova, 2015; Duke & duCellier, 1993) making it suitable as a protein source. The seeds also contain high amounts of some important omega 6 and 9 essential fatty acids including palmitic, stearic, oleic and various linoleic acids isomers (Berry, 2015). Moreover, the Kalahari melon seeds also contains variable amounts of some phytogenic compounds that have therapeutic effects, such as antioxidant, anti-infammatory and analgesic effects (Chen *et al.,* 2014; Gill *et al*., 2010). The high protein content in Kalahari melon press cake makes it a potential alternative protein sources in broiler diets. However, the high amounts of fibre and some secondary plant metabolites my hinder its utilisation in animal diets. The effects of high fibre content can be mitigated by inclusion of exogenous of enzymes to poultry diets, individually or in combinations providing vital flexibility in poultry diet formulation (Disetlhe *et al*., 2018). For example, xylanase has ability to attack and disrupt cells wall material of Kalahari melon thus releasing encapsulated carbohydrates, such as starch, free sugars and soluble non-starch polysaccharides (NSP), while amylase increases the digestion of released carbohydrates (Fang *et al* ., 2007). On the other hand, protease breaks down a portion of protein that may escape digestion in the gut, ultimately increasing protein digestion efficiency (Mahmood *et al*., 2017) while phytase may mitigate the effects of some secondary plant metabolites. Inclusion of commercial enzymes as feed additives in poultry, to enhance the productive performance, is therefore a well-established feeding strategy to improve digestibility and nutrient availability in poor quality ingredients (Alagawany & Attia, 2015; Abd El-Hack *et al*., 2017, 2018; Alagawany *et al*., 2017, 2018b, Disetlhe *et al.*, 2018). Despite the desirable proximate composition of Kalahari melon oil cake, currently there are no attempts to assess the efficacy of Kalahari melon (*C. lanatus*) oil cake press cake inclusion in broiler diets and the effect of inclusion of exogenous enzymes on its utilisation by broilers.

1.3 Justification

Conventional protein sources are becoming expensive due to a variety of factors including reduced production due to climate change and increased demand due to the competition between humans and livestock for the same protein resource. Therefore, Kalahari melon oil cake could be used as a non-conventional protein source because of the availability, low-cost and non-human feed, Kalahari Melon oil cake can play an important role in reducing the pressure on the demand for soya bean meal. The Kalahari Melon plants are drought tolerant and can survive in harsh light of the desert environment. These attributes show the significance of Kalahari melon in crop diversification as it utilizes land, which ordinarily is not suitable for conventional crops cultivation. The dry press cake contains some important nutrient suitable for animal diets. Nevertheless, the seeds are also rich in fat with the amount of 5, 21% and protein 15, 06% and are an excellent source of energy (African Origin Oil, 2014). Recently, more attention has been focused on the potential utilisation of agricultural by-products in developing new functional ingredients for food enrichment and environmental sustainability (Salgado *et al.*, 2001). Often, agricultural waste products are sources of phytochemicals with functional properties, such as phenolics, alkaloids and tannins, which have radical scavenging ability in a biological system. Therefore, utilization of agricultural waste such as the Kalahari melon oil cake may reduce environmental pollution, considering the nutrients and phytochemicals contents (Seidu *et al*., 2015). Moreover, assessment of the inclusion of enzymes in poor quality ingredients such as Kalahari melon oil cake may provide more approaches to least cost diet formulation. Ultimately, this may reduce over dependence on soya bean meal and the costs of broiler production.

1.4 Objectives

The major objective of the study was to assess the efficacy of Kalahari melon (*C. lanatus*) press cake inclusion in broiler diets and the effect of inclusion of exogenous enzymes on its utilisation by broilers.

The specific objectives were:

- To evaluate the effect of enzyme inclusion on digestibility, protein utilisation and growth performance in broilers fed diet containing Kalahari melon oil cake.
- To evaluate the effect of enzyme inclusion on serum biochemistry and immune development in broilers fed diets containing Kalahari melon oil cake.
- To evaluate the effect of enzyme inclusion on bone development in broiler fed diets containing Kalahari melon oil cake.
- To evaluate the effects of enzyme inclusion on meat quality and fatty acid profiles of broilers fed diets containing Kalahari melon oil cake.

1.5 Hypothesis

Ho: Inclusion of enzyme has no effect on digestibility, protein utilization, and growth performance in broiler fed diet containing Kalahari melon oil cake.

Ho: Inclusion of enzyme has no effect on serum biochemistry and immune development in broilers fed diets containing Kalahari melon oil cake.

Ho: Inclusion of enzyme has no effect on bone development in broiler fed diet containing Kalahari melon oil cake.

Ho: Inclusion of enzymes has no effect on meat quality and fatty acids profiles of broiler fed diets containing Kalahari melon oil cake.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Globally, the poultry industry has become an important source of animal protein for human consumption with increasing per capita consumption of broiler chicken meat. The human population growth and the need to meet shortfalls in the supply of quality nutrients to people for good health and well-being have increased the demand for animal protein. FAO, (2003) reported that the annual consumption of meat is expected to increase from 25.5 to 37 kg and 88 to 100 kg per person in developing and developed countries respectively between 1997 and 2030. This increase in the demand for animal protein has challenged geneticists and breeders to focus on producing animals within the least possible time (Raha, 2007). As a result, broiler chickens with their shorter production cycle, potential for rapid growth and greater feed efficiency are seen as ideal to supplying the quality protein required by the increasing human population. Broilers are produced within the least possible time compared to other meat-producing animals (Mbuza *et al*., 2017) and through genetic selection broilers weighing 1.5 to 1.8 kg in 35 to 39 days of age are produced. Broiler meat is in high demand than red meat due to its low fat, low sodium, and cholesterol content (Ramiah *et al.,* 2014) and this is important in reducing the risks of becoming overweight, developing cardiovascular diseases and type 2 diabetes mellitus in human beings (Marangoni *et al.,* 2015).

Broiler chickens (*Gallus gallus domesticus*) are domesticated fowls that are raised for meat production and have become important in the agricultural sector of many countries. In South Africa broiler chickens are the main suppliers of affordable protein to consumers than all other animal proteins combined (Department of Agriculture, Forestry & Fisheries, 2012; National Agricultural Marketing Council & Bureau for Food & Agricultural Policy, 2016). Moreover, there is a negative relationship in the supply and demand of traditional ingredients which is expected to worsen over the coming decades, thus providing a compelling reason for exploring the usefulness of unconventional feedstuffs in feed formulations. In the current scenario, developing countries are undertaking considerable efforts to utilize diversified sources of feed ingredients, in particular protein materials. Soybean meal, fishmeal and meat meal have been used as the key sources of protein for poultry diets globally. Mainly, soybean and fish meal are the most preferred protein sources used in the broiler diet due to their well-balanced amino acid composition (Iji *et al*., 2017; Teguia *et al*., 2002). However, these protein sources increased production costs, consequently affecting the profit margin of the poultry farmers. The limited quantity and continuous rising prices of high-quality feed ingredients immensely challenge poultry producers. This enormous challenge remains the most significant determinant of profit margins in poultry production (Ibrahim *et al*., 2020). As a result, there is an increasing interest regarding the exploitation of alternative animal proteins feedstuffs to reduce feeding cost, improve products quality and diminish the impact of production on environment. In fact, there are some alternatives that can be utilised as substitute for soybean meal, fishmeal and meat meal in poultry diets. One such potential alternative is Kalahari melon (*C.lanatus*) oil cake.

2.2 Anti-nutrients in feed ingredients

Antinutritional factors are the class of components found in ingredients that can disrupt the digestion and absorption of nutrients. These components can form insoluble complexes with nutrients like Ca, Zn, Fe etc., and prevent their absorption. Phytic acid and tannins are a type of anti-nutrients that can inhibit enzyme and block normal metabolism of nutrients in feed. The other types of enzyme inhibitor are lectins and trypsin inhibitor which can be found in legumes while another form of antinutrients like non-starch polysaccharides(NSP) increases the viscosity of the feed. NSP is the major component of plant cell wall, e.g., β-glucans, xylose, and pentosans. The soluble NSP are believed to increase the digesta viscosity in the intestinal tract and decrease the nutrients utilization in broilers (Choct & Annison, 1992). Due to lack of endogenous enzymes to breakdown these NSP, the digestibility of nutrients is reduced, leading to poor performance in broilers. NSP degrading enzymes can increase metabolizable energy and decrease digesta viscosity (Adeola & Bedford, 2004). In response to increased digesta viscosity, there is an increase in transit time of digesta which in turn brings negative impact on broiler microbiota and growth (Choct & Annison, 1992; Vahjen *et al*., 1998).

2.2 Nutrients requirements of broiler chickens

Broilers are characterised by high nutritional requirements (Alvarenga *et al*., 2015) and greater feed efficiency (Mbuza *et al.,* 2017) to sustain their rapid growth. In this way broiler diets are made up of a variety of feedstuffs that provide vital nutrients for bird's rapid developments, reproduction and overall health. Hence the requirement for precise knowledge on the chemical composition and metabolizable energy of feedstuffs to allow for the formulation of nutritionally and economically balanced diets (Popescu & Criste, 2003; Ravindran *et al.,* 2010; Mariano *et al*., 2012) which are profound to maintaining a sustainable poultry industry. However, attention is given to the protein and energy levels of these feedstuffs as they both play important roles in poultry nutrition and are the most expensive nutrients to supply in diets for poultry. Jafarnejad and Sadegh (2011) stated that the protein is an essential constituent of body tissues whereas energy is needed for the functioning of the body. After energy, which account for 70-75% of feed cost (Saleh *et al.,* 2004; Van der Klis *et al.,* 2010), protein constitute the largest portion in poultry diet formulation (Beski *et al*., 2015). Animals and plants are important sources of protein and energy in poultry diet formulation (Ali *et al*., 2001; Lara *et al*., 2005). But due to scarcity of animal sources, plant sources such as soybean are used in large quantities although they are costly. Thus, choosing cheaper feed ingredients with proper nutrients levels that optimize growth, carcass quality and feed efficiency while allowing for profitable production are essential (Saleh *et al*., 2004; Rahman *et al*., 2010). One of such feed ingredient, which can be used in broilers diets formulation is Kalahari melon (*C.lanatus*) oil cake.

2.3 Conventional protein sources of broiler chickens

Poultry industry use conventional protein sources to provide essential amino acids (AAs) and achieve a better balance of needed AAs. Animal protein sources are more variable in their amino acids than plant proteins. Plant sources such as soybean meal, canola meal, sunflower meal, and cottonseed meal. Other sources include linseed meal, flaxseed meal and field peas. However, soya bean meal is the most commonly used protein source for broilers chicken which is expensive. While animal sources are a fish meal, which has a good balance of AAs, but must be used in small amount (2-5% of diet) to avoid fishy flavor in eggs and poultry meal. There is therefore, a need to explore alternative low cost protein sources that can be used in broiler diets.

2.4 Alternative protein sources

Alternative legumes that can partly minimize dependence on SBM include the different non-oilseed legumes (pulses) that are extensively cultivated in Africa, Europe and the world over. As an example, peanut is one of the legumes plants cultivated in tropical and subtropical regions mainly used as an oilseed, and its meal can be used as a protein source in animal diets (Sarbaz *et al*., 2018). Peanut meal (PNM) is fibrous since it contains about 10% crude fibre and multiple skins and shell residues (Davis & Dean, 2016). Due to the comprehensive extraction procedures, the residual oil content is highly variable, ranging from 3% or higher for a solvent-extracted meal to 10% in mechanically extracted cakes. More than 90% of the peanut oil's fatty acids (FAs) are unsaturated FAs, mainly linoleic and oleic (Bera *et al.,* 2019). Linseed is also one of the alternatives that contains a considerable level of protein (25–35%) and a high source of ω-3 polyunsaturated fatty acids (PUFA) and produces linseed meal (LSM) as a by-product after oil extraction. However, a few factors limit the inclusion of LSM in poultry diets in high proportions such as low lysine and methionine compared to other oilseeds, relatively high fibre content, and anti-nutritional factors (Iji *et al.,* 2017).

Plant or animal protein sources can be used in diets; however, plant-based feedstuffs are typically included in broiler diets because of their availability and low costs (Babatunde *et al*., 2021). According to De Santis *et al*. (2016), Faba bean (*Vicia faba* minor) has a great potential to be used to a higher extent in broiler chicken production. The protein-rich fraction of air-classified faba beans has been tested as an alternative for soybean protein concentrate in salmon feed, but there is a lack of information on the nutritive value of the starch-rich fraction bean starch concentrate (BSC) for broilers chicken. In Latin America, Mediterranean and North Africa regions, lupines have be shown to have great efficacy as an alternative dietary protein source in poultry. According to Petterson (2000) the most cultivated lupine species (*L.albus*) has the higher content of protein and has improved amino acids profile, making it a more favourable protein source for poultry. Petterson (2000) also stated that given the nutritional characteristics, yellow lupine have a good potential in feeding of broilers chicken, particularly as alternative to soybean meal protein concentration. Velvet beans (*Mucuna pruriens*), which is native to the tropical African and Asian regions has also been reported as a potential alternative protein source despite its characteristic high level of some secondary plant metabolites that may affect production in broilers (Vadivel *et al.,* 2010). In Southern Africa, some wild legumes often referred to as orphan legumes have been discovered and shown to be potentials sources of protein for both humans and livestock particularly in the resource poor areas. These legumes co6vuld have a special role, since they are usually protein-rich and because protein-energy malnutrition poses one of the most serious nutritional problems in developing countries (Aphane *et al.,* 2003; Bhat & Karim, 2009). They can therefore be integrated in approaches to alleviate hunger and poverty and mitigate the effects of climate change in sub-Saharan Africa. One important characteristic these leguminous plants is their ability to flourish in harsh environments. One of the wild melon thriving in South Africa is Kalahari melon oil cake *(C.lanatus*). Use of natural feed additives such as phytogenic plants with medicinal benefits has been suggested as possible alternatives to the commercial feed additives (Karori *et al.,* 2007; Bozkurt *et al.,* 2014; Omonijo *et al*., 2017). There is very little information available on its possible inclusion in animal diets. It is therefore important to investigate their nutritive value, chemical composition and the efficacy of its inclusion in animal diets.

