MORULA KERNEL CAKE AS A DIETARY COMPONENT IN COMPLETE DIETS FOR TSWANA SHEEP

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GENERAL ABSTRACT

Lack of locally produced high quality feed ingredients for livestock, especially to supply protein is an impediment to increased productivity in Botswana. Driven by the need to fill this gap, a series of experiments were conducted to evaluate the nutritive value and utilisation of Morula (*Sclerocarya birrea*) Kernel Cake (MKC) in diets of sheep. Therefore, this thesis reports results from those studies and the first study chemically characterised MKC in comparison to Sunflower Seed Cake (SSC). MKC was found to be a rich source of gross energy, protein, phosphorus and magnesium. Lysine was the limiting amino acid in MKC when compared to SSC. However, cysteine and tyrosine were greater in MKC than in SSC. On the other hand, summation of essential amino acids values resulted in a similar value between the two seed cakes (MKC and SSC).

Dry matter *in situ* degradability of MKC indicated that it is highly degradable in the rumen compared to the dry matter of SSC. Nevertheless, the potential degradabilities of CP were similar for both SSC and MKC. But the protein of MKC contained higher bypass protein than that of SSC. The major fatty acids in MKC were found to be palmitic acid, stearic acid and oleic acid. Two growth trials and nitrogen balance studies were conducted to understand the impact of graded inclusion of MKC in lamb's complete diets. The trials indicated that MKC was a good source of RDP but also RUP to the host animal that elicited desired growth performance. Additionally, the inclusion of 12% MKC was noted to give the best results as it was not harmful to the lambs and promoted improved growth and normal blood metabolites.

A follow-up fattening trial was done using the best diet from graded level study. The diet with 12% MKC inclusion level was compared with other two conventional protein supplementary sources used in complete diets of lambs (SSC and Lucerne (*Medicago sativa*)). SSC and Lucerne are imported from abroad at high cost to the country and subsequently to the livestock farmers. The growth performance of fattened lambs across the three protein sources treatments was similar. However, nitrogen retention was better in Lucerne based diet and lowest in sunflower seed cake-based diet. In addition, the *Longissimus dorsi* muscle from lambs in MKC treatment was rich in oleic acid and the cooked meat steaks tended to have numerically better sensory attributes than in the other two treatments. MKC diet was found to have higher gross margin analysis value than Lucerne diet (commercial diet). Therefore, MKC is suited for use in diets of ruminants to supplement both energy and protein in areas where it is locally available. The inclusion level of MKC in total diet dry matter should not exceed 12% to

minimise amount of ether extract in the total diet. A study on effect of Morula oil on rumen fermentation or microbiota should be conducted or a study on *in situ* ruminal degradation of diets used in the graded MKC inclusion in lamb diets.

Key words: **lambs, morula kernel cake, fatty acids, sunflower seed cake**

SIMPLE SUMMARY

The search for new feed ingredients is of paramount importance as current feed resources may be inadequate especially for the projected livestock population increase in the near future. The current research findings have shown that MKC is a potential ruminant feed ingredient rich in energy, protein and some important minerals like phosphorus and magnesium. The amount of total essential amino acid in MKC is comparable to that of sunflower seed cake (SSC). However, MKC was found to be limited in lysine when compared to SSC. The residual oil from MKC was found to be rich in unsaturated fatty acid which may provide health benefits if deposited in meat products eaten by humans. Unsaturated fatty acids are known to lower blood plasma cholesterol levels in humans when compared to the saturated fatty acids.

Feeding MKC up to 12% total diet dry matter in lambs had no harmful effects on feed intake, nutrient digestibility, growth, blood metabolites and health. Additionally, a diet with 12% MKC in total diet dry matter when compared to commercial diet (with Lucerne) or complete diet with SSC, resulted in similar growth performance of lambs to these diets. The quality of meat from the lambs that ate diet with MKC was comparable to the meat quality of lambs that ate diets with conventional protein supplements (Lucerne and SSC) used in the current study. However, meat of lambs that ate MKC diet was very rich in oleic acid and cooked meat steaks tended to have enhanced sensory attributes when compared to the meat steaks from other dietary treatments. MKC had higher gross margin value than commercial diet (Lucerne diet) in the current study, thus suggesting that it might be used as an alternative protein or energy supplement to minimise feeding costs during fattening period for ruminants. Additionally, the feed costs accounted for 61% of the total cost of production. The amount of MKC included in total diet for ruminant animals should not exceed 12% to minimise fat content in the total diet to avoid compromising nutrient digestibility especially for fibre.

DECLARATION

I, Leonard Boitumelo Baleseng, declare that this thesis hereby submitted for the degree of Doctor of Philosophy in Animal Science (Ruminant Nutrition) at the Department of Animal Science, Faculty of Animal and Veterinary Sciences, BUAN, is my original work and has not been submitted to another University for an award of a degree or diploma. All the information obtained from various sources that I have used have been acknowledged accordingly by a complete list of references.

Author's Signature Date

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APPROVAL

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LIST OF ABBREVIATIONS

a: rapidly degradable fraction

AD: apparent digestibility

ADF: acid detergent fibre

ADG: average daily gain

ADL: acid detergent lignin

AFRC: agricultural and food research centre

ANOVA: analysis of variance

b: slowly degraded fraction

BCS: body condition score

BEN: basal endogenous nitrogen

BUAN: Botswana university of agriculture and natural resources

BWP: Botswana pula (currency)

c: degradation rate of fraction “b”

CRD: completely randomised design

CP: crude protein

CD: commercial diet

DE: digestible energy

DAR: department of agricultural research

DM: dry matter

DMI: dry matter intake

DP: dressing out %

EBW: empty body weight

ED: effective degradability

EDTA: ethylene diamine tetraacetic acid

EE: ether extract

FAME: fatty acid methyl esters

FCR: feed conversion ratio

GC: gas chromatography

GE: gross energy

GLM: general linear model

GM: gross margin

HCW: hot carcass weight

ME: metabolisable energy

MKC: morula kernel cake

MKD: morula kernel cake diet

MUFA: monounsaturated fatty acid

MP: metabolisable protein

N: nitrogen

NB: nitrogen balance

NDF: neutral detergent fibre

NFTRC: national food technology and research centre

NFE: nitrogen free extract

NR: nitrogen retention

NRC: national research council

OM: organic matter

PD: potential degradability

PUFA: polyunsaturated fatty acid

RDP: rumen degradable protein

RMSE: root mean standard error

RUP: rumen undegradable protein

SAS: statistical analysis system

SSC: sunflower seedcake

SCD: sunflower seedcake diet

SFA: saturated fatty acids

UFA: unsaturated fatty acid

USD: American dollar (Currency)

CHAPTER 1

1.0 Introduction

1.1. General Introduction

Ruminant livestock naturally survive by eating herbaceous plants, grasses and sometimes browse plants. Uddin et al. (2015) explained that symbiotic rumen microbes degrade ingested nutrients in the rumen to produce volatile fatty acids and synthesise microbial protein as an energy and protein source respectively for the host animal. However, ruminant livestock are also versatile as they can eat either an all-roughage diet or a partially grain-based diet (Stock & Britton, 1991). According to Herrero et al. (2013), globally, total biomass consumed by livestock in the year 2000 was 4.7 billion tonnes of which 79% were consumed by ruminant animals. This data signifies the importance of livestock in the livelihoods of humankind as they can convert cellulose into valuable animal food protein like meat, milk and eggs. It also points to the fact that, without ruminants, this biomass, some from arable agriculture, may accumulate and result in pollution and moribund or lack of vigour in rangelands. Nowadays, most livestock feeding systems place a lot of emphasis on performance measures like weight gains, feed efficiency and carcass quality that are of great economic importance (Bures & Burton, 2012). On the other hand, it is acceded that sustainable agriculture must be practised in the production of livestock food proteins to meet human demand with minimal damage to the environment (Davis et al., 2016). It is envisaged that meat production in Sub-Saharan Africa (SSA) will increase by 2.7% per annum until year 2030 (Desiere et al., 2018) and obviously, this projection will have a bearing on livestock feed demand in SSA.

In tropical and subtropical countries, most ruminants are raised on natural pastures to get nutrients needed for maintenance, growth, reproduction and fight against disease (Madibela et al., 2018). However, nutrition is a major limitation to livestock production, especially in countries like Botswana, due to inadequate feed resources and seasonal fluctuation of available biomass and quality (Mine et al., 2002). This is attributable to the semi-arid climate that is characterised by inadequate and highly variable rainfall (Batisani & Yarnal, 2009) that creates a risky environment for plant growth in general. In recent times, shortage of quality animal feed for livestock has been aggravated by climate change as evidenced by changes in the onset of rainfall season (Kgosikoma et al., 2018), hence negatively affecting productivity of natural pastures. Therefore, the negative effects of climate change are also presenting new challenges

to livestock farmers. Farmers who rely on dry land farming to cultivate fodder crops may be subjected to delayed cropping season or complete crop failure in worst-case scenario. This will have spill-over effects on availability of supplementary feed to livestock.