2.5 Nutrient value of Kalahari melon oil cake

2.5.1 Proximate composition

Kalahari melon seeds oil has been used traditionally in Southern Africa as a cosmetic product, primarily as a face and body scrub, which is said to impart a blemish-free complexion to the skin (Vermaak *et al*., 2011). The seeds are also rich in fat, protein and are an excellent source of energy (African Origin Oil, 2014). Moreover, the seed essential oils contain large amounts of some essential fats including palmitic, stearic, oleic, and various linoleic acids isomers (Berry, 2015). In particular, conjugated linoleic acids have been reported to have a wide range of health-beneficial effects, including anticarcinogenic, antiatherogenic, anthelmintic, antidiabetic, and immune stimulatory effects (Suksombat *et al.,* 2006; Liu *et al*., 2017). They also have positive effects of fatty acid composition on meat and egg yolk and bone parameters (Kolakshyapati *et al.,* 2019). Indeed, poultry diets are made up of a variety of feedstuffs, including cereal grains, soybean meal, animal by-product meals, fats, and vitamin and mineral premixes. These feedstuffs, along with water, provide the energy and nutrients (proteins, amino acids, starch, fats, minerals, and vitamins) that the bird requires for development, reproduction, and health (Rhekis, 2002). The energy for maintaining the bird's general metabolism and for producing meat and eggs is provided by the energy-yielding dietary components, primarily carbohydrates and fats, but also protein (Hofman, 2000). As concern proteins, their dietary requirements in feedstuffs are linked to the amino acid’s contents. Indeed, amino acids obtained from dietary proteins are used by animals to fulfil a diversity of functions as primary constituents of structural and protective tissues and precursors of many important non-protein body constituents (NRC, 1994). The recommended protein intake is in the range 180 to 240 g of total protein per kilogram of feed for chickens according to their stage of life

(Lachapelle, 1995; Rhekis, 2002). Essential amino acids must be mixed with diet because the chickens (Picard *et al*., 1993) cannot

Table 2. 1 Chemical composition of Kalahari melon oil cake compared to Soya bean meal

Parameter	Kalahari Melon	Soya bean meal
Moisture	7	8
Dry matter	92.9	92.0
Organic matter	93.3	92
Crude Protein	35.6	44.4
Crude Fibre	6.4	3.5
Carbohydrates	7.2	13
Energy value (kcal per 100 g)	516	208.7

synthesize them. The most essential amino acids namely lysine and methionine are often insufficient in the diet and are thereby limiting amino acids (Mendonca & Jensen, 1989; Lachapelle, 1995). The Cucurbits seeds from *C.lanatus* which are considered as valuable sources of proteins (Shayo *et al*., 1997; Loukou *et al*., 2007; Ojieh *et al.,* 2008; Ayssiwede *et al*., 2011; Ogbe & George 2012; Shazali *et al*., 2013; Ngoran *et al.,* 2015), according to these authors, proteins contents of seeds from *C.lanatus* varied from 25 to 30%. *C. lanatus* cake is good source of protein for animal and it is comparable to cotton cake, linseeds cake and neem seeds cake (Pal & Mahadevan, 1968). However, in comparison with soybean meal, *C. lanatus* cake contains high mounts of fibre (up to 17.4%) and some antinutritional factors which may reduce its utility in broiler diets. Therefore, strategies that also include exogenous enzymes inclusion in diets may be needed to improve digestibility and utilisation of such ingredients.

2.5.2 Secondary plant metabolites

Anti-nutrients are plant compounds which reduces the nutritional value of plant food, usually by making an essential nutrient indigestible when consumed by animals or human (Geo-pie project, 2004) though they have the advantage of reducing the gastrointestinal as a result of delaying blood glucose response on carbohydrate-containing foods. As most plant-based protein crops, Kalahari melon contain secondary plant metabolites (SPM) that might limit the possible inclusion level in broiler diets. Kalahari melon contains some albumins, globulins, prolamines, alkali-soluble and acid-soluble compounds which are reported to be some of the anti-nutritional factors (Bower *et al.,*1988). Chavan and Kadam (1989) also reported that Kalahari melon is known to contain protease inhibitors that limit the edibility and reduce the nutritional quality of legume proteins. Tannins and trypsin inhibitors are also SPM that are associated with Kalahari melon (Bower *et al.,*1988). Tannins reduce bioavailability of proteins and energy but through plant breeding this effect has been reduced in white-flowered cultivars and is not an issue when these are used. Trypsin inhibitors (TI) impair the protein utilization and might also cause an overactive and thereby enlarged pancreas (Bower *et al.,* 1988). However, trypsin inhibitors are heat labile and hence heat treatment may negate the negative effect of trypsin inhibitors in broiler diets. Kalahari melon have been reported to have high antioxidant potential. The Kalahari melon was discovered to be a rich natural source of lycopene, a carotenoid with antioxidant properties and possible health benefits. According to Veazie *et al*. (2003) the Kalahari melon cultivars exhibited a wide range of lycopene concentrations with seedless and seeded red-fleshed types having 36-78 µg/g lycopene, and orange or yellow watermelons having less than 5 µg/g lycopene. Under-ripe and over-ripe melons had as much as 20% less lycopene than fully ripe melons for 2-10 days lowered lycopene content by 6-10%.

2.5.3 Potential effects on C. lanatus inclusion in diets on nutrient digestibility, growth performance and meat quality

Broiler rations are, almost completely formulated from two basic ingredients: corn, which is an excellent energy source, and soybean meal, which provides high-quality proteins and with great amino acid content (opalinski *et al.,* 2006). However, Kalahari melon oil cake, while containing high amount of fiber and lower amount of protein compared to that of soybean meal, Kalahari melon is a potential source of energy and proteins for efficient growth in animals. The high amount of oil content in the Kalahari melon cake could provide additional energy, while the amount of fats could stimulate efficient utilisation and digestions of diets. Kalahari melon cake also contain some important phytogenics, which can alter intestinal morphology, permeability and gut microflora for improved digestion and assimilation of nutrients resulting in improved growth. Moreover, phytogenics may alter, post-mortem processes resulting in desirable alterations in meat quality. The high amounts of essential fats including oleic, stearic, palmitic and various linoleic acids isomers (Berry, 2015) could be beneficial to the quality of meat. In particular, conjugated linoleic acids have been shown to have a variety of health-promoting properties, including anticarcinogenic, antiatherogenic, anthelmintic, antidiabetic, and immune stimulatory properties (Suksombat *et al.,* 2006; Liu *et al*., 2017).

Figure 2. 1: Kalahari melon (*C.lanatus*) meal (photographed by Mcobokazi S.P,2022)

2.6 Strategies to improve digestibility of KM oil cake

2.6.1 Thermal treatment

Food legumes are commonly used as a source of protein and carbohydrates in human and animal diet in many countries of the world. Food legumes have poor nutritive values, due to the presence of some anti-nutritional factors (Marrow,1991). Tannins inhibit the availability of protein, whereas phytic acid reduces the bioavailability of some essential minerals (Duhan, Chauhan, & Kapoor, 1989; Van der Poel, 1990). Rehman and Shah (2001) discovered that the elimination of tannins after pressure improved the protein digestibility of black grams. According to Kataria, Chauhan, and Punia (1989) pressure cooking reduced the anti-nutrients food legumes more effectively than normal cooking. The findings by Kadam, Smithard, Eyre, and Armstrong (1987) revealed that autoclaving and boiling in water improved the protein quality of winged beans due to reduction in the levels of antinutrient. Mbofung, Rigby, and waldron (1999) stated that starch that starch digestibility of cowpea was improved by using steam cooking methods, while in earlier study, Yu-Hui (1991) discovered that simple boiling method improved the protein and starch digestibility of cowpeas. Food legumes are usually cooked either by pressure cooker or simple boiling method. Simple boiling method improves the nutritional quality of food legumes due to the reduction in antinutrients. However, cooking of food legumes is also related to heating temperature and time, initial moisture and amount of water added during the cooking process. There is scarce information about the improvement in nutritional quality of food legumes as a result of cooking in a pressure cooker at different conditions.

2.6.2 Inclusion of enzymes

The inclusion of exogenous enzymes in the diets of broilers to improve digestibility and utilisation of poor protein sources has been gaining more space and has become a great strategy in improving digestibility of feeds, reducing the anti-nutritional effects and promoting the productivity indexes in animals (Hooge *et al*., 2010). The possibility of using exogeneous enzymes in non-ruminant diets has provided nutritionist with a very important tool to improve feed digestibility, reduce environmental contamination and lower feed cost, thus allowing for more flexibility in diet formulation. Numerous studies have confirmed the inclusion on exogenous enzymes in diets containing poor quality protein sources can effectively improve the utilization of such diets and ultimate performance in broiler (Ravn *et al*., 2018; Disetlhe *et al*., 2018, 2019; Zhou *et al*., 2009). Protease is an enzyme that is responsible for proteolysis. Howevers, studies on exogenous supplementation of protease enzyme improved the digestibility in broilers (Cowieson *et al*., 2017).

Commercial use of feed enzymes in poultry diet started in late 1980's and early 1990's due to their ability to digestion and utilisation of high fiber diets. Yildiz *et al.,* (2018) stated that xylanase-based enzyme supplementation improved growth performance and meat quality in broilers while xylanse

–amylase-protese enzyme mix was also shown to have the same effect (Disetlhe *et al*., 2018, 2019). Hence, there is well documented evidence that enzyme inclusion in diets containing unconventional protein sources may improve the utilisation of these protein sources. The possibility of using exogenous enzymes in non-ruminants' diets has thus provided nutritionist with a very important tool to improve digestibility and utilisation of unconventional feed sources allowing for more flexibility in least cost diet formulations.

2.6.3 Effect of enzymes on growth performance and meat quality

The use of enzymes in diets has provided a very intrinsic way to increase the digestibility of poor nutrient sources, allowing for greater flexibility in the formulation of low-cost diets (Zakaria *et al*., 2012). The variation in reaction and effectiveness of exogenous enzymes rely on the type of feed and nutritional compositions of the feed. Various enzymes like phytase, xylanase, glucanase, protease, amylase and other enzymes have been included in broiler diets based on the nutritional profiles of resources (Ghazi *et al*., 2002; Yu *et al*., 2002; Gracia *et al.,* 2003). Canola meal has low protein content and a high fibre content compared to SBM, requires the use of enzyme complexes including phytases, proteases and carbohydrases which may help optimum utilisation of phosphorus, protein and energy for quality enhancements of Canola meal (Slominski & Campbell, 1990; Simbaya *et al*., 1996; Kocher *et al*., 2000). Proteins play an important role in metabolism as blood plasma proteins, enzymes, hormones and antibodies each with a distinct function in body (Pond *et al.,*1995). However, protein is also one of the most expensive ingredients in poultry diets. Soybean protein is favoured because of its well-balanced essential amino acid profile, which allows it to fit into almost any diet (Ravindran, 2013). Recently, research has focused on the effect that proteases have in broiler diets on the overall production performance, nutrient digestibility and meat quality (Fidelis *et al*. 2010; Angel *et al.* 2011; Frietas *et al*. 2011; Dosković *et al*. 2015). Enzyme supplementation may possibly reduce the inconsistency in nutritional value between feedstuff and may improve quality of the feed formulations. Gracia *et al.* (2003) reported that α-amylase inclusion in SBM and maize diet significantly improved digestibility. Furthermore, α -amylase can reduce the relative weight of the pancrease without having any effects on the gizzard, the liver or the small intestines (SI). Reports on enzyme inclusion vary in findings with some reports demostrating that benefits are realised more in the grower or finishing phase, while other reports state the opposite (Dusel *et al.,* 1998; Fontes *et al*., 2004; Gao *et al.,* 2008). According to these authors (Nitsan *et al*., 1991; Dunnington & Siegel, 1995), during the early ages of broilers, the production of endogenous digestive enzymes is generally low and may restrict feed digestion. It has been previously reported that exogenous enzymes can hydrolyze and improve nutrient absorption, growth, and performance of the birds. The addition of the enzyme supplement to a broiler diet based on wheat, soybean meal, canola meal and peas resulted in a significant improvement in digestibility of protein, starch and NSP and therefore, improved growth performance (Mohammed *et al*., 2018)

2.7 Summary

Feed cost represents the highest total costs in intensive broiler production largely because feedstuffs used to formulate diets are also in high demand for human consumption. This higher price means broilers production is dependent on expensive feedstuffs which are used in diet formulation hence the calls for exploration of cheaper and locally available feed ingredients with potential to replace conventional feedstuffs such as soybeans without affecting broilers performance. One such locally available feedstuff, which shows potential to replace conventional feedstuffs due to its good nutritional qualities, is Kalahari melon oil cake (*C.lanatus*). *C.lanatus* has good quality protein and oil, which are comparable to soybean (*Glycine max*) and peanuts (*Arachis hypogaea*). It is, therefore, imperative to assess the inclusion of Kalahari melon oil cake in broiler diets and the effect of enzymes inclusion in diets its utilisation in broilers.

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CHAPTER 3

EFFECT OF ENZYMES INCLUSION ON GROWTH PERFORMANCE PROTEIN UTILIZATION EFFICIENCY AND NUTRITIONAL STATUS IN BROILER FED DIET CONTAINING KALAHARI MELON OIL CAKE

Abstract

The study investigated the effect of enzymes inclusion on protein utilisation and growth performance in broiler fed diet containing Kalahari melon oil cake. Five isoenergetic and isonitrogenous diets were formulated with the inclusion of Kalahari melon cake at 10% in place of soybean meal and various enzymes combinations to the grower and finisher diets. The following diets were formulated: Control (commercial diet), commercial diet + 10% Kalahari melon,, commercial diet + 10% Kalahari melon + protease, commercial diet + 10% Kalahari melon + xylanase and β-glucanase and commercial diet + 10% Kalahari melon + xylanase, amylase and protease). Four hundred unsexed day-old broiler chicks were random distributed to the five dietary treatments, replicated 8 times. The chicks were offered commercial starter diet from 1 to 14 days. The experimental diets were offered during the grower and finisher phase. The feed intake, weight gain and protein utilization efficiency were measured. From the results, Broilers fed control diet had the highest average daily feed intake in the grower phases, whilst birds fed the KMP diet had the lowest. Similarly, the birds fed the KMP diet had the lowest average daily gain compared with the other treatments. With regards to the feed conversion ratio, the birds fed the KMP diet had the highest value while those fed the KMXAP had the lowest value. In the finisher phase, the birds fed the control diet had the highest ADFI and ADG respectively compared with the other treatments. The birds fed the control diet also had the lowest FCR. Like the grower phase, the birds fed the KMP had the lowest values for ADFI and ADG respectively. In grower phase, protein consumed was higher in birds fed KM followed by control. Protein efficiency ratio and specific growth rate was however, highest in birds fed the KMXAP diets. In all instances, the birds fed the KMP diets had the lowest values for protein consumed, protein efficiency ratio, specific growth rate and growth efficiency in the grower phase. In the finisher phase, the birds fed the control diet had the highest PC although not significantly different from all other treatments apart from the KMP diet. Similarly, the birds fed the control diet had the highest value for PER followed by those fed KMXB diet. SGR was however highest in birds fed the KMXB diet followed by those fed the KMXAP diet. As with the grower phase the birds fed the KMP diet consistently had the lowest values for all parameters. In conclusion, inclusion of enzymes on diet containing Kalahari melon oil cake positively influenced protein utilisation and growth performance parameters. Desirably, enzyme inclusion on diets containing Kalahari melon oil cake was able to mitigate the fiber content and secondary plant metabolite, that could have affected the growth performance and protein utilisation.

Keywords : serum biochemistry, canola meal, fibre, feed, supplementation.

3.1 Introduction

In poultry production, feed costs amount to 70 - 75% of the total production costs mainly due to the use of conventional feedstuff such as maize and soybean meal (SBM) (Alagwanya *et al*., 2018) with the latter ingredient being the most preferred plant protein source for both human and animal diets. This has inevitably resulted in competition between humans and animals for the same plant protein source. Coupled with this, soya bean production has been on the decline across the world due to combined effects of climate change, recurring droughts and the debilitating effects of wars involving some soya bean producing countries (Ciurescu *et al*., 2019). Ultimately, there has been as unprecedented growth in feed prices that is affecting animal production. Imperatively, there has been some growing attempts to explore unconventional feed sources to meet animal nutritional requirements.

According to Fu *et al*. (2021), use of unconventional feed materials could be a potential escape route to the sustainable of the poultry industry. A variety of non-conventional protein sources are being explored across the world with some including canola meal having already been integrated into the broiler diet formulation programmes. Nevertheless, many of the proposed alternative protein sources are being limited by having high fibre and low protein content compared to soyabean meal, as well as the presence of some anti-nutritional factors (ANF) which can reduce feed digestibility and slow down growth rate in broiler chickens. The use of feed additives such as enzyme cocktails can improve the utilisation of such protein sources. The inclusion of exogenous enzyme complexes such as astra XP (protease), XB (xylanase and *B-glucanase)* and astra XAP an enhanced combination of xylanase, amylase and protease have been observed to improve the digestion of high fibre and secondary plant metabolites (glucosinolates and sinapine) while providing vital flexibility in poultry diet formulations (Disetlhe *et al.*, 2018). Enzyme supplementation improves production efficiency by increasing the digestion of low quality products and reducing nutrient loss through excreta, removing anti-nutritional factors, improving nutrients availability and increasing overall animal performance.

One of the potential protein sources that has received very little attention is the Kalahari melon (KM) oil cake. The cake is produced as a by-product of the cold pressing of essential oils that are normally used in the cosmetic industry (Marume *et al*., 2020). It has relatively lower nutritional value relatively to SBM mainly due to the high fibre content of the cake, lower levels of lysine and high non-starch polysaccharides concentrations (Biesiada-Drzazga *et al*., 2010; Havilei *et al*., 2020) It can thus be postulated that inclusion of enzymes in diets containing Kalahari melon (*Citrallus lanatus*) cake at lower levels can improve its utility and animal performance. Therefore, the objective of this study was to evaluate the effect of enzymes inclusion on digestibility, protein utilization and growth performance in broiler fed diets containing Kalahari melon oil cake.

3.2 Material and Methods

3.2.1 Study site

The study was conducted at North-West University poultry unit in Rooigrond. The study site is situated in North West province of South Africa. The geographical coordinates are 25° 55′ 0″ South, 25° 48′ 0″ East at an altitude of 1290 metres above sea level and temperature ranges from 3 ºC to 37 ºC and rainfall ranges between 300 to 700 mm annually. The study area is semi-arid (tropical area).

3.2.2 Sourcing of Kalahari melon meal, ingredients, and enzymes

The Kalahari melon meal (KM) was obtained from Highlands Essential Oil SA (PTY) Ltd. The company is involved in the production of plant essential oils from the Kalahari melon seeds mainly for use in the production of cosmetic products. The remaining cake from the extraction of essentials oil cake was analysed for chemical composition at the GMP Laboratories (Labworld). The enzyme complex (xylanase, amylase and protease) Astra XAP was obtained from Chemuniqué SA while protease was obtained from Nutroteq (PYT) Ltd. All other ingredients used in the formulation of diets were obtained from Nutroteq (Pvt) Ltd.

3.2.3 Dietary treatment and experimental design

Five isoenergetic and isonitrogenous diets were formulated with the inclusion of Kalahari melon cake in place of soybean meal and various enzymes combinations. The control was a commercial diet whose major protein source was 100% soybean (SBM), whilst the other four diets contained 10% Kalahari melon oil cake (KM) in place of soybean (SBM). The dietary treatments were formulated as follows: 1. Control (commercial broiler diet); 2. KM (10% Kalahari melon oil cake in place of SB meal); 3. KMP (10% Kalahari melon oil cake + 1.50 g/kg protease); 4. KMXB (10% Kalahari melon oil cake + 1.50 g/kg Astra xylanase, β-glucanase; 5. KMXAP (10% Kalahari melon oil cake + 1.50 g/kg Astra xylanase, amylase and protease). The experimental diets are shown in Table 3.1 while Table 3.2 shows the chemical composition of the diets and Table 3.3 shows the chemical composition of Kalahari melon and Soya bean meal . A total of four hundred day-old chicks (cobb 500) obtained from Chicken Ranch (Pty) Ltd were randomly distributed to the five dietary treatments replicated 8 times with a pen housing 10 birds as the experimental unit. The study was arranged in a completely randomized design. The pens (measuring 2.1 x 1.5 x 1.7 m) were designed to meet the welfare standards for optimum productions of broilers.

Table 3.1 Ingredients composition (%) of experimental diet fed to broiler chickens for grower and finisher phase

	Dietary treatment									
	Grower					Finisher				
Ingredients	Con	KM	KMP	KMXB	KMXAP	Con	KM	KMP	KMXB	KMXAP
Yellow maize-fine (	43.98	43.98	43.98	43.98	43.98	48.22	48.22	48.22	48.22	48.22
kalahari melon oil cake	0	10	10	10	10	0	10	10	10	10
Prime gluten 60 (yellow)	1.8	1.8	1.8	1.8	1.8	1.3	1.3	1.3	1.3	1.3
Fullfat soya	5.1	5.1	5.1	5.1	5.1	1.5	1.5	1.5	1.5	1.5
Soyabean meal (local)	37.16	27.75	27.74	27.74	27.74	33.12	23.28	23.28	23.28	23.28
Limestone powder-fine	1.45	1.45	1.45	1.45	1.45	1.30	1.30	1.30	1.30	1.30
MCP/mono cal KK	0.72	0.72	0.72	0.72	0.72	0.50	0.50	0.50	0.50	0.50
Salt-fine	0.32	0.32	0.32	0.32	0.32	0.33	0.33	0.33	0.33	0.33
Koeksoda	0.17	0.17	0.17	0.17	0.17	0.13	0.13	0.13	0.13	0.13
Choline powder	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075
Lysine	0.279	0.279	0.279	0.279	0.279	0.262	0.262	0.262	0.262	0.262
L-threonine	0.041	0.041	0.041	0.041	0.041	0.030	0.030	0.030	0.030	0.030
Methionine	0.187	0.187	0.187	0.187	0.187	0.161	0.161	0.161	0.161	0.161
PX P2 Br grower with phytase	0.167	0.167	0.167	0.167	0.167	0	0	0	0	0
PX P3 Br finisher with phytase	0	0	0	0	0	0.167	0.167	0.167	0.167	0.167
Coxistac	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Olaquindox	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.4	0.04
Protease	0	0	0.3	0	0	0	0	0.3	0	0
Astra XB	0	0	0	0.3	0	0	0	0	0.3	0
Astra XAP	0	0	0	0	0.3	0	0	0	0	0.3

Dietary treatment: Con: commercial broiler diet without Kalahari melon oil cake; KM: Experimental broiler diet containing 10% Kalahari melon oil cake; KMP: Experimental broiler diet containing 10% Kalahari melon 3% of protease; KMXB: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% of xylanase, β-glucanase; KMXAP: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% XAP, XAP is a combination of xylanase, amylase and protease; SEM: standard error of means.

Table 3. 2 Nutrient composition (kg) of experimental diets fed to broiler chickens for grower and finisher phase

Kalahari melon oil cake diet composition		
Parameters	Grower phase	Finisher phase
Volume	100	100
Dry matter	88.234	88.171
Protein	20.538	18.781
Ash	4.219	4.119
Fat EE	3.043	3.124
Fibre	8.928	8.844
PTR ME KC	2.866	2.985
Calcium	1.000	1.000
Total P	0.549	0.568
AP PTRY	0.458	0.461
K	0.950	0.866
NA	0.180	0.160
DCAB PTRY	2.53	2.24
LYS TOT	1.163	1.051

Table 3. 3 Chemical composition of Kalahari melon oil cake compared to Soya bean meal

Parameter	Kalahari Melon	Soya bean meal
Moisture	7	8
Dry matter	92.9	92.0
Organic matter	93.3	92
Crude Protein	35.6	44.4
Crude Fibre	6.4	3.5
Carbohydrates	7.2	13
Energy value (kcal per 100 g)	516	208.7

3.2.4 Animal management

On the day of arrival, the chicks were randomly placed in pens measuring 2.1 x 1.5 x 1.7 m in broiler house. During the first 3 days of brooding the ambient temperature in the house was kept between 32.5 and 33°C but was gradually reduced to 26°C at 14 days of age. These temperature requirements were met using the infra-red lights that were used until 14. Stress pack was given to the chicks for 3 days. The birds were phase-fed starting with the provision of commercial starter ration from day 1 to 14. Experimental diets were offered during the grower (day 15-28) and finisher (day 29-42) phase. Water was provided ad-libitum. Experimental diets were formulated according to the commercial feed formulation standards to meet the nutrient requirements for the grower and finisher phases. The chickens were monitored regularly for behaviour and health. Sick animals were removed from the study and euthanized. Mortality was recorded and autopsies was conducted on dead chickens to establish the cause of death. The experimental procedure was approved by the NWU-AnimProdREC and the ethics number granted is NWU-00808-21-A5.

3.2.5 Feed intake and growth performance

Feed intake was measured daily and weight gain was measured weekly. All birds from forty pens were weighed at the beginning of the trial at day 14 (Initial body weight) and subsequently weighed weekly (21, 28, 35 and day 42) using (TSW equipment weighing scales/Adam equipment). Feed offered to the birds and feed refusal were collected and weighed each morning before feeding. The average daily feed intake (ADFI), Average daily gain (ADG) and feed conversion ratio (FCR) for each feeding phase were calculated as:

ADFI = Feed offered - feed refusal)/ (14 days)

ADG = (Finish weight - Start weight)/ (Age (days))

FCR = (Feed intake)/ (Weight gain)

3.2.6 Protein utilization efficiency

Protein consumed (PC g/bird) was calculated by multiplying the concentration of crude protein (CPd) in the diet (g/kg DM consumed) by feed intake over the feeding phase, whilst protein efficiency ratio (PER g/kg) was calculated by dividing mean body weight gain (BWG) by the mean protein consumed. Specific growth rate (SGR), which is percent growth per feeding phase and growth efficiency (GE) were also calculated using the following formulas:

$PC = FI \times CPd$

$PER = (BWG) / (PC)$

$SGR = ((\text{final weight} - \text{initial weight}))/(14\ d) \times 100$

$GE = (BWG)/(\text{Initial weight})$

3.2.7 Blood collection and serum biochemical analysis

On day 39, 1 broiler was randomly selected from each pen for blood collection. Blood was collected from the brachial vein using a 23-gauge needle and syringe into red top vacutainer tube without anti-coagulant. The blood was centrifuged at 3000 rpm and serum was separated for analysis of biochemical indices. The enzymes were analysed using a clinical chemistry analyser (Gilford Impact, 404lE, Ciba Coming Diagnostic Corp., Gilford Systems, and Oberlin, OH 44774). A UV–VIS spectrophotometer (SPECORD 50 PC, Analytik Jena AG) was used to perform the enzyme (Alanine transaminase, Alkaline phosphatase(IU/L)) assays (using respective commercial kits (Ciba Coming Diagnostic Corp., Gilford Systems, Oberlin, OH 44774) according to the procedures outlined previously by Ogunsanmi *et al.* (1994). The total protein (TP) and albumin, cholesterol and mineral content were quantified using an auto-analyser (Hitachi-704, Boehringer Mannheim Ltd, and Germany).

3.2.7 Statistical analysis

Data on growth performance, protein utilization and serum biochemical parameters were analysed using GLM procedure of SAS (2010) with diet as only fixed effect (model 1). Data on cumulative weight gain was measured on a weekly basis and were analysed using mixed model procedure SAS (2010) that took into consideration the effect of both diet and week measurement (model 2). PDIFF option of SAS (2010) was used to perform pairwise comparisons of the least square means.

The statistical models was as follows:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij} \quad (1)$$

Where: Yij = observation (growth performance, protein utilisation and serum biochemical), μ = population mean constant common to all observations, Ti = effect of diet, and εij = random error term.

$$Y_{ijk} = \mu + T_i + W_j + (T * W)_{ij} + \varepsilon_{ijk} \quad (2)$$

Where: Y_{ij} = observation (Cumulative weight gain parameter), μ = population mean constant common to the observation, T_i = effect of diet, $T*W_{ij}$ = effect of diet interacting with week and εij = random error term. For all tests, the level of significance was set at ($P < 0.05$).

3.3 Results

3.3.1 Feed intake and growth performance

The results of the effects of exogenous enzymes on feed intake and growth performance parameters of broilers fed diets containing Kalahari melon meal are shown in Table 3.3. From the results, diet had a significant (P < 0.05) effect on growth performance parameters in the grower and finisher phases. Broilers fed control had the highest (P < 0.05) ADFI in the grower phases, whilst birds fed the KMP diet had the lowest. Similarly, the birds fed the KMP diet had the lowest ADG compared with the other treatments. With regards to the FCR, the birds fed the KPM diet had the highest (P < 0.05) value while those fed the KMXAP had the lowest value. In the finisher phase, the birds fed the control diet had the highest ADFI and ADG respectively compared with the other treatments. The birds fed the control diet also had the lowest FCR. Like the grower phase, the birds fed the KMP had the lowest values for ADFI and ADG respectively. Nevertheless, the birds fed the KMXAP had the highest FCR compared with the other treatments. With regards to the cumulative weight gain, birds fed the KMP diet consistently had the lowest gains throughout the study (Figure 3.1). Birds fed the control diet had the highest gain in week 3 of the study. Nevertheless all treatments appeared to have the same gain in the final week apart from the birds fed the KMP diet. Overall, a similar trend was observed with the birds fed the KMP diet having the lowest ADFI and ADG, and highest overall FCR.

Table 3. 4 The effect of enzymes inclusion in Kalahari melon meal on average daily feed intake (g/bird), average daily gain (g/bird) and feed conversion ratio in broiler chickens

	Dietary Treatment						
Phase	CON	KM	KMP	KMXB	KMXAP	SEM	*P*-value
Grower							
ADFI (g)	93.33[b]	93.57[b]	80.58[a]	92.88[b]	91.56[b]	2.21	0.001
ADG (g)	45.06[b]	44.94[b]	36.69[a]	44.90[b]	45.88[b]	4.72	0.025
FCR	2.08[b]	2.08[b]	2.19[b]	2.06[b]	1.19[a]	0.05	0.023
Finisher							
ADFI (g)	143.02[b]	139.09[b]	135.95[a]	139.61[b]	142.88[b]	2.22	0.025
ADG (g)	141.45[c]	125.81[b]	119.78[a]	136.09[c]	127.43[bc]	3.72	0.002
FCR	1.02[a]	1.13[b]	1.15[b]	1.03[ab]	1.33[c]	0.03	0.013
Overall							
ADFI (g)	117.66[b]	116.33[b]	108.10a	116.25[b]	117.22[b]	1.93	0.011
ADG (g)	93.26[c]	85.36[b]	78.24[a]	90.50[c]	86.66[bc]	2.12	0.006
FCR	1.55[a]	1.61[b]	1.67[b]	1.56ab	1.26[c]	0.05	0.024

[a b c] means in the same row with different superscripts are significant different ($P < 0.05$) . Dietary treatment: Con: commercial broiler diet without Kalahari melon oil cake; KM: Experimental broiler diet containing 10% Kalahari melon oil cake; KMP: Experimental broiler diet containing 10% Kalahari melon meal 3% of Protease; KMXB: Experimental broiler diet containing 10% Kalahari melon meal and 3% of xylanase, β-glucanase; KMXAP: Experimental broiler diet containing 10% Kalahari melon meal and 3% XAP ,XAP is a combination of xylanase, amylase and protease; SEM: Standard error of means, ADFI; average daily feed intake, ADG; average daily gain , FCR; feed conversion ratio.