Forages in the tropics and subtropics are usually low in available energy and protein, especially during the dry season. Evidence from past research (Lanyasunya et al., 2005) suggests that deficiency in energy, protein and minerals (phosphorus) in livestock results in poor growth, low conception rate and poor body condition. Therefore, some farmers use pricy conventional oil seedcakes and cereal grains to supplement their livestock (Habib et al., 2013). Supplementation of low quality forages with oilcakes is a simple way to augment the nutritional content of poor forages. Oil seedcakes such as soybean meal, cottonseed meal and sunflower seed meal have a crude protein (CP) range of 26% (sunflower meal) to 50% (soybean mean) (NRC, 2000). The rate of degradation of protein in the rumen for soybean meal is 6.8%/h (Todorov et al., 2016), cottonseed cake 5%/h and sunflower seed meal 5%/h (Kamalak et al., 2005). Lately, most feeding systems, especially in developed countries, have been using feed formulations that satisfy protein requirements of microbes (RDP) and host animal (RUP), thereby maximising animal performance, reducing gaseous nitrogen loss to the environment and increasing economic profitability. The amount of nitrogen excreted through urine or faecal route indicates the amount of nitrogen wasted but could be used by the animal for protein accretion. Therefore, excessive feeding of protein should be avoided to reduce environmental pollution by proper feed formulations and adoption of feeding techniques like phase feeding. Additionally, the challenge in most developing countries is lack of information on rumen degradation kinetics and nutritive value of local feeds (Habib et al., 2013). With increasing human population, it is prudent to use livestock feeding practises that will improve animal productivity and produce quality animal products like meat and milk at the most cost-effective way while promoting environmental sustainability.

Modern consumers of animal protein demand meat of high quality from the meat industry. According to Sapkota et al. (2007), ingredients used in animal feed have an influence on animal food products and ultimately on human health. Santos et al. (2019) explained that meat is appreciated by its sensory attributes, nutritional composition, technical parameters (pH, water holding capacity), absence of chemical and microbial residues and ethical considerations (animal welfare) during slaughter. This expectation by modern consumers still needs to be met,

even when new feed products are introduced in the production system in order to promote sustainable consumption.

To promote sustainable livestock production, there is need to use alternate non-traditional local feed ingredients in supplementary feeding strategies. The identified potential feed ingredients should be sustainable, cheaper and nutritious to livestock. According to Ben et al. (2004), the increasing human demands for diversity of foods have resulted in the availability of several agro-industrial by-products that are available in African and Asian countries. Some of these agro-industrial by-products like MKC could be candidates in livestock feeding but are still not being fully taken advantage of. Morula (*Sclerocarya birrea*) Kernel Cake (MKC) is rich in protein, energy and minerals (Malebana et al., 2017). Other researchers (Mlambo et al., 2011a; Mdziniso et al., 2016; Mthinyane et al., 2017) have demonstrated that it has potential as a feed ingredient for livestock and poultry.

1.2. Problem statement

The production indicators of ruminant livestock in Botswana are not satisfactory in terms of birth rate, off-take and death rate (Statistics Botswana, 2019). Nutrition is the major factor curtailing animal production especially that Botswana's climate is semi-arid. Therefore, during the dry season, natural pastures are dormant and there is hardly any precipitation. This results in pastures being low in nutritive content and in short supply. Most livestock farmers at this time of the season are obliged to supplement their livestock with pricy imported conventional feed supplements to augment meagre nutrients supplied by dormant grasses from natural pastures. Therefore, a search for locally available source of supplementary feeds is necessary. Potential source of such supplementary feeds includes local plants and their by-products. For instance, the use of MKC in livestock and poultry diets is uncommon mainly because of paucity of information on its nutritive value, digestion and utilization by the animal. Additionally, to date no attempt have been made locally to incorporate MKC in animal feed formulations. However, this is very important to explore especially that MKC might help lower the cost of livestock production including positively affecting product (meat) quality.

1.3. Justification

Feed costs account for 60-70% of the total cost of production (Thirumalaisamy et al., 2016). Therefore, the continuous search and nutritional characterisation of cheaper and locally available potential protein and energy supplements is a welcome development for the livestock

farming community in general. In Botswana, the banning of use of Blood meal (BM) and Meat and Bone Meal (MBM) as a livestock feed ingredient from Botswana Meat Commission (BMC) abattoir created a shortage in protein source that disadvantaged local farmers and its effects are still evident. A case of Bovine Spongiform Encephalopathy (BSE) reported in the UK in 1996 (Brookes, 2001) resulted in the discontinued use of MBM as feed ingredient in European Union (EU) countries and other countries selling their beef or livestock meat products to the EU countries. Discontinued use of MBM resulted in most local farmers supplementing their livestock with plant-protein supplements or energy supplements obtained from South Africa and other neighbouring countries which came at a cost.

Most of the plant protein supplements currently imported into Botswana are soybean meal, cottonseed cake, sunflower seed meal and groundnut cake (Ministry of Agriculture; personal communication). According to Coffey et al. (2015), it is envisaged that the world's population will reach 9 billion people by 2050. Therefore, an increased human population will obviously result in increased demand for animal food proteins such as eggs, milk and meat. In Botswana, the majority of livestock farmers are resource limited practicing communal farming which is anchored on free feed obtained from natural pastures. Therefore, most of them cannot afford to buy conventional plant protein sources sold by local feed suppliers or retailers. This necessitates for local animal scientists to identify novel protein or energy feed ingredients that can be used to match the animal feed demand envisaged to produce animal food protein (meat or eggs) needed to feed the projected high human population in 2050.

MKC is a by-product from local oil extraction from morula fruits (seed) that are sourced from communal women groups who harvest morula fruits to sustain their households. The resultant cake is an alternative feed ingredient that should be evaluated for use in the diets of ruminants as a protein source as per preliminary studies done by other researchers (Mlambo et al., 2011a; Malebana et al., 2017). Most potential feed resources that are by-products that come from local industrial processing like MKC are not fully exploited and sometimes are likely to become an environmental hazard if they are not disposed of properly. In fact, Seo et al. (2015) stated that in the past, by-products from processing crops and food products have always received much attention as feed alternatives because of the consistent production. Hence, local availability of MKC will be driven by growth of the "emerging oil extraction industry" which currently has a strong appetite for growth. Therefore, there is need to rigorously investigate and characterise

MKC to determine both its potential use and/or limitation in complete ruminant diets. This will not only promote livestock productivity but also sustainable utilisation and conservation of natural resources at the same time supporting rural development where resources are located.

1.4. Objectives

1.4.1. General objective

The overall objective of the research was to evaluate the use of MKC as an alternative protein source in complete diets of ruminants. The specific objectives of the study were:

- to determine the chemical composition and rumen degradation of MKC in comparison with sunflower seed cake (SSC).
- to evaluate animal performance and nitrogen balance with MKC as a sole protein source in complete diets of sheep.
- to investigate the effects of graded inclusion of MKC on body weight, morphometric measurements and blood metabolites of sheep.
- to investigate the effects of inclusion of MKC in diets of sheep on growth, meat attributes and gross margins in comparison to other conventional protein sources.

1.5. Hypotheses

- There is no significant difference in the chemical composition and rumen degradation of MKC in comparison with SSC.
- There is no significant difference on animal performance and nitrogen balance for sheep fed graded levels of MKC as a sole protein supplement in complete diets of sheep.
- Inclusion of graded levels of MKC in sheep complete diets elicits similar performance on body weight, morphometric measurements and blood metabolites of sheep.
- Inclusion of MKC at 12% of total diet dry matter elicits similar animal performance, meat attributes and gross margins as the other conventional protein sources (sunflower seed cake or Lucerne).

CHAPTER 2

2.0 Review of literature

2.1. Introduction

Non-conventional feeds, as defined by Aruwayo (2017), are feed ingredients that are traditionally not used for feeding livestock and are relatively new to many farmers or local feed manufacturers. The diets for ruminants in most African and Asian countries are based on rangelands, crop residues, agro-industrial by-products and other non-conventional feed resources (Ben et al., 2004). The search for new feed ingredients is very important as the current biomass base or feed resources may not be enough for envisaged increase of livestock population. On the other hand, the use of grains in livestock and poultry diets creates a competitive conflict with human needs and this exacerbates the already inadequate animal feed supplies (Vasta et al., 2008).

Despite the challenge of limited feed resources and pricy conventional protein supplements in most African countries, consumers still expect to get healthier livestock food products (meat and milk) in the market. As an example, meat fatty acid composition is very important to the health of consumers and meat colour determines the inclination to purchase of the product from the retail outlets (Vasta et al., 2008). Therefore, this calls for animal scientists to look for alternative feed ingredients with a good nutritious profile suited for proper animal growth and a comparatively cheaper production cost. For instance, one promising non-conventional feed resource like hemp (*Cannabis sativa*) cake, has been nutritionally profiled (Semwogerere et al., 2020) and found to be rich in crude protein (34.1%) and other nutrients. Ingredients with high nitrogen usually supply rumen microbes with enough nitrogen that is needed for their use to digest cellulose, multiply and later supply the host animal with microbial protein as they later die. In the current research, MKC (*Sclerocarya birrea*) as promising feed ingredient is investigated. Therefore, the current literature review explores general principles of feed utilisation on the animal, summarises the nutritional profile of main conventional protein supplements, non-conventional protein supplements, concepts on meat quality and feed evaluation techniques, summarises preliminary studies done on MKC and lastly discusses the economics of feeding.