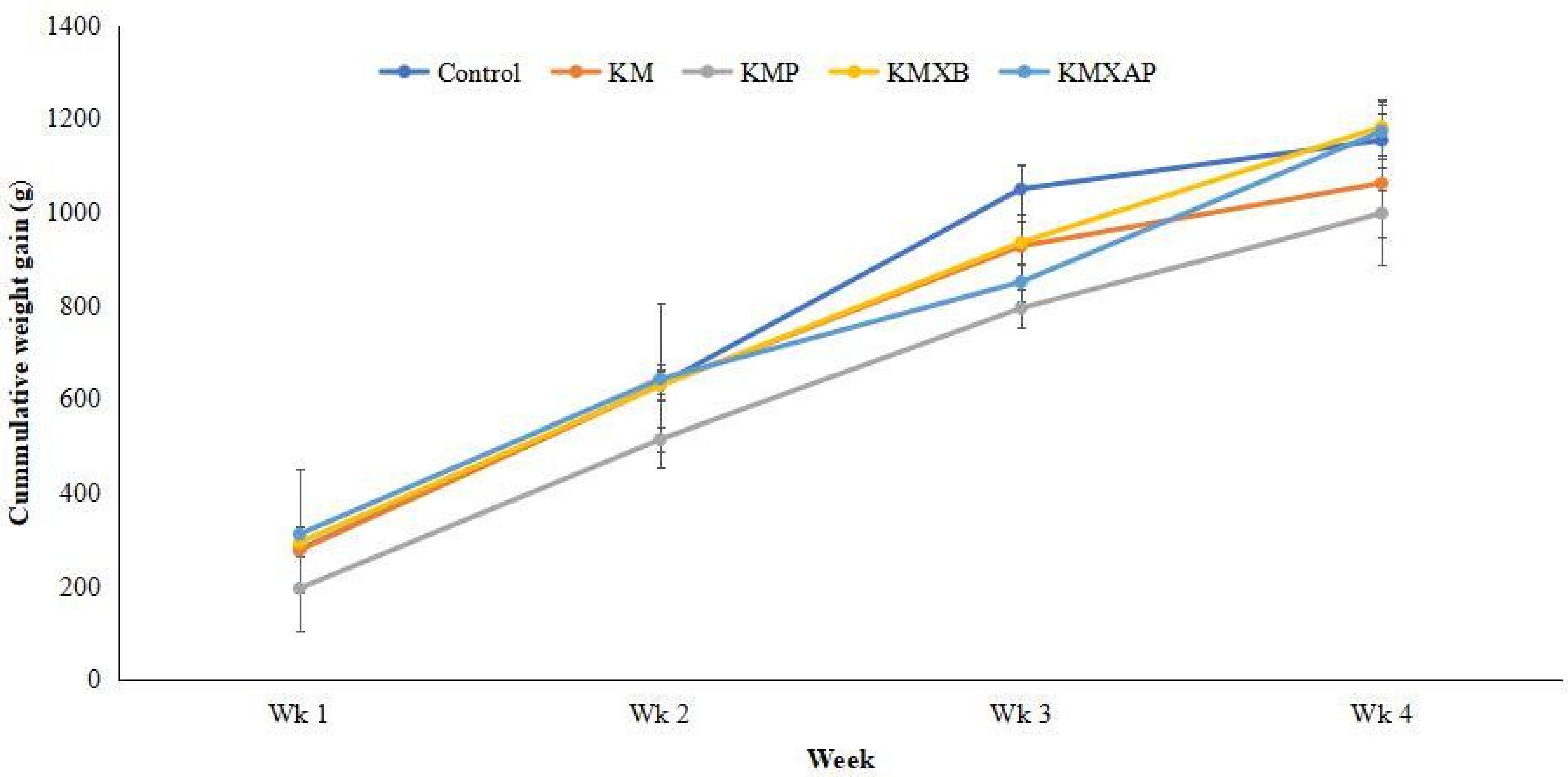

Figure 3. 1: Cumulative weight gains of broiler chickens fed based diets

3.3.2 Protein utilisation and growth efficiency

The effects of diets on protein utilisation and growth efficiency parameters are shown on Table 3.4. Dietary treatment had significant effect ($P < 0.05$) on protein utilisation and growth efficiency parameters in both grower and finisher phases. In grower phase, PC was higher ($P < 0.05$) in birds fed KM followed by control. PER and SGR was however, highest in birds fed the KMXAP diets. In all instances, the birds fed the KMP diets had the lowest values for PC, PER, SGR and GE in the grower phase. In the finisher phase, the birds fed the control diet had the highest PC although not significantly different from all other treatments apart from the KMP diet which had the lowest value. Similarly, the birds fed the control diet had the highest value followed by those fed KMXB diet. SGR was, however, highest in birds fed the KMXB diet followed by those fed the KMXAP diet. As with the grower phase, birds fed the KMP diet consistently had the lowest values for all parameters. Overall, the dietary treatment only effected PC and PER respectively, whereby both PC and PER were highest ($P < 0.05$) in birds fed control whilst they were lowest in those fed the KMP diet.

3.3.3 Serum biochemistry analysis

The results of the effects of exogenous enzymes on serum biochemical parameters of broiler fed diet containing Kalahari melon meal are shown on Table 4.1. From the results, diet had a significant ($P < 0.05$) effect on serum biochemical parameters except glucose, creation, urea, albumin, ALT, total bilirubin, cholesterol, amylase and lipase. Broiler fed control had the highest ??? ($P < 0.05$) SDMA, whilst birds fed KMP had the lowest. Birds fed control had the highest amount of SDMA followed by diet KMXAP, whilst KMP had the lowest. Birds fed KMP had the highest amount of calcium. Amongst the different treatment groups, KMXAP had the highest amount of total protein (120 g/l), which was therefore, followed by the control diet, whilst KMP had the lowest amount of total protein (96.62 g/l). Birds fed KM had the

Table 3. 5 Effect of enzyme inclusion in Kalahari melon-based diet on protein utilization and growth efficiency

	Dietary treatment						
Phase	Con	KM	KMP	KMXB	KMXAP	SEM	*P*-value
Grower							
PC (g/bird)	19.16^{b}	19.21^{c}	16.55^{a}	19.07^{b}	18.80^{b}	0.43	0.004
PER (g/kg)	2.35^{b}	2.34^{b}	2.21^{a}	2.35^{b}	2.46^{c}	0.17	0.015
SGR(%)	45.06^{b}	44.94^{b}	36.69^{a}	44.90^{b}	45.88^{b}	3.40	0.013
GE	0.11^{b}	0.11^{b}	0.09^{a}	0.10^{b}	0.11^{b}	0.006	0.001
Finisher							
PC(g/bird)	26.86^{b}	26.12^{b}	25.53^{a}	26.22^{b}	26.83^{b}	0.43	0.042
PER(g/kg)	5.26^{c}	4.82^{b}	4.68^{a}	5.18^{c}	4.74^{b}	0.17	0.005
SGR(%)	37.29^{bc}	30.85^{a}	34.50^{b}	39.50^{c}	37.83b^{c}	3.40	0.031
GE	0.13^{b}	0.12^{a}	0.13^{b}	0.13^{b}	0.12^{a}	0.00	0.011
Overall							
PC (g/bird)	23.01^{c}	22.67^{b}	21.04^{a}	22.6^{b}	22.82b^{c}	0.30	0.006
PER (g/kg)	3.80^{b}	3.58^{a}	3.45^{a}	3.77^{b}	3.60^{a}	0.12	0.012
SGR(%)	41.18	37.90	35.60	42.20	41.85	2.40	NS
GE	0.12	0.11	0.11	0.11	0.11	0.006	NS

a b c means in the same row with different superscripts are significant different ($P < 0.05$) . CPd = Crude protein diet, PC = Protein consumed , PER = Protein efficiency ratio , SGR = Specific growth rate , GE= Growth efficiency; Control (commercial broiler diet) ; KM (10% Kalahari melon oil cake) ; KMP (10% of Kalahari melon oil cake + 0.3 g/kg astra P (protease)) ; KMXB (10% of Kalahari melon oil cake + 0.3 g/kg Astra XB (xylnase, B-glucanse); KMXAP (10% of Kalahari melon oil cake + 0.3 g/kg Astra XAP (xylanase, amylase, proteases).

Table 3. 6 The effect of dietary Kalahari melon oil cake and exogenous enzyme on blood chemistry of broilers

	Dietary treatments						
Parameters	Con	KM	KMP	KMXB	KMXAP	SEM	*P*-value
Glucose(mg/dL)	9.63	9.85	9.51	9.84	10.15	0.28	NS
SDMA	45.75^{d}	19.00^{b}	14.25^{a}	19.87^{b}	28.50^{c}	2.48	0.006
Creatine(μ/L)	9.00	9.00	9.00	9.00	9.00	0.00	NS
Urea(mmol/L)	1.08	1.12	1.11	1.17	1.08	0.05	NS
Phosphorus(mmol/L)	4.98^{b}	3.97^{a}	4.96^{b}	5.20^{c}	4.89^{b}	0.39	0.012
Calcium (mmol/L)	1.30^{bc}	1.06^{a}	1.35^{c}	1.28^{b}	1.41^{d}	0.10	0.002
Total protein(g/L)	108.37^{c}	104.87^{b}	96.62^{a}	109.87^{c}	120.00^{d}	6.13	0.016
Albumin(g/L)	49.12	38.75	44.75	49.00	49.12	5.51	NS
Globulin	55.87^{b}	65.50^{c}	51.25^{b}	58.12^{b}	38.37^{a}	4.48	0.042
Albumin/Globulin	0.53^{ab}	0.48^{a}	0.71^{b}	1.03^{c}	0.70^{b}	0.13	0.002
Alanine transaminase	119.12	117.12	116.00	117.75	115.00	6.19	NS
Alkaline phosphatase(IU/L)	92.87^{b}	77.37^{a}	187.25e	112.50^{c}	135.25^{d}	31.39	0.001
Total bilirubin (IU/L)	304.37	239.37	290.50	373.37	300.62	51.06	NS
Cholesterol(mg/dL)	2.11	2.02	2.61	2.06	2.28	0.23	NS
Amylase	281.37	307.12	311.00	296.00	280.12	44.74	NS
Lipase	37.37	33.87	43.75	23.75	32.50	10.65	NS

$^{a\ b\ c}$ means in the same row with different superscripts are significant different (P < 0.05) Dietary treatment: Con: commercial broiler diet without Kalahari melon oil cake; KM: Experimental broiler diet containing 10% Kalahari melon oil cake; KMP: Experimental broiler diet containing 10% Kalahari melon 3% of protease; KMXB: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% of xylanase,β-glucanase ; KMXAP: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% XAP ,XAP is a combination of xylanase, amylase and protease; SEM: Standard error of mean ; parameters: SDMA; symmetric dimethylarginine, ALB/GLOB; ALT; alanine transaminase.

highest amount of GLOB whilst KMXAP had the lowest. Birds fed KMXB had the highest amount of ALB/GLOB followed by KM. However, birds fed KMP had the highest amount of ALKP followed by the KMXAP. With regards to liver enzymes, it was found that the higher values for alkaline phosphatase (ALP) were obtained in all diets containing the Kalahari melon meal with the inclusion of enzymes compared to control diet.

3.4 Discussion

3.4.1 Feed intake and growth performance

In broiler chickens, growth performance is the most significant economic factor in determining the profitability of production. The findings from the current study showed that inclusion of KM at 10% in the diet had no effects on the growth performance parameters particularly during the grower phase. This could probably be explained by the fact that the inclusion KM level was insignificant to cause major changes to diets and ultimately the performance of the birds. KM contains high amounts of fibre compared to soya bean meal and if included in high amounts, it may have resulted in significant alteration of the dietary composition that would subsequently

influenced performance of the birds as observed elsewhere (Kalmendal *et al*., 2011). In the finisher phase, the effects of inclusion of KM in the diets was apparent. While there was no reduction in ADFI, the ADG in birds fed the KM diet only was significantly lowered. This could be due to the incremental levels of fibre as the feed intake increased with the growth of the birds. The results of this study are concurrent with recent study of Khosravinia *et al*. (2015) who reported that dietary low protein diet reduced FI in broiler with a resultant decline in ADG.

With the inclusion of enzymes, particularly XB, the ADG of the birds was significantly improved. Moreover, the birds fed the KMXB had far much better FCR compared to all other treatments. This can be explained by the fact that XB had been proven to mitigate the negative effects of selected non-starch polysaccharides found in high fiber ingredients such as the KM, while supporting the effect release of valuable nutrients. In particular, XB has been shown to overcome the anti-nutritional effects of arabinoxylan and ß-glucan characterising the KM while promoting consistent digestibility improvement, which enhances animal performance. This is consistent with observation from other studies (Khosravinia *et al.*,2015; Mahmood *et al.*, 2017, Disetlhe *et al.*, 2018). The lower performance of the birds fed the KMP diet was expected. Inclusion of protease in high fibre diets has been observed to cause disruption of protein-starch interaction that in return would have resulted in better starch digestibility (Mahmood *et al.*, 2017). Overall, the current study indicated that the body weight gain and feed intake of birds fed with KMXB diets comparable to those of the birds fed the control diet.

3.4.2 Protein utilisation efficiency and growth efficiency

The protein utilisation efficiency parameters, PER in particular, have often been used in the comparisons of the food values of different proteins (Boye *et al.*, 2012). Nevertheless, there is limited information of the use of the paremeters in determining feed value of different protein sources in large animal species apart from rats and fish. It is generally accepted that the rate of growth of weanling rats under standardized conditions provides a reliable measure of the value of dietary protein (Boye *et al.*, 2012; Sarwar *et al.*, 1984; Cruz *et al.*, 2003). Quiet a number of rodent studies have determined the "protein efficiency ratio" (PER, weight gain divided by the amount of protein consumed) of different protein sources in rodent diets, or other similar indexes such as the "relative protein efficiency ratio" or "(relative) net protein ratio," to compare weight gain between groups receiving a reference protein, or to compare the weight loss of a group receiving a protein-free diet and various indices were generated from different protein sources. In the current study, the lower PER observed the diets containing the KM only could be indicative of a reduced protein value for the KM based diet. With inclusion of enzymes, particularly the XB and XAP, an increase in the PER was observed. This is confirmatory of the effects if the enzymes in breaking down cell wall matrices of the fibrous KM, resulting in increased release of nutrients bound in cell walls while increasing surface area for efficient digestion, with subsequent enhancement of performance of the broilers as observed in other studies (Christian & Mellor, 2011; Dehghani-Taftia & Jahaniana, 2016; Ragaa & Korany, 2016; Disetlhe *et al*., 2018). The overall PER values obtained in the current study ranging from 3.45 to 3.80 were however above those obtained in rodent studies and the discrepancy can be attributed to the effects of different animal species used in the studies (Boye *et al.*, 2012; Cruz *et al*., 2003).

3.4.3 Serum biochemistry analysis

Assessment of serum blood biochemistry in poultry gives valuable data about their performance, nutritional and health status (Islam *et al*., 2019). According to Mahmud *et al*. (2016), the animal blood profiles is influenced by the animal's age as well as other factors such as diet. The findings from the current study showed that inclusion of KM meal at 10% in the diet had no effects on serum biochemistry parameters. In additionally, from currently study it was found that KM contains high amount of fibre (54.05%) and low protein content compared to soybean meal ,these results were corresponds to the results produced by (Shayo *et al*., 1997.; Loukou *et al*.,2007 ; Ojieh *et al*., 2008 ; Ayssiwede *et al*., 2011 ;Ogbe & George 2012 ; Shazali *et al*., 2013; Ngoran *et al*., 2015) proteins contents of seeds from *C.lanatus* varied from 25 to 30%. Neverless, fibres include other carbohydrates such as cellulose, hemicellulose, pentosans, all of which are poorlydigested by poultry (Kimsé 2016). This indicates that treating kalahari melon meal with exogenous enzymes in the currently study did not affect the health status of the birds. The study of serum biochemistry showed significant difference in SDMA level among the treated groups compared to the control. The SDMA has often been used in some animal species to detect kidney impairment with no reports ever made on its used in broilers. The inclusion of KM oil cake in the diet somewhat reduced the levels of SDMA in the blood which could be positive to the health of the birds. With regards to other serum metabolites values obtained in the current study are consistent with observation everywhere. The serum activities of alanine transaminase (ALT), and alkaline phosphatase (ALP) have been recognized as sensitive serological indicators in the impairment of the hepatic tissues and biliary system, and the serum level of total protein (TP) is the indicator of protein synthesis Abdel-Wahhab and aly (2005), however, TP measurement of ALT enzymes is indicative of liver status in poultry and is therefore a good indicator to determine the safe level of feed additives (Alagawany, 2017). This might be due to higher fibre content and low protein content in KM meal than in soybean meal and also the presence of anti-nutritional factor.

3.5 Conclusion

From the results of this study, it can be concluded that inclusion of enzymes on diet containing Kalahari melon oil cake positively influenced growth performance and protein utilisation efficiency parameters. Moreover, the inclusion of exogenous enzymes positively affected some of serum biochemical parameters apart from glucose, creatine, urea, albumin, total bilirubin, cholesterol amylase and lipase. Desirably, enzyme inclusion on diets containing Kalahari melon oil cake was able to mitigate the effects of fibre content, that could have affected the growth performance and protein utilisation and the nutritional status of the broilers. Overall, Kalahari melon oil cake was shown to have a great potential as a protein source in broiler diets in the presence of enzymes and can therefore be an alternative substitute for soybean meal (SBM) at lower levels.

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CHAPTER 4

EFFECT OF ENZYME INCLUSION ON MEAT QUALITY AND FATTY ACIDS PROFILES IN BROILER FED DIET CONTAINING KALAHARI MELON OIL CAKE

Abstract

The objective of the study was to assess the effects of enzyme inclusion on meat quality and fatty acids profiles in broiler fed diet containing Kalahari melon oil cake. Five isoenergetic and isonitrogenous diets were formulated with the inclusion of Kalahari melon cake in place of soybean meal and various enzymes combinations for grower and finisher phase at Control, 10% kalahari melon cake , 10% kalahari melon cake + protease,, 10% kalahari melon cake + xylanase and *β-glucanase* and 10% kalahari melon cake + xylanase, amylase and protease were formulated. Four hundred unsexed day-old broiler chicks were random distributed to five dietary treatment replicated 8 times. The chicks were offered commercial starter diet from 1 to 14 days. The experimental diet were offered during the grower and finisher phase. Meat quality and fatty acids were mesured. Broilers fed KMXB had the highest final weight, whilst KMP had lowest. Broilers fed control had the highest breast weight. Similarly, broilers fed control had the highest drum weight and thigh weight, whilst KMP had the lowest. No effect of diet was observed on hot carcass weight, cold carcass weight, carcass yield, breast index, thigh index and drum index. From the results , diet has a significant effect on internal organs. Diet had a significant effect on meat quality parameters with broilers fed KMXAP diet having lighter meat color, whilst those fed the control having darker meat color at both 0h and 24h after slaughter. Broilers fed KM had a higher cooking loss, whilst broilers fed control had the lowest. Similarly, broilers fed control had the lowest drip loss while broilers fed KMXAP had a higher shear force. However diet did not affect meat pH, meat colors and water holding capacity. With regards to fatty acids, diet had no effect on individual fatty acids apart from the Docosanoic and Vaccenic fatty acid which was highest in birds fed the KMXAP diet, acid Eicosapentaenoic acid which declined with inclusion of KM and enzymes in diet. Diet also affected total n-3 fats which were highest in the birds fed the control diet, and the n-6/n-3 ratio which increased with inclusion of KM and enzymes in the diet. Overall. Kalahari melon oil cake can be used as a partial substitute of soybean meal without affecting the intrinsic quality of broiler meat.

Keywords : Additives, carcass, intestine, metabolites, polyunsaturated fats.

4.1 Introduction

Globally, there has been rapid increase in the demand for chicken meat with the increasing population. Currently the world population stands at 8 billion and is projected to grow to around 8.5 billion by 2030, 9.7 billion in 2050 and 10.4 billion in 2100 (Singh *et al*., 2016; UN DESA, 2022). The increasing world population coupled with growth in income particulalry in developing countries is put pressure on the animal production sector due to the rising demand for the high quality animal protein (Mottet & Tempio, 2017). Chicken, which constitutes more than 90% of the demands in the poultry market, is considered a cheap and excellent source of protein, providing a healthier dietary protein alternative to the red meat (Yang *et al.,* 2022). According to Campos (2019), poultry products are quickly becoming one of the most-consumed sources of protein around the world. Consequently, global poultry meat demand and production are expected to continue rising with current estimates predicting that by 2026, poultry meat will account for 45% of global meat consumption.

With the increase in world population and income, particularly in the developing world, there has been an associated increase in demand for quality, healthy and wholesome meat and meat products. Generally, in comparison to beef, mutton and pork, chicken products are considered to be healthier, containing high amounts of protein, low fat content and relatively high concentrations of desirable polyunsaturated fatty acids (PUFAs), which are beneficial to the health of consumers (Brenes *et al.,* 2010). A variety of factors may influence meat quality in broiler chicken. In particular, nutritional alteration have been observed to significantly changee the quality and composition of meat (Yang *et al*., 2022). The most commonly used protein source in broiler diets in the soya bean meal. However, due to the increasing costs of soya bean meal due to effects of climate change and reduced rainfall among other factors, alternative protein sources such as Kalahari melon oil cake and various feed additives including enzymes, that may improve utilisation of such new protein sources are being explored

Kalahari melon oil cake is a potential protein source for broilers although with lower protein value and high fibre compared to the soya bean meal. Its intergration in broiler diets has the potential to influence meat quality and composition. Moreover, the inclusion of enzymes to improve its utility in broiler diets has the potential to alter meat quality characteristics. Apart from being a rich source of fat and protein Kalahari melon oil cake contains large amounts of some essential fats including palmitic, stearic, oleic and various linoleic acids isomers (Berry, 2015) with some desirable health-beneficial effects, including anticarcinogenic, antiatherogenic, anthelmintic, antidiabetic and immune stimulatory effects (Suksombat *et al*., 2006; Liu *et al*., 2017). Nevertheless, not empirical eveidence is availble on the effects of inclusion of the cake in broiler diets on meat quality. Therefore, the objective of this study was to evaluate the effect of enzyme inclusion on meat quality and fatty acid profile in broiler fed diet containing Kalahari melon oil cake.

4.2 Materials and methods

4.2.1 Study site, ingredients, treatments, experimental design and animal management.

The study was conducted at North-West University poultry unit in Rooigrond (Section 3.2.1). The sourcing of Kalahari melon meal, ingredients and enzymes were explained in Section 3.2.2. while the dietary treatments and experimental design was explained in Section 3.2.3. Animal management and ethical approval were highlighted in Section 3.2.4

4.2.2 Slaughter procedure

At the end of this trial, all birds were deprived of feed for 13 hours to ensure the emptying of the crop, (Ari *et al*., 2013) and weighed to obtain the slaughter weight (SLW). Thereafter, all broilers were taken to Rooigrond Braaikuikens abattoir A3/32 (Mafikeng, South Africa) for slaughter. Upon delivery at the abattoir, the broilers were grouped according to dietary treatment (Control, KM, KMP, KMXB and KMXAP), put into a metal rack that holds them upside down for stunning. Chickens were slaughtered by cutting the jugular vein with a sharp knife and were bled for up to 2 minutes. Subsequently, the chickens were de-feathered and eviscerated. The hot carcasses (without neck, giblets, and feet) was obtained immediately after. The carcasses and internal organs were taken back to the NWU farm laboratories for determination of internal organ, carcass and meat quality paremeters after being stored in the chiller for 24 hours.

4.2.3 Carcass traits and internal organs

After the slaughter, carcasses from the different treatments were identified by placing them in tagged plastics bags for each treatment. After, evisceration the following internal organs were removed and weighed: gizzards, livers, hearts, spleens and intestines. Length of small intestines (jejunum, duodenum and ileum) were also measured and recorded. Thereafter, carcasses were weighed to obtain the hot carcasses weights (HCW). The carcasses were chilled overnight for 24 h and reweighed to obtain the cold carcass weight (CCW). The dressing out percentage was calculated as the ratio of HCW over the slaughter weight. The breast and thighs were subsequently separated and weighed, and the breast and thigh ratios were also calculated. Breast (pectoralis major muscle) samples were also collected at 24 hours post-mortem and kept frozen (−20°C) pending the evaluation of meat quality traits. Some breast samples were also obtained, vacuum packed and sent for analysis of fatty acids at the Food Science Division, Stellenbosch University (SA).

4.2.4 Meat pH and temperature

Meat pH (pH_u) and temperature measurements were taken immediately after slaughter then 24 hours post-slaughter on the breast muscle (central area of the breast) using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA) equipped with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland) (Stanford *et al*., 2003).

4.2.5 Meat colour

Colour of the meat (L* = Lightness, a* = Redness and b* = Yellowness) was measured using a Minolta colour-guide (Spectrophotometer CM 2500c, Konika Minolta, Osaka, Japan), with a 20 mm diameter measurement area with innovative 45º a: 0 geometry optics. The colour meter was calibrated before measurements using a white tile for standard white colour calibration. Colour recording was done in triplicate of 6 samples of each treatment on the surface of a freshly cut slice of the breast muscle allowed to bloom for 1 hour on a polystyrene tray at 4°C.

4.2.6 Meat drip loss

Drip loss measurement was determined using a method adapted from Zhang *et al.* (2009). Pieces of muscle from the pectoralis major muscle (PPM) weighing ~ 2 grams (wet weight, w1) were hooked and suspended using wire steel in a plastic bottle and sealed properly so that the samples did not touch the sides of the bottle. Bottles were stored in a refrigerator at 4°C for 72 hours. The suspended samples were stored in a cold room at 4°C for 72 hours. The meat samples were reweighed to obtain weight after drip (w2). The difference in weight of each sample before and after drip was conveyed as percentage drip loss and calculated as follows:

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

where; w1 is initial weight, and w2 is weight after drip.

4.2.7 Meat water holding capacity

The WHC of the meat measurements were done in duplicate samples on the surface of a freshly cut slice of the pectoralis major muscle (PMM) (8-16 grams) and was determined as the amount of water expressed from fresh meat held under pressure (60 kg pressure) using the filter-paper press method developed by Grau and Hamm (1957). The water from the fresh meat was taken up by a pre-weighed filter paper and calculated as a percentage. Water holding capacity was calculated using the equation:

WHC (%) $\frac{(initial\ weigh - weight\ after\ pressing}{initial\ weight} \times 100$

4.2.8 Meat cooking loss

Raw breast muscle samples chilled overnight at 4°C chiller were individually weighed to obtain initial weight (w1) of PMM after thawing. The samples were then placed in foil plate and oven broiled (dry heating) at 180°C for 30 minutes. The broiled samples were then removed from the oven and left to cool for 20 minutes. The samples were then re-weighed to obtain the cooked weight (w2) of the PMM). The cooking loss was calculated based on the difference between the weight of raw meat and cooked meat using the following equation:

Cooking loss (%)

Where: w1 is weight of raw meat. w2 is weight after cooking.

4.2.9 Meat tenderness

The breast muscle samples that were previously cooked at 180°C for 30 minutes and used for determination of cooking loss were used for shear force evaluation. The subsamples of 2 cm high × 2 cm width × 12 cm length dimension were sheared perpendicular to the fibre direction using a Meullenet - Owens Razor Shear Blade (A/MORS) mounted on a Texture analyser (TA XT plus, Stable Micro Systems, Surrey, UK). The reported value in Newtons (N) represented the average of the peak force measurements of each sample.

4.2.10 Proximate and fatty acids

Total extracted fat from breast samples was determined gravimetrically and expressed as mg fat (w/w) per 100 g breast sample. The fat free dry matter (FFDM) content was determined by weighing the residue on a pre-weighed filter paper, used for Folch extraction, after drying. By determining the difference in weight, the FFDM could be expressed as mg FFDM (w/w) per 100 g tissue. The moisture content of the muscle was determined by subtraction (100% - % lipid - % FFDM) and expressed as mg moisture (w/w) per 100 g tissue. Fatty acid methyl esters (FAMEs) from the breast muscle were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thicknesses). Fatty acid methyl esters were subsequently identified by comparing the retention times of FAME peaks from samples with those of standards obtained from

Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). All other reagents and solvents were of analytical grade and obtained from Merck Chemicals (Pty Ltd, Halfway House, Johannesburg, South Africa). Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample. The following fatty acid combinations were calculated: omega-3 (n-3) fatty acids, omega-6 (n-6) fatty acids, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and n-6/n-3 ratio.

4.2.11 Statistical analysis

Data on carcass characteristics, internal organs, meat quality parameters and fatty acid profiles of broiler meat were analysed using GLM procedure of SAS (2010). The following statistical models were as follows:

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

Where: Y_{ij} = observation (carcass characteristics, internal organs, meat quality parameters and fatty acid profiles), μ = population mean constant common to all observations, T_i = effect of diet, and ε_{ij} = random error term, for all tests, the level of significance was set at (P < 0.05).