2.2. Nitrogen (N) metabolism in ruminants

McDonald et al. (2011) explained that feed proteins ingested by ruminants are hydrolysed into peptides and amino acids by rumen microbes. However, some amino acids are broken down into ammonia, organic acids and carbon dioxide. Also, non-protein nitrogen from feed is also converted to ammonia in the rumen. The rumen ammonia, peptides and free amino acids are then used by rumen micro-organisms to make microbial proteins. Excess rumen ammonia is absorbed through the rumen wall and transported to the liver where it is converted to urea. Urea is then transported to the kidney for excretion and some of the urea is recycled back to the rumen through the rumen wall and saliva produced from salivary glands (McDonald et al., 2011). Rumen microbes, as they grow and multiply in the rumen, die and/or get washed to the lower gut where they provide the host animal with microbial protein which is digested and the amino acids are absorbed in the small intestine together with rumen bypass protein as metabolisable protein (NRC, 2000; McDonald et al., 2011). These amino acids which constitute 50% to 80% true protein are absorbed in the small intestine and used for various functions like maintenance and production.

2.3. Metabolisable protein

In an effort to increase feeding efficiency, modern feeding systems partition protein requirements into those of rumen microbes and the host animal. Protein that is absorbed in the small intestine is called metabolisable protein and it is a summation of digestible bacterial crude protein, digestible rumen undegradable protein (RUP) and endogenous protein (NRC, 2001). Therefore, metabolisable protein (MP) is used by the animal for maintenance, growth, gestation and lactation (Lardy et al., 1997) and has been found to positively modulate immune function in parasitised ewes (Madibela et al., 2009). The nutrients required by rumen microbes for growth and multiplication include carbohydrates, proteins, sulphur (trace minerals) and vitamins (Uddin et al., 2015). According to Plascencia and Zinn (2014), microbial protein contains approximately 50% true protein of which 80% is digested in the intestines. The microbial protein is a good source of methionine and lysine. Consequently, understanding the kinetics of the ruminal degradation of various feed proteins including MKC (*Sclerocarya birrea*) as a potential feed ingredient is crucial in formulating diets with an adequate amount of rumen degradable protein (RDP) and RUP for rumen microbes and the host animal respectively (NRC, 2001). Overfeeding of RDP or MP produces surplus ammonia which is absorbed and converted to urea in the liver and finally excreted in the urine. It is very important

that protein in the animal diets meet but does not exceed animal requirements to avoid nitrogen pollution and unnecessary escalation of feed expenses. Nitrogen excreted to the environment signifies valuable nitrogen that could have been used for growth or lactation by the animal.

The amount of RDP and RUP (as a % of crude protein) in various protein supplements is different. Likewise, the degradation of protein from various feed sources as stated in NRC report (2001) is limited by protein 3-dimensional structure, differences in intra and inter-molecular bonding, inert barriers such as cell walls and anti-nutritional factors like tannins. Moreover, rumen degradability of feed proteins is dependent on microbial proteolytic activity, ruminal pH and ruminal retention time. Ruminal retention is dependent on the amount of NDF in feed (NRC, 2001).

According to Hacker (1981), the number of microbes produced in the rumen depends in part on the amount of carbohydrates (cellulose, hemicellulose, starch and soluble sugars) fermented and the amount of adenosine triphosphate (ATP) made available to the microbes for multiplication and growth. Therefore, synchronising the supply of fermentable carbohydrates and protein (RDP) in the diet is very important to promote microbial protein synthesis (NRC, 2001) which will promote increased dry matter intake and ultimately improved animal performance.

Lardy et al. (1997) concluded that, feeding the correct type of supplement at the proper time, may lower the cost of supplementation coupled with adequate animal performance. As an example, Anderson et al. (1988) demonstrated the importance of supplementary RUP to steers grazing smooth brome (*Bromus inermis*) pastures during the spring and fall season. The treatments were control supplement with corn starch and molasses (energy supplement) and RUP supplement with blood meal and corn gluten meal or blood meal, corn gluten meal and soyhulls. The steers received 582g supplemental dry matter (DM) per day with levels of RUP as; 0 kg/day, 0.11 kg/day, 0.23 kg/day and 0.34 kg/day in four treatments. A linear increase in ADG was observed with an increasing level of RUP. The authors observed that, even if actively growing cool-season grasses had a high protein content, they were deficient in metabolisable protein due to insufficient RUP but over supplied the RDP. MKC as a potential feed ingredient should be characterised for RDP and RUP to be effectively used in ruminant feeding systems.

Understanding of both protein metabolism and energy metabolism is very important as both nutrients (energy and protein) are the main drivers of animal productivity.

2.4. Energy metabolism in ruminants

In tropical and subtropical countries, energy utilisation is one of the limiting factors affecting livestock production, especially during the dry season. This is because as the plant matures and the season transits into the dry season, more lignin is laid down and energy gets locked in the lignocellulosic biomass. Diets poor in energy supply affect animal biological processes culminating in loss of weight, poor reproduction and flock reproduction efficiency. MKC has anti-nutrients such as saponins and tannins (Malebana, 2018) which, when supplied at low levels in animal diets, may positively enhance energy utilisation by modifying rumen fermentation. Energy is produced during the oxidation of feed ingested by the animal.

Gross energy (GE) is the total amount of energy in a given feed (NRC, 2000; McDonald et al., 2011). However, during digestion of the feed by the animal, some energy is lost in the form of faeces. The difference between GE and faecal energy (FE) is called digestible energy (DE). Further losses of energy occur in the form of gaseous (carbon dioxide and methane) energy and urine energy during metabolism. The energy lost because of methane production is about 5 to 15% of the digestible energy in feed (Kataria et al., 2015). Therefore, the reduction of enteric methane production may generally improve livestock production by cutting energy inefficiency.

According to Sejian et al. (2011), understanding the effects of diet on enteric methane production is very important to develop programs based on diet manipulation as methane mitigation strategies. Kataria et al. (2015) stated that dietary oils like coconut oil, sunflower oil and mustard oil have been found to mitigate enteric methane production in ruminants. Vegetable oils have polyunsaturated fatty acids that are toxic to cellulolytic microbes and protozoa that produce hydrogen used in methanogenesis. Therefore, inhibition of methanogenesis will promote high production of propionate instead of acetate and methane (Sejian et al., 2011) hence increased energy supply. Methane gas is classified as a greenhouse gas and a lot of research is on-going looking at the use of plant extracts, organic acids and genetic selection of animals (Sejian et al., 2011) to mitigate enteric methane production in ruminants. Furthermore, the difference between DE and gaseous plus urinary losses is called

metabolisable energy (ME). Also, additional losses are incurred from ME because animals continuously produce heat during rumination or mastication. Therefore, the difference between ME and heat of increment (HI) produces net energy (NE) which is used for maintenance or production (McDonald, et al., 2011). Residual oil/lipids from Morula kernel after extraction may provide an opportunity to manipulate rumen fermentation to curtail methane yield.

2.5. Fat in Ruminant diets

Lipids play an important role in the diets of ruminants, mainly because they increase the energy density of the diet (Palmquist & Jenkins, 2017) as well as help with the absorption of fat-soluble vitamins (Cetingul & Yardimci, 2008). Moreover, addition of fats in animal diets may alter lipid profile of meat and improve its nutritional characteristics in terms of essential fatty acids such as alpha-linolenic acid, linoleic acid, docosahexaenoic acid and eicosapentaenoic acid. Therefore, the use of MKC in ruminant diets may also bring additional benefits of producing meat products rich in oleic acid, as reflected in composition of MKC, as profiled by Malebana et al. (2017). Oleic acid is known for reducing cardiovascular diseases in humans (Xu et al., 2020). Most animal forages, grains, by-products and seedcakes used for livestock feeding contain some amount of lipid. Lipids or fats are a group of substances that are insoluble in water but soluble in organic solvents like chloroform, hexane and certain alcohols (Nelson & Cox, 2005). Most naturally occurring fats in forages are in the form of triglycerides, phospholipids and glycolipids (Russel, 2002). Triglyceride is a major lipid type that occurs in oilseed cakes, animal fats, cereal grains and other by-products feed. Triglycerides comprise of a single glycerol molecule with three carbons linked to three fatty acid molecules by ester linkages. Phospholipids are minor components of most feeds and they form cell membranes of all animal cells. Phospholipid is a three-carbon glycerol molecule attached to two fatty acid molecules and one phosphate group (Nelson & Cox, 2005). Glycolipids are similar to triglycerides except that they have two or more sugars linked to glycerol instead of fatty acids (Nelson & Cox, 2005).

Ruminants consume feeds with ample unsaturated fatty acids but produce meat and milk that are highly concentrated with saturated fatty acids (Duckett et al., 2009). Lipids in the rumen undergo lipolysis and biohydrogenation. Lipolysis is the hydrolysis of ester linkages of glycerol and fatty acids by ruminal microbes to produce volatile fatty acids from glycerol and liberate free fatty acid (Russel, 2002; Bremer, 2014). The released fatty acids in the rumen are then

transported to the small intestine, which is the primary site for absorption of the fatty acids. Biohydrogenation is the process of saturating the double bonds of the unsaturated fatty acids in the rumen with hydrogen atoms, thereby producing single bonded saturated fatty acids that will flow to the small intestine. Fatty acids with double bonds are more toxic to the rumen bacteria than saturated fatty acids (Russel, 2002). According to Russel (2002), the rumen has two distinct groups of bacteria, namely "A-group" and "B-group" which are responsible for biohydrogenation. The "A-group" converts linoleic acid (C18:2) to oleic acid (C18:1) while the "B-group" substantially converts oleic acid (C18:1) to stearic acid (C18:0) as such stearic acid is the major saturated fatty acid (SFA) in the digesta leaving the rumen to the intestine.

The digestibility of fats in the intestine is determined by the amount, the chain length, the degree of unsaturation of dietary fatty acids and the extent of rumen biohydrogenation (Chilliard, 1993). The forms of lipids entering the jejunum range from free fatty acids attached to feed particles, microbial phospholipids, triglycerides and glycolipids (Bauman et al., 2003). The esterified lipids will be hydrolysed by intestinal and pancreatic lipases (Bauman et al., 2003). Fat absorption occurs in the jejunum after micelle formation. Micelle formation is made possible by secretions from the gall bladder (bile salts and lecithin) and pancreases (phospholipase) (Bauman et al., 2003). The moment that micelles are formed, they are absorbed into the epithelial cells of the jejunum by passive diffusion (McDonald et al., 2011). However, the bile salts are recycled and transported to the liver (Pond et al., 1995; McDonald et al., 2011). The absorbed fat is transformed into chylomicrons which pass into lacteals of villi, diffuse into lymph and finally enter the bloodstream through the thoracic duct. Furthermore, medium and short chain fatty acids are usually absorbed into portal blood stream without undergoing the processes of both micelle formation and bile secretions (McDonald et al., 2011).

Nevertheless, in ruminants, diet, age and fat depot determine fatty acid composition in various storage tissues (Smith & Smith, 2016). Also, Daley et al. (2010) stated that there is no consistent difference in the total SFA content of beef between grass-fed and grain-fed cattle. However, Richardson (2006) noted that concentrated feeds tend to reduce the amount of valuable omega-3 fatty acids in the meat and increase the less valuable omega-6 fatty acids when compared to the grass or all forage diet. Additionally, dietary fat content can affect the digestibility of various feed ingredients. Dietary fat concentrations should not be more than 3% of diet dry matter (DM) in forage-based diets (Hess et al., 2007) as greater concentrations in

the diet might impede or suppress fibre digesting microbes in the rumen (Russel, 2002). In high concentrated diets, diet fat concentration can be as high as 9.4% dry matter (DM) inclusion before fibre digestion in the rumen is drastically affected (Atkinson et al., 2006).

2.5.1. Importance of fatty acids

Fatty acids minimise methane driven energy loss as they also act as hydrogen sink during rumen biohydrogenation (Chilliard, 1993). In some animals, triglycerides that are stored under the skin serve as an energy store and insulation against low temperatures. Arachidonic acid (C20:4) is involved in the formation of blood clots and gastric acid secretion (Nelson & Cox, 2005). Additionally, diets rich in linoleic acid and arachidonic acid result in better follicular development and increase ovulation rate in female animals (Centingul & Yardimci, 2008). On the other hand, dry matter intake depression in ruminants is caused by fatty acid long chain length and the degree of unsaturation (Palmquist & Jenkins, 2017). Thus, long chain fatty acids (C18 fatty acids as an example) inhibit the growth of cellulolytic bacteria and fungi responsible for fibre digestion (Slavov, 2017). Plant protein supplements, besides having varying levels of fat content, also have phytochemicals that affect animal metabolism either positively or negatively.

2.6. Phytochemicals

Phytochemicals are feed additives of plant origin and sometimes they are referred to as plant secondary metabolites (Tedeschi et al., 2021). The phytochemicals type and amount vary from one plant species or genera to the next (Kennedy & Wightman, 2011). The plants use these secondary metabolites for defence against herbivory therefore they are not used by the plant as a primary metabolic requirement. In this regard, the majority of phytochemicals are found in most parts of the plants including leaves, seeds, fruits, bark and root (Jeronimo et al., 2016). According to Valenzuela-Grijalva et al. (2017), phytochemicals may affect animal metabolism by using any of the following mechanisms: improving animal feed intake; modulating ruminal fermentation; improving nutrient digestion and absorption or reducing intake and digestibility of protein. Additionally, Valenzuela-Grijalva et al. (2017) stated that the proposed mechanism of action is dependent on a given phytochemical (its structure), dosage level and animal species ingesting the phytochemical. The phytochemicals are categorised as phenolics, terpenes and alkaloids (Villalba et al., 2016).

Tannins are phenolic compounds found in forages, shrubs, cereals and medicinal herbs with a concentration ranging from < 3% to 5%, as recommended for animal diets (Aboagye & Beauchemin, 2019). Tannins are classified into hydrolysable and condensed tannins. Hydrolysable tannins are soluble in water and get easily hydrolysed by animal enzymes when compared to condensed tannins. Therefore, hydrolysable tannins, when released by enzyme hydrolysis in the rumen, may enter the blood and cause organ damage (Jeronimo et al., 2016). In contrast, condensed tannins are resistant to rumen enzymatic hydrolysis and hardly cause organ damage. Condensed tannins in low concentrations in animal diets reduce bloating, improve ADG, decrease urinary nitrogen, decrease methane production and reduce internal nematode load. On the other hand, in high concentrations, condensed tannins decrease DMI, protein digestibility and body weight (Aboagye & Beauchemin, 2019). This is attributable to high tannin content in the diet binding to either free proteins, organic matter, carbohydrates or digestive enzymes thereby decreasing nutrient digestion in the gastro intestinal tract (Aganga and Tshwenyane, 2003).

Terpenes, as described by Tedeschi et al. (2021), are bitter in taste, non-ionic, non-volatile emulsifiers and are structurally diverse saponins of low molecular weight. According to Tekeli et al. (2007), saponins are terpenes that have properties similar to soaps and detergents. Patra and Saxena (2009) concluded that saponins act as rumen modifiers as they greatly change composition of rumen microbes which may increase both efficiency of microbial protein synthesis and nitrogen retention. Tekeli et al. (2007) explained that the detergent action of saponins mainly kills rumen protozoa, thereby modifying rumen fermentation through increased population of both bacteria and fungi. However, Patra and Saxena (2009) stated that saponins can also selectively kill some bacteria, protozoa and fungi which may affect rumen metabolism positively or negatively. In fact, defaunation of protozoa has spin-off effects of reducing enteric methane production as a result of interfering with symbiotic relationship between methanogens and protozoa (Patra and Sexena, 2009). Das et al. (2012) pointed out that saponins may boost the immune response in animals or result in haemolysis of red blood cells when ingested by animals at toxic level.

Alkaloids, as described by Kennedy and Wightman (2011), are cyclic nitrogen containing compounds found in over 20% of the plant species. Alkaloids functionally act as feeding deterrents and toxins to herbivores by interfering with a variety of neurotransmitter systems,

causing liver damage, muscle cramps and death (Tedeschi et al., 2021). However, alkaloids also have beneficial properties like being antioxidants, cancer-preventative and anti-inflammatory (Tedeschi et al., 2021). Livestock are known to limit the intake of plants with alkaloids as they have a bitter taste.

Essential oils are not needed daily in animal diets as the name may suggest and they are not oils per se. Therefore, Tekeli et al. (2007) defined essential oils as a complex mixture of several different chemicals that are responsible for the characteristic smell of spices. Furthermore, Tedeschi et al. (2021) stated that essential oils control rumen fermentation by promoting the growth of the microbial population that improves nutrient utilisation while reducing fermentative waste products such as methane and ammonia. However, some essential oils can also have a negative effect on the growth of fibre digesting rumen microbes. Chaudhary et al. (2016) demonstrated that *in vitro* thyme oil supplementation reduced the population of both methanogens and cellulolytic bacteria. Therefore, reduction of cellulolytic bacteria negatively impacted *in vitro* dry matter digestibility (IVDMD). On the other hand, Ahmed et al. (2014) reported that supplementation with graded inclusion of essential oil blends (eucalyptus, cinnamon, peppermint, thyme and lemon) in complete diets of sheep did not affect digestibility of dry matter and organic matter. Additionally, Amin et al. (2021) stated that essential oils are generally considered as potential alternatives to antibiotics since they have antimicrobial, anti-inflammatory, antiparasitic and immune enhancing properties.

All these phytochemicals, as they are part of plants make-up in rangelands, influence voluntary feed intake in grazing ungulates.