4.3 Results

4.3.1 Carcass traits

The results of the effects of exogenous enzymes on carcass traits of broiler fed diets containing Kalahari melon meal are shown on Table 4.1. From the results, diet had no effect on all carcass traits apart from the final weight, breast weight, thigh weight, drumstick weight and wing weight. Broilers fed KMXB had the highest final weight, whilst KMP had lowest. However, broilers fed control had highest (P < 0.05) breast weight, whilst KMP treatment had the lowest weight. Similarly, the drum and thigh weights of broilers fed control diet were highest ($P < 0.05$), whilst KMP had lowest weights. Moreover, broilers fed KM had highest ($P < 0.05$) wing

Table 4. 1 Effect of enzyme inclusion on broiler fed diet containing Kalahari melon oil cake on carcass trait of broiler chickens

	Dietry treatments						
Carcass traits	Control	KM	KMP	KMXB	KMXAP	SEM	*P*-value
Final weight(g)	1562.27^{c}	1464.27^{b}	1396.93^{a}	1599.50^{c}	1576.50^{c}	67.70	0.041
HCW(g)	1363.75	1305.00	1274.37	1281.87	1348.12	61.61	NS
CCW (g)	1274.75	1284.37	1288.75	1288.75	1312.50	59.43	NS
Carcass yield %	83.23	88.34	87.56	81.58	83.51	4.46	NS
Breast weight(g)	270.41^{c}	263.75^{b}	234.70^{a}	255.62^{b}	261.87^{b}	10.11	0.001
Drumstick weight(g)	93.54^{b}	95.00^{c}	84.79^{a}	90.83^{b}	92.29^{b}	2.82	0.023
Wing weight (g)	77.29^{c}	77.91^{c}	71.25^{a}	74.37^{b}	77.70^{c}	1.85	0.003
Thigh weight (g)	107.08^{c}	97.29^{b}	93.64^{a}	101.25^{b}	96.25^{c}	3.57	0.043
Breast index (%)	18.33	18.99	17.94	18.65	18..94	0.83	NS
Thigh index (%)	7.31	6.99	7.26	7.39	6.91	0.32	NS
Drum index (%)	6.41	6.79	6.51	6.66	6.65	0.26	NS

$^{a\ b\ c}$ means in the same row with different superscripts are significant different *(P* < 0.05).) Dietary treatment: Con: commercial broiler diet without Kalahari melon oil cake; KM: Experimental broiler diet containing 10% Kalahari melon oil cake; KMP: Experimental broiler diet containing 10% Kalahari melon 3% of protease; KMXB: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% of xylanase, β-glucanase; KMXAP: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% XAP, XAP is a combination of xylanase, amylase and protease ; HCC:hot carcass weight ; CCW:cold carcass weight; SEM: Standard error of mean;

weight compared to KMP. For all parameters, the broilers fed the KMP diets consistently had the lowest values compared to all other treatments.

4.3.2 Internal organs

The results of the effects of exogenous enzymes on internal organs parameters of broiler fed diet containing Kalahari melon meal are shown in Table 4.2. From the results, diet has a significant ($P < 0.05$) effect only on liver, jujenum, colon and caecal weights. Broilers fed control had significantly ($P < 0.05$) higher liver and jejunum weights as well as caecum weight, whilst broilers fed KMP had lowest. Broilers fed KMXAP had highest ($P < 0.05$) proventriculus weight whilst broilers fed KMXB had lowest. In addition, broilers fed KM had highest ($P < 0.05$) colon weight compared all other broilers. Nevertheless, diets did not affect gizzard, spleen, duodenum, ileum and small intestines length.

4.3.3 Meat quality

The results of the effects of exogenous enzymes on meat quality parameters of broilers fed diets containing Kalahari melon meal are shown in Table 4.3. From the results, diet had a significant ($P < 0.05$) effect on all meat quality parameters apart from meat pH, some meat color parameters and water holding capacity (WHC). Immediately after slaughter, broilers fed KMXAP had the highest value for lightness (L^*), whilst those fed the control diet had the lowest. Coversely, the broilers fed the control diet had the highest value for redness, (a^*). At 24h post slaughter, the broilers fed the KMXAP diet still had the lightest meat, whilst those fed the control diet had the lowest value for yellowness (b*). Cooking loss was highest in broilers fed KM diet, whilst those fed the KMXB had the highest drip loss. Cooking loss and drip loss were lowest in broilers fed control diet. Broilers fed KMXAP had higher ($P<0.05$) shear force value, whilst broiler fed KMP had the lowest.

Table 4. 2 Effect of enzyme inclusion on broiler fed diet containing Kalahari melon meal on internal organs

	Dietary treatments						
Parameters	Con	KM	KMP	KMXB	KMXAP	SEM	*P*-value
Gizzard (g)	34.19	35.14	32.53	33.43	34.58	1.20	NS
Liver (g)	38.60d	34.99^{ab}	34.76^{a}	36.21^{c}	34.99^{ab}	1.10	0.005
Spleen (g)	2.20	2.15	1.96	2.04	2.05	0.12	NS
Proventriculus (g)	7.01b	7.03b	7.04^{b}	6.68^{a}	7.45^{b}	0.23	0.015
Duodenum (g)	16.62	16.02	17.75	16.97	16.45	0.56	NS
Jejunum(g)	29.11^{d}	25.70^{bc}	23.12^{a}	24.83^{b}	27.45^{c}	1.11	0.041
iIeum (g)	22.46	21.17	21.76	21.80	21.96	1.00	NS
Caecum weight (g)	10.89^{c}	9.83^{ab}	9.35^{a}	10.71^{c}	10.33^{c}	0.52	0.011
Colon weight (g)	1.57^{ab}	1.77^{b}	1.55^{ab}	1.64^{b}	1.36^{a}	0.12	0.031
Small intestine length (mm)	181.08	176.17	177.56	179.18	179.61	2.91	NS

$^{a\ b\ c}$ means in the same row with different superscripts are significant different ($P < 0.05$).) Dietary treatment: Con: commercial broiler diet without Kalahari melon oil cake; KM: Experimental broiler diet containing 10% Kalahari melon oil cake; KMP: Experimental broiler diet containing 10% Kalahari melon 3% of protease; KMXB: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% of xylanase, β-glucanase; KMXAP: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% XAP ,XAP is a combination of xylanase, amylase and protease; SEM: Standard error of mean

Table 4. 3 Effect of enzymes inclusion on broiler diet containing Kalahari melon oil cake on meat quality of broiler chickens

	Dietary treatments					
Meat quality parameters	CON	KM	KMP	KMXB	KMXAP	*P*-value
Meat pH_0	6.22 ± 0.05	6.14 ±0.05	6.10± 0.05	6.02 ±0.05b	6.09 ± 0.05	NS
Meat $temperature_0$	21.35 ±0.34	21.27 ±0.34	20.84 ±0.34a	21.12 ±0.34	21.75 ±0.34	NS
Ultimate pH_u	5.78 ± 0.03	5.80 ± 0.03	5.79 ± 0.03	5.80 ± 0.03	5.77± 0.03a	NS
Meat $temperature_{24}$	12.92 ±0.15^b	12.90 ±0.15^b	12.79 ±0.15^b	12.54 ±0.15^{ab}	12.32 ±0.15^a	0.041
Meat color (0 hrs)						
*L**	39.25 ± 1.00^a	40.23 ±1.02^b	42.02 ± 1.00^c	42.81 ±1.00^c	43.17 ±1.00^{cd}	0.002
*a**	2.81 ± 18.53	1.20 ± 18.93a	1.20± 18.53	1.22 ±18.53	1.30 ±18.53	0.002
*b**	1.80 ±0.22a	2.19 ±0.23	2.50 ±0.22	2.29 ±0.22	2.03 ±0.22	NS
Meat colour (24 h)						
*L**	41.90 ±0.51^a	45.15 ±0.51^c	44.57 ±0.51^c	45.39 ±0.51^{bc}	45.85 ±0.51^c	0.045
*a**	2.00 ±0.12	1.72 ±0.12a	1.83 ±0.12	1.78 ±0.12	1.78 ±0.12	NS
*b**	2.68 ±0.21a	3.11 ±0.21^b	3.81 ±0.21^b	3.83 ±0.21^b	3.29 ±0.21^b	0.003
Cooking loss (%)	23.30±0.49^a	25.43±0.49^c	23.90±0.49^{ab}	24.78±0.49^b	25.36±0.49^c	0.012
WHC (%)	17.21±1.40	17.32±1.40	20.90±1.40	17.72±1.40	18.69±1.40	NS
Drip loss (%)	14.27±0.72^a	16.60±0.72^b	16.29±0.72^b	18.20±0.72^c	16.14±0.72^b	0.005
Shear force(N)	5.14±0.24^b	5.58±0.24^b	4.47±0.24a	5.16±0.24^b	6.42±0.24c	0.005

$^{a\ b\ c}$ means in the same row with different superscripts are significant different ($P < 0.05$). Dietary treatment: explained in Table 4.2; L -lightness, a – redness, b – yellowness, SEM: Standard Error of Mean

4.3.4 Fatty acid profiles

The results of the effects of exogenous enzymes on fatty acids profile of broiler fed diets containing kalahali melon meal are shown on Table 4.4. From the results , diet had no effect on all fatty acids profile except docosanoic,vaccenic and Eicosapentaenoic acid. Broiler fed KMXAP diet had the highest values (P<0.05) for docosanoic acid, whilst broilers fed control had the lowest. In addition, broilers fed the KMXAP diet had the highest (P<0.05) concentrations for vaccenic acid while those fed the KMP had the lowest. With regards to Eicosapentaenoic acid, significant higher (P<0.05) values of were observed in broilers fed control diet compared to KM containing dietary treatments. Palmitic acid was the most common fatty acid observed in all dietary treatments followed by stearic acid. The results of the effects of exogenous enzymes on fatty acids profile of broiler fed diets containing Kalahari melon meal are shown on Table 4.5. From the results, diet had no effect on all total fatty acids profiles except for total n-3 and n-3/n-6 . Broilers fed control had the highest (P<0.05) total n-3 fatty acids, whilst broilers fed KMP had the lowest. Total n-3 fatty acids decloined with the inclusion of KM oil cake in the diet. Nevertheless, the n-6/n-3 ration increased with the inckusion of the KM oil cake and enzymes in the diet. Broilers fed KMP had the higheest (P<0.05) n-6/n-3 ratio, while the control diet had the lowest. All the KM containing diets had higher values for n-6/n-3 ratios when compared to the control diet.

Table 4. 4 Fatty acids composition of kalahali melon oil cake

	Dietary Treatments						
Individually fatty acids	Con	KM	KMP	KMXB	KMXAP	SEM	*P*-Value
SFAs							
Octanoic	0.02	0.02	0.04	0.01	0.01	0.1	NS
Decanoic	0.03	0.03	0.04	0.03	0.02	0.009	NS
Lauric	0.06	0.08	0.09	0.06	0.05	0.02	NS
Myristic	0.87	1.42	1.33	0.92	0.58	0.56	NS
Pentadecanoic	0.10	0.18	0.15	0.14	0.08	0.06	NS
Palmitic	41.72	64.67	58.97	42.26	30.61	23.88	NS
Heptadecanoic	0.11	0.12	0.10	0.15	0.12	0.02	NS
Stearic	11.56	19.64	16.40	14.83	10.43	6.23	NS
Eicosanoic	0.11	0.24	0.18	0.20	0.12	0.08	NS
Heneicosanoic	0.02	0.04	0.04	0.03	0.03	0.01	NS
Docosanoic	0.09^{d}	0.13^{a}	0.15^{b}	0.13^{a}	0.16^{c}	0.01	$P<0.05$
Tricosanoic	0.02	0.03	0.03	0.03	0.02	0.006	NS
lignoceric	0.03	0.04	0.04	0.03	0.03	0.005	NS
Total SFA	54.77	86.70	77.62	58.88	42.33	30.86	NS
MUFAs							
Myristoleic	0.25	0.35	0.37	0.19	0.13	0.15	NS
Palmitoleic	9.82	13.92	13.20	7.19	5.56	5.48	NS
Eicosenoic	1.81	2.98	2.21	1.90	1.43	0.91	NS
Erucic	0.04	0.04	0.04	0.04	0.04	0.005	NS
Nervonic	0.03	0.02	0.02	0.02	0.02	0.007	NS
Eicosadienoic	0.24	0.42	0.35	0.34	0.29	0.08	NS
Docosadienoic	0.02	0.02	0.03	0.03	0.03	0.005	NS
Oleic	60.88	102.57	83.93	62.40	44.97	36.50	NS
Linoileic	22.96	51.44	37.18	33.45	22.29	16.83	NS
Vaccenic	0.10^{d}	0.09^{c}	0.06^{a}	0.07^{b}	0.14^{e}	0.02	$P<0.05$
Total MUFA	96.18	171.90	137.49	105.68	74.93	59.77	NS
PUFAs							
γ-Linolenic	0.26	0.34	0.20	0.28	0.18	0.08	NS
C20	0.58	0.60	0.60	0.55	0.51	0.08	NS
Arachidonic	3.26	3.34	3.29	3.54	3.71	0.21	NS
Docosahexaenoic	0.31	0.30	0.22	0.31	0.31	0.03	NS
Eicosatrienoic	0.03	0.03	0.03	0.03	0.03	0.007	NS
Eicosapentaenoic	0.18^{c}	0.10^{a}	0.10^{a}	0.11^{b}	0.11^{b}	0.01	$P<0.05$
Total PUFA	4.65	4.72	4.47	4.84	4.86	0.33	NS

$^{a\ b\ c}$ means in the same row with different superscripts are significant different ($P< 0.05$).

Dietary treatment: explained in Table 4.2

Table 4. 5 Effect of enzyme inclusion in Kalahari melon based diet on total fatty acids profiles of broiler chickens meat

	Dietary Treatment						
Parameters	Con	KM	KMP	KMXB	KMXAP	SEM	*P*- value
Total SFA	54.77	86.70	77.62	58.88	42.33	30.86	NS
Total MUFA	96.18	171.90	137.49	105.68	75.93	59.77	NS
Total PUFA	4.65	4.72	4.47	4.84	4.86	0.33	NS
Total n-6	4.11	4.28	4.11	4.38	4.40	0.30	NS
Total n-3	0.53[c]	0.43[b]	0.36[a]	0.46[b]	0.46[b]	0.03	$P < 0.05$
PUFA:SFA	0.10	0.09	0.16	0.12	0.12	0.02	NS
n-6/n-3	7.79[a]	9.98[b]	11.17[c]	9.55[b]	9.70[b]	0.44	$P < 0.05$

[a b c] means in the same row with different superscripts are significant different ($P < 0.05$). Dietary treatment:explained in Table 4.2; Total n-6-: omega 6 fatty acids; Total n-3: omega 3 fatty acids; SEM: Standard error of mean

4.4 Discussion

4.4.1 Carcass traits

Dietary alterations through inclusion of new ingredients and feed additives such as commercial exogenous enzyme have been reported to improve digestibility, enhance growth and subsequently enhance carcass composition and meat quality. (Sharma *et al*. 2018). Kumanda *et al.* (2019) reported that an increase in carcass weights in broiler chickens when high fibre dietary susbrateand enzymes were included in broiler diets. In the current study, although there was a slight increase in the slaughter weight of chickens with the inclusion of KM and enzymes this did not translate to an increase in HCW and CCW respectively. The results are however indicative of the fact that inclusion exogenous enzymes and KM can provide the same performance as with the control diets containing soya bean meal. The lack of dietary effects on carcass yield and all other carcass cuts, in the current study , is in concordance with the findings reported by Hajati *et al.* (2009) who also observed a lack of significant effect on the same carcass traits fed Prosopis juliflflora pods meal treated with exogenous enzymes. Similarly, Zanella *et al*. (1999) reported no effect of xylanase, amylase, and protease combination on carcass weight, abdominal fat and breast meat weight. The effect of protease on broiler performance and nutrient digestibiliy, however, has been inconsistent (Naveed *et al.,* 1998; Ghazi *et al*., 2003; Rutherfurd *et al*., 2007; Boguhn & Rodehutscord, 2010; Angel *et al*. 2011; Romero *et al*., 2013, 2014). The number of studies examining the effect of enzyme combination on carcass characteristics are limited. Café *et al.* (2002) reported that abdominal fat was consistently increased by the xylanase, amylase, and protease combination and suggested that birds fed the diets containing enzymes obtained a greater amount of net energy from their diets.