2.7. Voluntary feed intake

The amount of biomass consumed daily by the animal is crucial as it determines the amount of nutrients availed to the animal for proper growth, development and productive purposes. Proper feeding of livestock will avoid either malnourishment or obesity and, in the process contribute to minimising feed costs (NRC, 2001). Factors controlling voluntary feed intake can be categorised into feed related factors, animal factors and environment factors (McDonald et al., 2011) and are as follows:

2.7.1. Feed factors

- Forages rich in cell wall material (NDF) are degraded slowly in the rumen which leads to low daily DMI (McDonald et al., 2011). High energy diets are controlled metabolically. For instance, the presence of fermentation products such as acetic acid and propionic acids may limit DMI by stimulation of cholecystokinin hormone from hypothalamus which will make the animal to stop eating (Van Soest, 1994).
- Sward structure: according to Decruyenaere et al. (2009), sward characteristics such as leaf blade morphology, thickness of cuticle, leaves size, stem physical properties and amount of dead biomass can stimulate or limit animal foraging behaviour. Additionally, Givens et al. (2000) noted that plant density and height determine intake through their effect on ease of holding and bite size. Also, forage contaminated by animal dung reduces its intake (Preston & Leng, 1987). Leaf to stem ratio also plays a key role in feed intake as some animals like cattle prefer to eat leaves over twigs (Givens et al., 2000).
- Nutrient deficiency: Fisher (2002) stated that dietary nutrient imbalances can lead to an increase in feed intake in an attempt to compensate for limiting nutrients and on the other hand getting rid of surplus nutrients. Additionally, feed intake may be depressed when rumen microbes are starved of proteins, minerals (especially sulphur, phosphorus, sodium and cobalt) and vitamins which they use when digesting fibre (Zereu, 2016) in the rumen.

2.7.2. Animal factors

- Rumen capacity: the amount of feed ingested by the animal is limited by the volume of the reticulo-rumen. Therefore, bulky feeds are consumed until reticulo-rumen capacity is reached (Pond et al., 1995). Physical regulation is anchored on fill effects. Feeds with low digestibility negatively affect feed intake as they take long in the rumen to be digested and have a slow passage rate (NRC, 2001).
- Physiological status of the animal: Zereu (2016), in a review paper, stated that feed intake in growing and pregnant animals is very high in order to meet the high nutrient demand. However, in ruminants feed intake increases in early pregnancy as the foetus grows but in the last trimester feed intake declines due to reduced space available for rumen expansion (McDonald et al., 2011). Lactating ruminants achieve high DMI

during the lactation period. In fact, lactating animals consume 50% more herbage than non-pregnant, non-lactating animals (Hacker, 1981).

- Fatigue: Preston and Leng (1987) stated that ruminants get tired or are fatigued from searching for feed, ingesting, chewing and ruminating their feed. Fibrous feed may be ruminated several times to extract the nutrients and in the process, consumption of fresh feed will be delayed until rumination is completed.

2.7.3. Environmental factors

- Diseases and parasites: according to the Merck Animal Health report (2017), parasites' impact on animals includes a decrease on feed intake or nutrient absorption. Therefore, reduced feed intake results in the animal ingesting less protein, energy, vitamins and minerals, all of which are very important for proper growth or reproduction of animals. In addition, Cowley et al. (2019) stated that diseased animals have decreased feed intake and nitrogen retention due to the response of the animal's immune system to inflammation by mobilising essential and non-essential amino acids from the body to fight disease, as an example.
- Temperature: high temperatures reduce feed intake in animals but during cold temperatures, animals consume a lot of feed (Hacker, 1981). In fact, Decruyenaere et al. (2009) stated that climatic conditions influence foraging behaviour of ruminants. At temperatures higher than 25^{o}C, ruminants graze early in the morning or at night which may reduce normal daily feed intake. Therefore, as stated in NRC (2000), feed intake increases as the temperature falls below the thermoneutral zone and decreases above that zone. The thermoneutral zone is temperature between the lower critical temperature and upper critical temperature comprising of cool zone, thermal comfort and warm zone.
- Day length: day length affects the pattern of grazing. NRC (2000) stated that the effect of day length on feed intake is not yet fully understood. However, voluntary feed intake tends to increase by 1.5% to 2% during the long day length when compared to short day length. For instance, McDonald et al. (2011) pointed out that sheep consume less feed during short day length and this coincides with periods of shortage of fodder during winter in the tropics.

Since the magnitude of feed intake in conjunction with nutrient concentration determines nutrient supply, the amount of nutrient absorbed will therefore influence productive processes

such as growth. There is limited research in feeding MKC to ruminants; therefore, there is lack of information on its influence on feed intake and subsequent growth.

2.8. Growth

Hudson et al. (2010) define growth as an increase in height, length or body weight change through the processes of hyperplasia and hypertrophy. Owens et al. (1993) also stated that cumulative weight (growth) in animals plotted against time (age) follows a sigmoid curve comprising of the following sequential stages of growth: prepubertal, self-accelerating, post pubertal and self-inhibiting stage. Body weight and physical body measurements (morphometric) are important growth indicators in livestock. According to Taye et al. (2016), measured animal weights are very important as they are used in decision-making relating to the application of medication, supplementary feeding and marketing. As stated by Abd-Allah et al. (2018), parameters used for body physical measurements can include body length, wither height, rump height, heart girth, head length and neck circumference.

The body physical measurements technique is simple and less expensive to practise to estimate animal growth based on external conformation. Cam et al. (2010) and Agamy et al. (2015) stated that skeletal growth is estimated using body length, wither height and rump height. In contrast, tissue growth is estimated using heart girth, paunch girth and neck circumference (Cam et al. 2010). Regarding organ's growth, Pond et al. (1995) indicated that, during growth, various organs grow at different rates. In agreement, Suliman et al. (2007) stated that the head, hide, four (4) feet, alimentary tract, heart and liver are early maturing body parts and are less influenced by diet. In contrast, body fat depots are late maturing tissues and as such are mainly influenced by dietary energy density. Therefore, when evaluating a potential feed ingredient like MKC, it is very critical to collect data on various growth parameters of an animal to ascertain how nutrients provided by MKC effect growth in a holistic manner. Lima et al. (2018) reported diet effect on nutrient intake, lamb weight and body physical measurements in a study of Santa Ines lambs. The treatments in their growth study were sunflower seed cake with inclusion levels of 0%, 10%, 20% and 30% of total diet DM, replacing soybean meal in a totally mixed ration (TMR). Sunflower seed cake inclusion in the diet linearly decreased the intake of dry matter, crude protein and total digestible nutrients. There was also a linear decrease on hot carcass weight and cold carcass weight. The researchers attributed the reduction in production parameters to an increase in fibre and lipid content as the sunflower cake inclusion level

increased in the TMR diets. Besides the influence of feed intake alluded above, other factors come into play in assessing growth in ruminants.

Factors affecting growth in animals

- Breed: the genetic composition of an animal determines its potential growth rate. Different breeds have different mature body size (Owens et al., 1993; Alemneh & Getabalew, 2019). Hutu et al. (2020) explained that growth is determined by numerous genes such as those dealing with growth factors and other genes that give an animal tolerance and resilience to certain diseases. However, Owens et al. (1993) stated that factors like nutrition or environment, if they are of suboptimal standard, may impede animal growth at cellular level leading to failure of animals to reach their genetic potential.
- Nutrition: the level and quality of feeds in terms of nutrients provided such as proteins, carbohydrates, minerals and vitamins are the most vital factors influencing growth at either uterine or post-uterine stage (Hutu et al., 2020). According to Owens et al. (1993), certain tissues grow and mature before others. For instance, growth starts with nervous (neural) tissue and proceeds to bone, muscle tissue and ends by adipose tissue growth.
- The hormones: the anterior pituitary gland controls growth by the production of the somatotropic hormone (growth hormone) as well as effects growth by coordinating the activities of other secretory glands (Irshad et al., 2012; Hutu et al., 2020). The growth hormone also triggers the release of somatomedins from the liver and is responsible for protein synthesis in concert with growth hormone resulting in the production of lean tissue (Irshad et al., 2012). Additionally, sexual glands produce hormones that are also vital in growth and development (Irshad et al., 2012). The testes produce androgens that stimulate growth in muscles by increasing protein accretion accompanied by a decrease in fat deposition (Alemneh & Getabalew, 2019). On the other hand, the ovaries produce estrogen that is effective in promoting the deposition of body fat than protein accretion in muscles (Alemneh & Getabalew, 2019). Therefore, the differences in growth rate and development of tissues in animals are linked to the sex of animals. Thus, male animals usually grow faster, mature later and have carcasses that are more muscular and with less fat than females (Irshad et al., 2012). However, Owens et al. (1993) noted that protein deposition would be limited by inadequate provision of amino acids and energy in the diet.

- Movement or exercise: helps in the activation of the metabolism. This results in a balanced growth of various tissues and organs (Hutu et al., 2020).

In order to monitor animal performance besides measuring only morphometric parameters, it is important to assess the blood chemistry of animals under different feeding regimes. This is important for non-conventional feeds which may contain deleterious chemicals that would affect the health and physiology of animals.

2.9. Blood sampling

Blood metabolites can be used for the assessment of the nutritional and health status of the animals. The blood metabolites level reflects the extent of metabolism of absorbed nutrients from feed (Maurya & Singh, 2015). Body weight and body condition scores are routinely recorded in livestock but when coupled with blood metabolites, they give a robust and accurate assessment of the nutritional status of an animal (Madziga et al., 2013). According to Ndlovu et al. (2007), blood metabolites give an instant indication of an animal's nutritional status at a given time. Madresh-Ghahfarokhi et al. (2018) also stated that metabolic profile tests are routinely used to complement dietary evaluation, especially in dairy cattle, to identify nutrition and management challenges.

Ndlovu et al. (2007) stated that the metabolic health of livestock can be ascertained through the profiling of glucose, non-esterified fatty acids, β-hydroxyl-butyrate, cholesterol, total proteins, albumin, haematology parameters, urea and minerals. Additionally, it should be noted that some blood metabolites are influenced by the physiological status and age of an animal. For instance, non-pregnant and non-lactating cows have a higher glucose concentration (Ndlovu et al., 2007). On the other hand, total proteins are lower in young animals but higher in mature animals (Ndlovu et al., 2007). Needless to say, the total protein and albumin in blood reflects the availability of protein. Therefore, the protein and albumin concentration in blood declines during malnutrition (Maurya & Singh, 2015). MKC as a potential feed ingredient should be validated through blood profiling of animals offered Morula products to establish whether or not compromises their health. Nowadays consumers prefer animal protein products that are not hazardous to their health and are not from animals subjected to cruelty. Consumer preference, together with meat quality, creates a marketing reference point that producers should be aware of.

2.10. Meat quality

Meat quality as described in a review paper by Valenzuela-Grijalva et al. (2017) refers to summation of chemical, physico-chemical, nutritional, sensory, health and food safety characteristics that would result in consumer acceptance and a better retail value of meat. Livestock producers must always comply with meat quality production standards to satisfy consumer demands and to remain competitive in the global market (Ramirez-Retamal & Morales, 2014). Meat quality can be assessed by the following parameters: pH, colour, tenderness, fatty acids and sensory attributes. In the literature, no information could be found about MKC meat sensory attributes; therefore, before being introduced as a viable ruminant feed ingredient, the meat quality of animals fed MKC should be assessed.

The ultimate pH of normal meat is less or equal to 5.7 (Ijaz et al., 2019). After slaughter, during conversion of muscle to flesh, adenosine triphosphate (ATP) and glycogen levels decrease due to glycolytic process occurring in the muscle (Ramirez-Retamal & Morales, 2014) which leads to lactic acid production. Therefore, lactic acid produced during glycolysis is responsible for lowering the pH from 7 to 5.7 or less (Ramirez-Retamal & Morales, 2014). Low glycogen stores before slaughter limit post-mortem glycolysis resulting in dark firm dry (DFD) meat with pH higher than 6.09 (Ijaz et al., 2019). Dark firm dry meat is the result of animals subjected to chronic stress before slaughter (Adzitey & Nurul, 2011). For instance, transportation of animals over long distances, long hours of food deprivation and overcrowding of animals in the lairage over a long period of time are some of the predisposing factors of DFD meat (Adzitey & Nurul, 2011). Chronic stress before slaughter also depletes glycogen stores; therefore, less glycogen is available for anaerobic glycolysis after death resulting in low acidification and leaving meat pH high (Adzitey & Nurul, 2011).

Dark firm dry meats are major causes for financial losses to most businesses or farmers as most consumers are unwilling to buy this meat (Adzitey & Nurul, 2011). The use of MKC in complete diets of ruminants may help prevent the occurrence of DFD related to nutritional deficiencies since MKC may provide nutrients that are insufficient from other ingredients in the complete diet, especially protein and energy. In the process, such animals consuming the diet are expected to grow optimally with adequate nutrients required for proper growth. Consumers use the colour of meat to discern the quality of meat (Purslow et al., 2019) and therefore take a purchasing decision at a retail store.

According to Ramirez-Retamal and Morales (2014), meat colour is determined by chemical status of myoglobin. When freshly cut, meat has a higher content of desoxymyoglobin and it gives meat a reddish purple colour (Moore et al., 2003). After exposure to oxygen, desoxymyoglobin changes to oxymyoglobin resulting in a desirable bright red colour (Moore et al., 2003). Further exposure of meat to oxygen changes oxymyoglobin to metmyoglobin due to oxidation (Moore et al. 2003; Kadim et al., 2003). Moreover, meat colour can be evaluated instrumentally using Commission Internationale de L'Eclairage (CIE) system (Ramirez-Retamal & Morales, 2014). Colour evaluation is done as L* brightness or lightness, a* for redness and b* for yellowness (Ramirez-Retamal & Morales, 2014). Atsbha et al. (2021) reported influence of nutrition on meat colour parameter (L*) whereby lambs fed complete diets with sesame (*Sesamum indicum*) seed cake had high L* value which was attributed to high intramuscular fat content. Therefore, in the present research, it was anticipated that MKC might also influence colour parameters given its high EE content which might promote marbling on meat.

Maltin et al. (2003) stated that most consumers use tenderness to gauge the eating quality of meat. Tenderness refers to the ease of chewing meat (Thu, 2006). There are several factors that can affect the tenderness of meat. Taylor (2003) explained that the collagen amount in a connective tissue contributes to toughness of meat. Therefore, meat from young animals is tender while it is tough in older animals due to a higher collagen content and more cross-links of the connective tissue for strength of the muscle (Taylor, 2003). The type of collagen in the muscle also contributes to the toughness of meat. For instance, synthesis of type-xii and type-xiv collagen decreases total collagen solubility and, in the process reduces meat tenderness (Astruc, 2014). On the other hand, type-iii collagen is associated with the tenderness of meat as elucidated by Astruc (2014). Also, Maltin et al. (2003) explained that animals with a high growth rate towards the end of the finishing phase tend to have a lower proportion of matured proteins in the muscle and a lower proportion of stable non-reducible cross-links resulting in tender meat. Taylor (2003) and Maltin et al. (2003) stated that the calpins are responsible for the cleavage of most muscle proteins (desmin and titin), thereby increasing the tenderness of meat. The amount of intramuscular fat in meat can also affect tenderness (Thu, 2006; Retamal & Morales, 2014). However, subjecting the animals to stress before slaughter can also reduce the tenderness of meat (Santos et al., 2019). Given the fact that MKC is rich in EE, it is expected that it may increase intramuscular fat in animal tissues, thereby improving the quality of meat.

Meat quality can also be determined by the composition of the fatty acid and cholesterol level (Smeti et al., 2018; Lage et al., 2020). Saturated fatty acids in red meat are associated with coronary heart disease in humans (Xu et al., 2020). The main saturated fatty acids in meat are myristic acid (C14: 0), palmitic acid (C16: 0) and stearic acid (C18: 0) (Lage et al., 2020). Also, myristic acid is regarded as a highly hypercholesterolaemic in human health (FAO, 2010; Parente et al., 2019) and therefore, meat products low in both myristic acid and palmitic acid are considered healthy. Monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in meat are beneficial to human health as they reduce the risk of coronary heart disease (Facciolongo et al., 2018). As a result, feed ingredients rich in MUFA and PUFA are likely to provide healthier animal products (meat or milk) for human consumption (Facciolongo et al., 2018). In ruminants, MUFA and PUFA in the diet are usually bio-hydrogenated in the rumen as they are very harmful to the rumen microbes (McDonald et al., 2011). Consequently, beef becomes rich in saturated fatty acids. Nevertheless, other researchers (Smeti et al., 2018) have reported that some PUFA and MUFA do escape rumen biohydrogenation and get absorbed in the small intestines for incorporation into animal products. Also, Lage et al. (2020) stated that fatty acids like margaric acid (C17: 0) negatively affect sensory traits like juiciness and flavour. For this reason, potential feed ingredients like MKC should not be assessed only on animal utilisation but also on other aspects like meat quality attributes with sensory traits included.

2.11. Sensory evaluation

Nowadays, meat consumers demand products of better nutritive value and sensory quality (Revilla et al., 2009). Sensory evaluation entails assessment of meat in terms of flavour, taste, appearance or smell through the senses (sight, smell, taste or touch) of the panellists (Ruiz-Capillas & Herrero, 2021). Therefore, sensory analysis by nature is both subjective but it is a scientific method used to assess and explain responses of panellists after they have evaluated food. However, subjectivity during sensory evaluation is minimised by training or preparation of panellists and labelling samples with random numbers (Ruiz-Capillas et al., 2021). It should also be pointed out that sensory quality plays a pivotal role in influencing consumer preferences and the actual buying of a meat product. It is anticipated that, since most people like morula fruits, meat products from MKC diets will therefore have enhanced sensory attributes.