4.4.2 Internal organs

Changes in size and structure of internal organs can be indicative of the effect of diet and its components on the development and function of the organs. The liver functions to detoxify chemicals and other toxic substances not required by the body. It is known that the size of the liver may increase in response to higher levels of some chemical compounds requiring detoxification (Pavastegan *et al*., 2017). The KM oil cake is known to contain some secondary plant metabolites such as suponins and albumins which may influence the live function if present in high amounts (Bower *et al*.,1988). Results from the current study showed that the liver weights for broilers fed KM containing diets were lower compared to the control indicating that the levels were insignificant to influence liver function. This could be explained by the fact that on oil essential oil extraction, much of the secondary plant metabolites were also removed from the cake. While the jejunum weight was significantly influenced by the inclusion of with lower values obtained in KM fed broilers, diet had no effect on the intestinal length. The lack of effect of diet on the intestinal length could be could be indicative of the absence of trophic effects of enzymes and the KM components in stimulating the proliferation of normal cells and tissues, enhancing healthy tissue turnover and maintenance (Li et al., 2016; Ivarsson & Wall, 2017). Enzymes and some secondary plant metabolites contained in some feed ingredient have been observed to elicit some intestinal morphological alterations with the ultimate effect of increasing retention time of feed for digestion processes and enhancing mucosal permeability for efficient nutrient assimilation (Jiang *et al*., 2007; Mahagna *et al*., 1994). The results from the current study are also consistent with the findings by Baraik (2010) who also found no effect from individual or combination of exogenous enzymes on internal organs of broiler chickens.

4.4.3. Meat quality

Meat colour is one of the most important quality attributes of poultry meat that influences consumer perceptions of meat and products, ultimately influencing purchasing decisions (Tang *et al*., 2007). A diversity of factors including diet may influence meat colour. From the current study, meat colour appeared to increase with the inclusion of KM and various enzymes combinations in the diet with the broilers fed the KMXAP diet having the lightest meat both immediately after slaughter and 24 hrs after slaughter. However, all the values for meat lightness were (L) were lower than the values classified for normal and pale breast meat Lokaewmanee and Promdee (2018) classified chicken breast meat colour as lighter than normal (L* >53), normal (48 < L* > 53) and darker than normal (L* < 48), but Corzo *et al*. (2005) reported broiler breast meat with a L* value of 55 as normal and a L* value of 60 as pale meat. While there was no effect of diet on meat pH, the parameter is critical as it often influence all other meat quality parameter such as meat color, shear force, cooking loss, drip loss and water holding capacity. Scheffler *et al*. (2015), reported that anaerobic glycolysis is a major metabolic pathway during the post-mortem period, which leads to the accumulation of lactate in meat, thus rapidly to decreasing its pH. The values for meat pH obtained in the current study were within the range reported in other studies for broilers (Disetlhe *et al*., 2020; Zhang *et al.,*2015). In this research, the results revealed that cooking loss was significant higher in the KM , whilst broilers fed control had lowest. The birds fed the control diet also had the lowest drip loss meaning that inclusion of enzymes and KM in diets may have enhanced the excessive lost of meat juices, which is undesirable. Generally, greater drip loss may induce a reduction in water-holding capacity and tenderness of meat. Nevertheless, in the current study shear force values declined with the inclusion exogenous enzymes , which could be attributed to the reduction of drip loss of the meat after 72 hours, since there is a positive correlation between drip loss and shear force (Zhang *et al*.,2015). In other reports, (Gil *et al*.,2008; Yu *et al*., 2021) reported that meat with great water retention properties after slaughter is juicy, which implies low shear force, high tenderness, and favorable taste.

4.4.4 Fatty acids profiles

The chemical composition of poultry meat, including the fatty acids, is a great concern to consumers due to their associations with health and cardiovascular diseases in humans (Waheed *et al.,* (2018). Analysis of fatty acid profiles is therefore one of the critical aspects in the evaluation of meat quality. From the results, the major fatty acids obtained in all dietary treatments were the palmitic and stearic acids which fall under the SFAs. This is consistent with observations from other studies (Laudadio and Tufarelli, 2010; Ahmed et al., 2015). Although there were no differences in terms of the palmitic acid and stearic acids across treatments, when present in high amounts, these fatty acids are of great significance due to their hypercholesterolemic properties which are related with coronary heart disease (FAO/WHO, 2009; Ahmed et al., 2015). Nevertheless, the inclusion of KM oil cake and enzymes in the diets appeared to increase the levels of vaccenic and while decreasing the levels of eicosapentaenoic acid and polyunsaturated fatty acid, which is undesirable (Sanz *et al*. 1999). According to Ahmed et al. (2015), PUFAs in the meat play an immense role in human nutrition as they are originators of eicosanoids, leucotriens, and thromboxanes, which regulate the cardiovascular system and immunological processes.

From the reults only total n-3 fatty acids and n-6/n-3 ratio were affected by diets. The PUFA: SFA and n-6/n-3 ratios are critical parameters used to evaluate the nutritional value meat (Ahmed et al., 2015; Ma et al., 2021). PUFAs and MUFAs are known to influence consumer health by reducing the risk of cardiovascular diseases. Dietary monosaturated fatty acids such as gondoic, oleic and nervonic acids may reduce the overall risk of stroke, cardiovascular conditions and mortality (Schwingshackl & Hoffmann, 2014). Generally meat with low PUFA:SFA ratio which is less than (0.4) and high n-6/n-3 ratio (> 5) ratio may be considered to be poor and unfavourable since they might encourage an escalation in cholesterolaemia. (Santos-Silva et al., 2002; Ukropec et al., 2003). In the current study, while there was no effect on in the PUFA/SFA ratio, the inclusion of KM oil cake and enzymes indiets resulted in an increase in n-6/n-3 ratio. Nevertheless the n-6/n-3 values for all treatments where above the recommdend value of less than 5 (Santos-Silva et al., 2002; Ukropec et al., 2003). The results of the study are however consistent with previous observations (Laudadio and Tufarelli, 2010)

4.5 Conclusion

In conclusion, the present study demonstrated that the KM oil cake and enzymes can be included in diets with some important beneficial effects of enzyme inclusion on carcass characteristics, meat quality and some important fatty acids for the benefit of human health. Desirably the inclusion of KM oil cake in diets resulted in comparable values for some PUFAs to those of the conventional diets. Therefore, Kalahari melon oil cake can be used as a partial substitute of soybean meal without affecting the intrinsic quality of broiler meat.

4.6 References

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CHAPTER 5

EFFECT OF ENZYME INCLUSION ON BONE DEVELOPMENT IN BROILER FED DIET CONTAINING KALAHARI MELON OIL CAKE

Abstract

The current study was consucted to determine the effect of enzyme inclusion on bone development in broiler fed diet containing Kalahari melon oil cake. As explained in cahpter 3, five isoenergetic and isonitrogenous diets were formulated with the inclusion of Kalahari melon cake in place of soybean meal and various enzymes combinations for grower and finisher phase. Four hundred unsexed day-old broiler chicks were random distributed to the five dietary treatment replicated 8 times. At the end of the feeding trial the birds were slaughtered for bone development and welfare assessment. From the results , diet had no effect tibia growth plate parameters of broiler chicken. Broilers fed KMXB had the highest tibia weight and tibia diameter proximal compared to other treatments. Broiler fed KM and KMP had the highest tibia width diameter, whilst control had lowest. Diet also affect latency to lie parameter of broiler chicken with broilers fed control diet having the highest standing, persistency followed by broilers fed KM diet. Broilers fed KMXAP had a higher tibia ash(%) , whilst KM had a lower tibia ash. It was concluded that the inclusion of enzymes on diet contain 10% of kalahari melon meal to broiler chicken diet can improve bone development and welfare in broiler chickens.

Keywords : biomenchanical, growth plate, minerals, physiology, tibia.

5.1 Introduction

Over the last few decades, the poultry industry has seen some tremendously increase in production and efficiency due to advancements in genetics, nutrition, and management practices (Mottet & Tempio, 2017). At the same time, nutrition has progressed in terms of comprehensive digestive physiology and metabolism, and more precise evaluation of the quality of dietary raw materials (Kiarie & Mills, 2019). The genetic improvements have culminated in the development of fast-growing broiler strains, with high feed conversion, reaching market weight at an earlier age than ever before (about 2 kg in 40 d) (Havensteinet *al et.*, 1994, 2003a,b; Dogan *et al.,* 2019; Mancinelli *et al*., 2020). However, the improvements in feed efficiency and high growth rates came at a cost, as fast and early growth have been linked to skeletal disorders, leg weakness, and impaired walking ability that can compromise the welfare of the birds (Julian, 1998; Scahaw, 2000; Meluzzi & Sirri, 2009; Kierończyk *et al*., 2017).

The utilization of ingredients with neutracical properties such as the KM oil cake and inclusion of feed additives such as exogenous enzymes, organic acids and probiotics may promote effective release and assimilation of nutrients particularly minerals in the gut, ultimately stimulating active bone growth (Ozturk *et al*., 2010; Kim *et al*., 2017). Together with the exogenous enzymes. The KM oil cake contains considerable amounts of secondary plant metabolites with the potential to induce intestinal morphological alterations and provoke some changes in intracellular divalent calcium levels, and act as dilators, increasing mucosal and cellular permeability (Pizzari *et al*., 2000; Johnsson *et al*., 2015). Increased permeability allows easier assimilation of minerals from the gut and their transfer from blood to the bone and cells, resulting in effective bone development (Stepchenko *et al*., 1991; Dinev, 2012).

Kalahari melon seed cake is a potential protein source in broiler diets as it contains considerable amounts of protein. In addition, it contains some saponin and albumins as well as large amounts of some essential fats including palmitic, stearic, oleic and various linoleic acids isomers (Berry, 2015), with wide range of health-beneficial effects, including anticarcinogenic, antiatherogenic, anthelmintic, antidiabetic and immune stimulatory effects (Suksombat *et al*., 2006, Liu *et al.*, 2017). Moreover, the essential fats have the potential to play a vital role in the bone health of poultry (Liu *et al*., 2003) it utilisation in broiler diets can be improved through inclusion of exogenous enzymes. There is currently no information on the potential effect of KM oil cake and exogenous enzymes inclusion in diets on bone development and welfare in broilers. Therefore, the objective of this study was to assess the effect of enzyme inclusion on bone development and and welfare in broiler fed diet containing Kalahari melon oil cake.

5.2 Materials and methods

5.2.1 Study site, ingredients, treatments, experimental design and animal management.

The study was conducted at North-West University poultry unit in Rooigrond (Section 3.2.1). The sourcing of Kalahari melon meal, ingredients and enzymes were explained in Section 3.2.2. while the dietary treatments and experimental design were explained in Section 3.2.3. Animal management and ethical approval were highlighted in Section 3.2.4 and the slaughter procedures are explained in Section 4.2.2

5.2.5 Latency to lie

At day 35, 1 broiler chicken per treatment was exposed to the latency to lie (LTL) test, as described by Berg and Sanotra (2003) and Caplen *et al*., 2014). This test is established on the fact that body interaction with water is a novel experience for broiler chickens. Each bird was individually placed into a plastic container filled with 3 cm water, the water temperature was measured at 32 °C for testing additional birds to maintain a similar temperature for all birds tested. The time spent standing before lying down for the first time and frequency of lying events per birds were recorded, according to the principle that the better leg health the broilers has, the longer it will stand up to avoid body contact with the water. If the chicken was still standing after 600 seconds which is equivalent to 10 minutes, the test was interrupted, which means the legs are strong and healthy.

5.2.3 Tibia gross lesion analysis

Tibia gross anatomy was conducted to assess the prevalence of rickets in broilers from different dietary treatments. The muscles around the tibia were carefully removed then the proximal end was dissected with very sharp scalpel to expose the epiphyseal plate. The growth plates of the tibia bones were examined visually for gross lesions.

5.2.4 Tibia linear parameters

At necropsy on day 42 days, 3 right tibiae from each treatment were surgically removed, de-fleshed and cleaned of all tissue, including cartilage caps (periosteum) by hand, and weighed to obtain the tibia weight (TW). Tibia diameter proximal end (TDPE), tibia width diameter (TWD), tibia length diameter (TLD) and tibia diameter distal end (TDDE) were determined using a Oxford 5Inch 150Mm Vernier Caliperfine adjustment.

5.2.5 Bone ash and mineral

Bone were crushed. The tibia pieces were collected, defatted and ashed. The samples were placed into a muffle furnace at 600 °C for 24 hours and cooled down in a desiccator before being weighed (final) to determine ash percentage. Tibia bone ash was

expressed as a percentage as follows:

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

The mineral content was analyzed in the Animal Health Laboratory (NWU) using the dry ashing macro and trace minerals methods and following the guidelines of the Agri-Laboratory Association of Southern Africa (AgriLASA, 1998). The ash was weighed and digested with 1 mL of 55% nitric acid overnight. After 24 hours, samples were then gently transferred to McCartney bottles without disturbing the sediment. The concentrations of Ca, Fe, Co, Mg, Mn, Cu and Zn were then determined using an ICP Mass Spectrometer (Perkin-Elmer, 1982, Nex ION 300Q). Macro-minerals were presented as mg/l

5.2.6 Statistical analysis

Data on LTL test and tibia parameters was analysed using

GLM procedure of SAS (2010). The statistical model was as follows:

$Yijk = \mu + Di + \varepsilon ijk$

Where: *Yijk* = response variables, μ = population mean, *Di* = effect diets and ε*ijk* = random error

assumed to be normally and independently distributed. For all statistical tests, significance was

declared at $P < 0.05$. Where significant difference was detected, mean separation was done using the PDIFF option of SAS (2010).

5.3 Results

5.3.1 Tibia growth plate analysis

The results of the effects of exogenous enzymes on tibia growth plate parameters of broiler fed diets containing Kalahari melon meal are shown on Table 5.1. From the results, diet did not affect the proliferative and hypetrophic zones of the growth plates of broiler chicken. Nevertheless, the hypertrophic zone of, broilers fed KMXAP appeared to higher than those of all other treatments inclusing the control. In contrast, the broilers fed the control diet appeared to have a higher value for the proliferative zone. Overall the birds fed the broilers fed fed KMXAP had the highest value for the growth plate, although not statistically significant.

Table 5. 1 Effect of enzyme inclusion on broiler diet containing Kalahari melon meal on bone growth of broiler chicken

	Dietary treatments						
Parameters	Con	KM	KMP	KMXB	KMXAP	SEM	*P*-value
Hypertrophic zone (mm)	5.00	5.00	5.00	4.33	6.06	0.64	NS
Proliferative zone (mm)	1.00	0.80	0.70	0.70	0.73	0.13	NS
Growth plate zone (mm)	6.00	5.80	5.93	5.03	6.80	0.65	NS

Dietary treatment: Con: commercial broiler diet without Kalahari melon oil cake; KM: Experimental broiler diet containing 10% Kalahari melon oil cake; KMP: Experimental broiler diet containing 10% Kalahari melon 3% of protease; KMXB: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% of xylanase,β-glucanase; KMXAP: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% XAP ,XAP is a combination of xylanase, amylase and protease; SEM: Standard error of mean;

5.3.2 Tibia linear parameters

The results of the effects of exogenous enzymes on tibia biomechanics of broiler fed diets containing Kalahari melon meal are shown on Table 5.2. From the results, diet had a significant ($P < 0.05$) effect on tibia biomechanics parameters of broiler chicken. Broilers fed KMXB had highest ($P < 0.05$) tibia weight and tibia diameter proximal compared to other treatments. Broiler fed KM and KMP had highest ($P < 0.05$) tibia width diameter, whilst control had lowest. Diet had no effect on both the tibia length and tibia diameter distal end respectively.

5.3.3 Latency to lie

The results of the effects of exogenous enzymes on latency to lie of broiler fed diets containing Kalahari melon meal are shown on Table 5.3. From the results, diet did not affect latency to lie parameter of broiler chicken. Nevertheless broilers fed the control diet appeared to have a high standing persistence as reflected by the longer time, followed by broilers fed the KM diet.

5.3.4 Tibia ash and mineral content

The results of the effects of exogenous enzymes on tibia ash and mineral content of broiler fed diets containing Kalahari melon meal are shown on Table 5.4. From the results, diet had a significant ($P < 0.05$) effect on tibia ash and some mineral content parameters of broiler chicken. Broilers fed KMXAP had higher ($P < 0.05$) tibia ash(%) , whilst KM had the lowest tibia ash. Broiler fed control had higher ($P < 0.05$) Mn, whilst broilers fed KMXAP had lower. Similar, broiler fed control diet had the highest ($P<0.5$) Zn, whilst broilers fed KMXAP had the lowest. Additionally, broilers fed KMP had the highest ($P < 0.05$) copper concentrations compared to to all other treatments.

Table 5. 2 Effects of enzyme inclusion on broiler fed diet containing Kalahari melon oil cake on tibia linear parameters of broiler chickens

	Dietary treatments						
Parameters	Con	KM	KMP	KMXB	KMXAP	SEM	*P*-value
Tibia weight (g)	16.89[a]	17.85[ab]	18.20[b]	20.09[b]	20.07[b]	1.08	0.04
Tibia diameter proximal (cm)	1.73[ab]	1.83[b]	1.60[a]	1.96[b]	1.93[b]	0.07	0.04
Tibia width diameter (cm)	0.56[a]	0.73[a]	0.73[a]	0.63[ab]	0.63[ab]	0.04	$P<0.05$
Tibia length (cm)	10.00	9.63	9.83	10.33	9.76	0.28	NS
Tibia diameter distal end	1.56	1.60	1.50	1.66	1.56	0.06	NS

[a b] means in the same row with different superscripts are significant different ($P < 0.05$). Dietary treatment: Con: commercial broiler diet without Kalahari melon oil cake; KM: Experimental broiler diet containing 10% Kalahari melon oil cake; KMP: Experimental broiler diet containing 10% Kalahari melon 3% of Protease; KMXB: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% of xylanase, β-glucanase; KMXAP: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% XAP ,XAP is a combination of xylanase, amylase and protease ; SEM: standard error of mean.

Table 5. 3 Effect of enzyme inclusion on diet containing Kalahari melon meal on leg disorder of broiler chicken

	Dietary treatments						
Parameters	Con	KM	KMP	KMXB	KMXAP	SEM	*P*-value
Time (min)	9.69	9.21	8.79	7.32	8.84	0.72	NS

Dietary treatment: Con: commercial broiler diet without Kalahari melon oil cake; KM: Experimental broiler diet containing 10% Kalahari melon oil cake; KMP: Experimental broiler diet containing 10% Kalahari melon 3% of protease; KMXB: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% of xylanase, β-glucanase; KMXAP: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% XAP ,XAP is a combination of xylanase, amylase and protease; SEM: standard error of mean.

Table 5. 4 Effect of enzyme inclusion on diet containing Kalahari melon meal on tibia ash and mineral content of broiler chickens

	Dietary treatments					
Parameters	Con	KM	KMP	KMXB	KMXAP	*P*-value
Tibia Ash%	97.65±0.14[b]	97.52±0.14[ab]	97.72±0.14[b]	97.29±0.14[a]	97.80±0.14[b]	*P*<0.05
Mn	87.20±6.18[d]	57.99± 5.05[c]	49.76 ±0.05b[c]	41.00± 5.05[b]	33.70± 6.18[a]	*P*<0.05
Fe	350.47± 20.19	343.31 ±24.73	351.17 ±24.73	298.11± 20.19	342.08 ±24.73	NS
Co	3.93± 0.59	2.42 ±0.48	2.91 ±0.59	2.05 ±0.59	2.57 ±0.84	NS
Cu	5.18± 1.46[b]	6.78 ±1.46[c]	8.84± 1.19[d]	4.69 ±1.19[a]	5.73 ±2,07[b]	*P*<0.05
Zn	172.99 ±17.39[e]	114.82 ±17.39[c]	115.29 ±17.39[d]	96.66 ±21.30[b]	82.27 ±21.30[a]	*P*<0.05

[a b c d e] means in the same row with different superscripts are significant different ($P < 0.05$). Dietary treatment: Con: commercial broiler diet without Kalahari melon oil cake; KM: Experimental broiler diet containing 10% Kalahari melon oil cake; KMP: Experimental broiler diet containing 10% Kalahari melon 3% of protease; KMXB: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% of xylanase, β-glucanase; KMXAP: Experimental broiler diet containing 10% Kalahari melon oil cake and 3% XAP, XAP is a combination of xylanase, amylase and protease; SEM: standard error of mean; Mn: manganese; Fe: iron; Co: Cobalt; Cu: copper; Zn: zinc.

5.4 Discussion

5.4.1 Tibia growth plate analysis

The growth plate (GP) is a dynamic tissue driving bone development through the proliferation, hypertrophy of the chondrocyte and matrix production. The GP biomechanical properties are often determined by the extracellular matrix (ECM) which is assumed to play a pivotal role for chondrocyte geometry and arrangement, thereby guiding proper growth plate morphogenesis and bone elongation (Prein et al., 2016). In fast growing broilers, bone development may be affected by many factors having both direct and indirect effects, nutrition being one of the most significant ones. Nevertheless, no effect of diet on the growth plate parameters was observed. Notably, fast growing broiler chickens have different requirements regarding mineral components and the composition of the feed mixture, depending on the species, age and productivity of poultry (Sesztáková *et al*., 2010). The observed hypertrophic and growth plate zone in broilers fed KMXAP could be attributed to the fact that KM meal had higher amount of some lipids and secondary plant metabolites, which may affect the bone development of broiler chickens as observed in other studies, which used products of similar nature in broiler diets (Yannicelli *et al*., 2002)

5.4.2 Tibia linear parameters

The tibia weight and linear measurements such as tibia diameter proximal, tibia length and tibia diameter distal end have been used as indicators of bone quality (Leblanc *et al.,* 1986; Krupski & Tatara, 2007; Charuta *et al*., 2013). From current study, results showed that broilers fed KMXB had higher tibia epiphyseal diameter, whilst broilers fed control had the lowest. Scott *et al*. (1982) found that lower bio-availability of Mn and Zn may lead to lower epiphyseal diameter . A low concentration of Zn in broiler diets was also reported to result in shorter tibia length (TL), due to its role in bone development by mechanisms influencing longitudinal bone growth at the growth plate (Starcher *et al*., 1980; WangWang *et al.,* 2002; Oviedo-Rondón *et al*., 2006). Tibia mechanical and biophysical measurements, such as bone mineral density (Watkins & Southern,1992; Rath *et al*., 2000; Onyango *et al*., 2003; Kim *et al*.,2006; Shim *et al*., 2012), bone mineral content (Akpeet al., 1987; Onyango et al., 2003; Kim *et al*., 2006), (Merkley, 1981; Ruff & Hughes, 1985; Park *et al*., 2003; Kim *et al*., 2006) have also been the used to assess bone health in poultry, because of their relationships with locomotion and leg pathologies. This is due to the fact that bone geometry responds to the changes in body weight and additional body mass increases the loading strain applied to the skeletal system, and therefore its composition and strength (Harner & Wilson, 1985; Cooper *et al.*, 1995).

5.4.3 Latency to lie

The LTL test as described by Weeks *et al.* (2002) is a prominent method that was developed to measure standing persistence, which is an indirect measure of bone development and bone strength in broilers. Water as an aversive stimulus has been shown to provide avoidance motivation, resulting in broilers with strong bones being able to stand for a longer period, whereas those with poor bone development might succumb to their weight and sit down sooner (Berg & Sanotra, 2003; Yang *et al.,* 2016). In the current study, diet did not affect the LTL, although the birds fed the control and the KM diets had a higher standing persistency than the other. The longer standing persistency in broilers fed diets with 10% Kalahari melon meal could be a result of the high amounts of phytochemicals contents (Seidu *et al*., 2015) on the Kalahari melon oil cake that could had facilitated the assimulation of minerals as shown by the reported high minreal concentration in the control and KM fed birds. The lower standing persistency in broilers fed diets containing exogenous enzymes could be due to the interference of the enzymes on mineral uptake.

5.3.4 Tibia ash and mineral

Bone ash is an indicator of bone mineralization (Shim *et al*., 2012). Findings from the current study showed that diet significantly affected tibia ash percentage .The is a positive relationship between tibia ash and bone strength. El-Husseiny *et al*. (2012) reported that improper bone development is one of the basic symptoms of Zn deficiency in the birds diet. From the current study, the lower mineral content in birds fed diets containing enzymes was evident and could have influenced bone development. In a study by Scrimgeour *et al*. (2007), Zn ions were reported to occur in the active centres of many enzymes such as alkaline phosphatase, thus, Zn is highly important to ensure correct ,mineralisation of the bone tissue bone metabolism as well as stimulation of DNA synthesis in osteoblasts, bone-forming processes and increase bone weight and calcium ions concentration (Ma & Yamaguchi, 2000). Both the excess and deficiency of Zn can cause a gradual decrease in the body weight, bone weight, and bone thickness, and can give rise to bone deformation, low mineralisation (Rath *et al.,* 2000). Mn is also an essential trace mineral and Mn required by the avain species., From the current fundings manganese (Mn) was suppressed in birds fed diets containing exogenous enzymes. Unfortunatley, in the current study, calcium and phosphorus, which are important minerals in bone formation, were not analysed due to ths issues with the mineral analysis equipment.

5.5 Conclusion

From the results, inclusion of KM and enzymes in the broiler diets appeared to positively influence bone growth. Nevertheless, inclusion of the Kalahari melon oil cake on its own improved the minerals assimilation, tibia bone strength of broiler chickens as reflected by the high latency to lie, bone ash and bone mineral content. In general inclusion of Kalahari melon oil cake can be included in broiler diets by up to 10% with significant improvements in performance and bone development of broiler chickens.

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CHAPTER 6

6 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

6.1 General discussion

The growth in poultry production is having a profound effect on the demand for feed and other raw materials for broiler production. Nevertheless, the price of corn and soybean like broiler feed ingredients, protein sources in particularly, keeps fluctuating with seasons and production (Singh *et al.*, 2018). Moreover, the competition between humans and animals for the same protein sources have also exacerbated the problem. However, there has been an increase on the global market due to the feed-fuel demand of soybean (Klir *et al.*, 2019; Tona, 2018). Thus, making production less profitable when soybean is included in livestock diets (Tona, 2018). This has stimulated many efforts focusing on exploration of alternative locally available protein sources for broiler diets particularly those that do not fall within the food matrix for humans. The current study, therefore, investigated the effect of inclusion of Kalahari melon oil cake and exogenous enzymes of performance in broilers.

In Chapter 3, the effects of exogenous enzymes inclusion in diets on growth performance and health was assessed in broilers. From the results, it was observed that inclusion of enzymes on diet containing Kalahari melon oil cake positively influenced growth performance and protein utilisation efficiency parameters. Moreover, the inclusion of exogenous enzymes positively affected some of serum biochemical parameters apart from glucose, creatine, urea, albumin, total bilirubin, cholesterol amylase and lipase. Desirably, enzyme inclusion on diets containing Kalahari melon oil cake was able to mitigate the effects of fibre content, that could have affected the growth performance and protein utilisation and the nutritional status of the broilers. Overall, Kalahari melon oil cake was shown to have a great potential as a protein source in broiler diets in the presence of enzymes and can therefore be an alternative substitute for soybean meal (SBM) at lower levels.

In Chapter 4, a follow up study was conducted to assess the effects of enzymes inclusion on carcass characteristics meat quality and fatty acid profiles. The study demonstrated the effect of enzyme inclusion on diet containing Kalahari melon meal on meat quality and fatty acids of broiler chickens depends on the commercial exogenous enzymes. The inclusion of exogenous enzymes and 10% of Kalahari melon meal improves some of the meat quality and fatty acids profiles of broiler meat. Moreover, Kalahari melon meal with or without enzyme inclusion is a strategy to improve broiler meat color by increasing pigments, total phenols and reduces bacterial counts without having affecting breast meat texture and sensory acceptance.

The welfare of birds is one of the critical aspects in broiler production. The high growth rates and heavy weights are associated with leg deformities in broilers. Therefore in Chapter 5, the effects of kalahari melon oil cake and enzymes inclusion of bone development in broilers was assessed. From the results, inclusion of KM and enzymes in the broiler diets appeared to positively influence bone growth. Nevertheless inclusion of Kalahari melon oil cake on its own improved the minerals assimilation, tibia bone strength of broiler chickens as reflected by the high latency to lie, bone ash and bone mineral content in general . Inclusion of Kalahari melon oil cake can be included in broiler diets by up to 10% with significant improvements in performance and bone development of broiler chickens.

6.2. Conclusions and recommendations

- Kalahari melon can be included in diet by up to 10 % without negatively affecting the performance of broilers.
- Enzyme inclusion on diets containing Kalahari melon oil cake was able to mitigate the effects of fibre content, that could have affected the growth performance and protein utilisation and the nutritional status of the broilers.
- Overall, Kalahari melon oil cake was shown improve bone development and welfare in broiler chickens
- Overall, Kalahari melon oil cake was shown to have a great potential as a protein source in broiler diets.
- The inclusion of enzymes in low protein high fibre diets such as the Kalahari melon oilcake is recommended to improve their utilisation in broilers.