2.11.1. Sensory evaluation techniques

According to Ruiz-Capillas et al. (2021), the techniques of sensory evaluation are classified broadly into, discriminative, descriptive, preference and hedonic. Discriminative techniques examine the difference between the two samples, eg. the duo-trio method, the triangular method and the mapping method. The duo-trio technique compares 2 or 3 samples by identifying similarities and differences. The triangular method is used to identify similarities and differences among the three samples under consideration. The mapping method is a new sensory technique that focuses on mapping the similarities between the samples and establishes the difference between the samples (Mihafu et al., 2020; Ruiz-Capillas et al., 2021). Descriptive techniques question how products or samples are different. It consists of an in-depth description of samples under observation and usually is done by trained personnel following the "guidelines for selection and training of assessors" (Morlein, 2019). The methods include check-all-that-apply (CATA) or rate-all-that-apply (RATA). Check-all-that -apply (CATA) uses a versatile multiple-choice questionnaire and RATA is a technique in which panellists use descriptors for defining intensity of responses (Ruiz-Capillas et al., 2021). This technique is used by trained panellists. Hedonic analysis on the one hand is concerned with the assessment of samples by consumer panellists. The panellists are chosen using demographics and socio-economic criteria. The consumer ratings are based on a hedonic scale (Morlein, 2019). The panellists use the scale to express their perception on the product or sample. For example, the 9-point hedonic scale that can be used is as follows; 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely (Morlein, 2019; Mihafu et al., 2020). The scale answers the question on "how do you like the sample". A hedonic analysis is a rapid test that provides information on the potential acceptability or rejection of a new product. Preference or choice tests answer the question of "which sample do you prefer". Choice selected is determined by the majority rule from the panellists whether negative or positive. The technique does not provide information on the magnitude of the liking or disliking from the panel (Ruiz-Capillas et al., 2021).

2.11.2. Attributes used for sensory evaluation

- Juiciness-Ribeiro et al. (2016) stated that the attribute of juiciness is influenced by moisture, intramuscular fat of meat and saliva produced during tasting. However, other attributes that stimulate salivation like appearance, aroma and flavour can interfere with

juiciness. MKC may positively affect cooked meat steaks juices given its high content of umami amino acids (especially glutamate) which are known to promote salivation (Ninimiya, 2015).

- Flavour-determines the eating quality of meat and the flavours arise from reactions between carbohydrates, proteins and lipids (Maillard-like reactions) during cooking (Wood et al., 1999). Therefore, meat with low carbohydrates as a result of pre-slaughter stress is bound to have a tasteless flavour. This is attributable to exhaustion of muscle glycogen during post-death glycolysis (Ijaz et al., 2019). Wood et al. (1999) and Pedroso et al. (2018) stated that in ruminants, fatty acid composition can be manipulated by diet to affect flavour of meat. Perhaps MKC may contribute positively to acceptable meat flavour when fed to animals.
- Sensory tenderness- refers to meat that requires low force to chew (Carlucci et al., 1998) due to less connective tissue. In general, the higher the insoluble collagen (in connective tissue) content, the tougher the meat (Tshabalala et al., 2003).
- Appearance- lighter meat in terms of colour is associated with younger animals and it is also regarded as a better-quality product (Ribeiro et al., 2016) than from older animals.

Morlein (2019) concluded that sensory science is a powerful tool that describes how a food item is perceived by the human senses in terms of likes or dislikes. Therefore, the current study is asking whether meat from lambs fattened from MKC-based diet can be accepted by consumers or not as a new meat product. Conventional oilseed cakes are the major plant protein supplements used in animal diets in most countries of the world. This is due to their richness in most nutrients used by animals for growth and development.

2.12. Conventional oil seedcakes

The protein value of the oilcakes is generally influenced by the extraction method, amino acid profile and amount of RDP and RUP. Oilseed cakes are the co-product of oil extraction from oilseeds (Atrrutia et al., 2020). The oil is extracted from oilseeds using solvents (hexane) or screw press method (McDonald et al., 2011).

Soybean meal (*Glycine max*) is the most used protein feed supplement with amino acid profile close to ideal (McDonald et al., 2011; Mthiyane et al., 2017). The most limiting amino acids in soybean meal are methionine and cysteine (McDonald et al., 2011). According to NRC (2000), soybean meal has 89% DM, 50% CP, 1.6% EE, 15% NDF and 7.2% ash. The meal has protease

inhibitors and haemagglutenin (McDonald et al., 2011). Soybean meal DM degradability constant values are “a” fraction; 29.7%, “b” fraction; 72.9%, “c” (rate of degradation) 7.4%/h, effective degradability ($ED_{0.05}$); 75% and CP degradability constants values “a” fraction; 15.8%, “b” fraction; 88.9%, “c”; 6.8%/h and effective degradability ($ED_{0.05}$); 69.2% (Todorov et al., 2016).

Cottonseed meal (*Gossypium spp*) has DM value of 90.2%, CP 46.1%, EE 3.2%, NDF 13.2% and ash 7% (NRC, 2000). The meal is deficient on cysteine, methionine, lysine and has anti-nutrients such as gossypol (antioxidant) and polymerization inhibitor (McDonald et al., 2011). Cottonseed meal DM degradability constants values are “a” fraction 19.5%, “b” fraction 50.4%, “c” 5%/h, effective degradability ($ED_{0.05}$); 44% and CP degradability constant values are; “a” 28.9%, “b” 44.7%, “c” 5%/h and effective degradability ($ED_{0.05}$); 50.5% (Kamalak et al., 2005).

Sunflower seed meal (*Helianthus annuus*) has DM value of 93%, CP 26%, EE 2.9%, NDF 40% and ash 8.2% (NRC, 2000). According to Wijayanti et al. (2020), sunflower seed’s meal limitation is its high fibre content. The meal is also deficient in lysine but rich in methionine when compared to soybean meal. Sunflower seed meal DM degradability constants are “a” fraction 21.6%, “b” fraction 49%, “c” 5%/h and effective degradability ($ED_{0.05}$); 45.5% and CP degradability constant values are; “a” 26.9%, “b”46.2%, “c” 5%/h and effective degradability ($ED_{0.05}$); 49.1% (Kamalak et al., 2005).

Conventional plant protein sources are very expensive for resource poor farmers and their supply in Botswana is met with imports. Therefore, it is imperative to search and screen potential unconventional feed ingredients from local natural resources to make livestock agriculture sustainable and profitable.

2.13. Non-conventional oilseed cakes

Non-conventional feed ingredients or feedstuffs as described earlier are traditionally not utilised for feeding livestock by most farmers. Known oil seedcakes from such non-conventional feedstuffs include but not limited to macadamia, baobab, hemp and sesame.

Macadamia oil cake (*Macadamia integrifolia*) nutritionally contains 95.3%, 18.5 MJ/kg, 18.5%, 55.4%, 8.5% and 3.3% for DM, GE, CP, NDF, EE and ash respectively (Mikasi, 2018). DM degradability constant values are “a’ fraction; 27.2%, “b” fraction; 70.5%, “c”; 1.5%/h

and ED (0.05); 56.8%. CP degradability constant values are “a’ fraction; 6.6%, “b” fraction; 69.8%, “c”; 0.1%/h and ED (0.05); 28%. Additionally, Macadamia oil cake is limited on methionine (Mikasi, 2018).

Baobab seed cake (*Adansonia digitata*) nutritionally contains 95.6%, 17.3 MJ/kg, 19.5%, 41.5%, 8.6% and 6.6% for DM, GE, CP, NDF, EE and ash respectively (Mikasi, 2018). DM degradability constant values are “a’ fraction; 21.3%, “b” fraction; 73.9%, “c”; 0.5%/h and ED (0.05); 63.2%. CP degradability constant values are “a’ fraction; 30.8%, “b” fraction; 69.2%, “c”; 0.1%/h and ED (0.05); 28%. Additionally, according to Babiker (2012), baobab seed cake is limited on both methionine and lysine.

Hemp (*Cannabis sativa*) cake nutritionally contains 92.9%, 34.1%, 39.5%, 11.6% and 6.8% for DM, CP, NDF, EE and ash respectively (Semwogerere et al., 2020). DM degradability constant values are “a’ fraction; 8.2%, “b” fraction; 50.6%, “c”; 2.4%/h and ED (0.05); 24.8%. CP degradability constant values are “a’ fraction; 6.5%, “b” fraction; 90.1%, “c”; 2.9%/h and ED (0.05); 39.4%. Additionally, it has anti-nutritional chemicals like condensed tannins, alkaloids, phytate and glucosinolates (Semwogerere et al., 2020).

Sesame meal (*Sesamum indicum*) cake nutritionally contains 94.2%, 32.2%, 48.4%, 3.5% and 12.1% for DM, CP, NDF, EE and ash respectively (Ghorbani et al., 2018). DM degradability constant values are “a’ fraction; 19.2%, “b” fraction; 64.1%, “c”; 16.6%/h and ED (0.04); 47.9%. CP degradability constant values are “a’ fraction; 13.9%, “b” fraction; 53.3%, “c”; 32.8%/h and ED (0.04); 39.4% (Ghorbani et al., 2018). Due to challenges in the supply of high-quality feedstuffs to livestock, exploration of non-conventional feeds in the Southern African Development Community (SADC) is underway. This desire is also bolstered by the need for sustainable management and utilisation of natural resources such as morula tree.

2.14. Importance of *Sclerocarya birrea* (Morula) tree

Sclerocarya birrea (English name: Marula; local name: Morula) is one of the best-known tree species (Moss, 1988) in Botswana. According to Xaba (2011), *S. birrea* is Africa’s highly valued indigenous fruit tree that is commonly found in homesteads in rural areas. The tree occurs in well drained sands and loams (Moss, 1988) in tropical Africa. It is a tree that grows to an average height of 10m in favourable conditions. Some trees, however, can reach 15m (Palgrave, 1981). The tree has compound leaves that are greyish green in colour and turn pale yellow before abscission (Roodt, 1998). The fruit is pale yellow when ripe, 30mm in diameter and is produced in large quantities in summer (Moss, 1988; Roodt, 1998).

S. birrea is a multipurpose tree that is highly valued in communities where it flourishes. The fruits contain four times as much vitamin C as orange juice (Roodt, 1998). Fruits can be eaten fresh when ripe (Roodt, 1998) or fermented to brew beer (Xaba, 2011). The fruit pulp can be consumed raw or boiled into a thick, black consistency to sweeten porridge. The wood is used for making fruit boxes, furniture, utensils and canoes (Xaba, 2011). In some countries in Southern Africa, the leaves of Morula tree are lopped (collected) for ruminant feeding (Mariod & Abdelwahab, 2012). However, *S. birrea* fruit is not fully exploited (Moss, 1988) by people and in some instances large quantities of the fruit are frequently found rotting under the trees or being consumed by livestock and wild animals.

The stone inside the fruit contains two or three edible kernels which contain 53%, 28% and 8% of oil, protein and carbohydrates respectively (Mariod & Abdelwahab, 2012). The seed kernel from the fruit can be eaten or used for oil extraction. The oil from the Morula kernel is used as skin care oil or for cooking (Xaba, 2011).

2.15. Production of morula kernel cake

The process of MKC production as described by Mlambo et al. (2011b) entail collection of morula nuts after removal of the fruit pulp during alcoholic beverage production or after consumption of pulp by individuals. The collected nuts are sun-dried for a couple of days or weeks and decorticated mechanically to remove kernels from cracked nuts. Thereafter kernels are roasted at temperature range of 45^{o}C to 47^{o}C to make oil extraction less difficult. Oil will then be extracted using hydraulic cold press method.

2.16. Chemical characterisation of morula kernel cake

2.16.1. Proximate composition

The CP content in MKC ranges from 39.1 to 48% with the mean value of 46% (Table 2.1). Currently, only one publication (Nkosi et al., 2019) has quantified the amount of CP that is RDP and/or RUP. In Table 2.1, EE value ranged from 29 to 41% with mean value of 28.5%. The high lipid content in MKC might be a hindrance for its use in ruminant diets (Mlambo et al., 2011b, Malebana et al., 2017), especially if fed at high amounts or as a sole feed. High lipid content in ruminant diets interferes with fibre digestibility (Church, 1988). The GE from MKC is 28.5 MJ/kg and is higher than the GE of maize grain (18.5MJ/kg; McDonald et al., 2011) of which is mostly used in concentrate diets. Therefore, MKC has a potential to also supplement

energy and partially replace maize in concentrate diets. The NDF in MKC ranged from 14.5 to 20.1%. NRC report (2001) documented that the amount of NDF in forage material is determined by the proportion or total amount of CP and EE. The mean NDF value in MKC is 18% which is much lower than NDF value (40%) of sunflower seed meal (NRC, 2000). Additionally, the amount of NDF recommended in ruminant diets ranges from 23 – 35% (Ensminger et al., 1990). On the other hand, MKC has a mean value of 13.4% for ADF. The mean values generated in Table 2.1 were an average value generated from 5 publications. Therefore, to control variation from individual studies or produce representative data on analysed parameters of MKC more data should be generated annually whenever oil is extracted from morula kernels to amass data that can generate MKC nutritional table values in future. Hiwilepo-Van Hal (2013) stated that variation in concentration of analysed nutrients of MKC may be attributable to origin of sample (soil type and climatic conditions), method of oil extraction and genotype of morula trees.

Table 2.1 : Proximate and fibre composition of morula kernel cake

Chemical composition of MKC (%DM)								
DM	OM	CP	EE	GE	ASH	NDF	ADF	Reference
ND	87.0	48	39.4	ND	ND	20.1	18.0	Mlambo et al. (2011a)
95.6	95.3	39.1	41.1	28.5	4.7	14.5	7.8	Malebana et al. (2017)
94.7	94.6	47	34.4	ND	5.4	ND	ND	Mthinyane et al. (2017)
90.1	94.4	47	39.4	ND	5.6	19.4	13.1	Mlambo et al. (2011b)
93.8	93.4	47.2	29.0	ND	6.6	ND	14.7	Mdziniso et al. (2016)
93.6	**92.9**	**45.7**	**28.5**	**-**	**5.6**	**18**	**13.4**	**Mean**

Note: DM=dry matter, OM= organic matter, CP=crude protein, EE=ether extract, GE=gross energy (MJ/kg), NDF=neutral detergent fibre, ADF= acid detergent fibre, ND= not done

2.16.2. Mineral composition

The amount of calcium across studies ranged from 0.1 to 0.2% with a mean value of 0.1% (Table 2.2). The phosphorus amount ranged from 1 to 1.1%. Therefore, the calcium and phosphorus ratio is 1:11. However, the recommended dietary calcium and phosphorus ratio range is 1:1 or 4:1 (NRC, 2005). This suggests that, when feeding MKC in ruminant diets, calcium supplementation should be considered to keep the calcium and phosphorus ratio at

required levels. There was only one publication that profiled macro minerals and micro minerals in MKC, whereas the other two publications reported on phosphorus and calcium only (Table 2.2). This necessitates the need to do more MKC mineral analyses to avail more mineral information and other nutrients that will be used to create feed table values to be used for feed formulations. The nutritive value of MKC will be improved as morula oil processing companies continue to improve the extraction procedures.

Table 2.2 : Mineral composition of morula kernel cake

Chemical composition of MKC (% DM)											
Ca	P	Mg	K	Na	Cl	Cu	Fe	Zn	Co	S	Reference
0.1	1.0	0.6	0.9	0.01	0.03	0.003	0.004	0.006	0.001	0.5	Malebana et al.2017
0.2	1.3	ND	ND	0.01	ND	ND	ND	ND	ND	ND	Mdziniso et al. 2016
0.1	1.1	ND	ND	0	ND	ND	ND	ND	ND	ND	Mthiyane et al. 2017
0.1	**1.1**	**-**	**-**	**0.01**	**-**	**-**	**-**	**-**	**-**	**-**	**Mean**

Note ND= not done.

2.16.3. Essential Amino acids

MKC is rich in arginine and has an adequate amount of methionine (Table 2.3). The amount of methionine is in the same range as that of sunflower seed meal (NRC, 2000). Malebana (2017) stated that lysine in MKC may not be adequate for growing monogastric animals. Only two research studies profiled amino acids of MKC (Table 2.3). Therefore, it is important to expand research in amino acids of MKC in diets of ruminants since Madibela et al (2009) has indicated that essential amino acids are involved in immune function (initiation and maintenance) in sheep infected with internal worms.

Table 2.3 : Essential amino acids in MKC

Amino acids (% DM)										
Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Reference
7.6	0.7	1.3	1.7	0.8	0.7	1.2	0.6	0.6	1.3	Malebana et al. (2017)
6.4	1.1	1.8	2.7	0.9	0.8	ND	1.0	ND	2.1	Mthiyane et al. (2017)
7	**0.9**	**1.6**	**2.2**	**0.9**	**0.8**	**-**	**0.8**	**-**	**1.7**	**Mean**

Note: Arg=arginine, His=histidine Ile=isoleucine, leu=leucine, Lys=lysine, Met=methionine, Phe=Phenylalanine, Thr=threonine, Trp=tryptophan, Val=valine, ND=not done.

2.16.4. Fatty acids

The main fatty acids in MKC are palmitic acid, stearic acid and oleic acid with mean values (% total fat) of 12.4%, 6.4% and 75% respectively, with traces of linolenic acid. The mean values (% total fat) for SFA, MUFA and PUFA are 15.0%, 83.4% and 5.2% respectively (Mariod & Abdelwahab, 2012; Mthiyane et al., 2017; Malebana et al., 2017). The high proportion of unsaturated fats in MKC may serve as an effective hydrogen sink in the rumen, thereby lowering enteric methane production (Church, 1988). Additionally, a variation in fatty acids concentration in MKC is mainly affected by the harvesting time (Mariod & Abdelwahab, 2012). That is, the concentration of fatty acids is high at the beginning of harvest season and decreases as the season progresses. However, there is still inadequate information of profiling fatty acids from MKC, especially from other ecological regions in Sub-Saharan Africa. Therefore, this calls for intensive efforts in analysis of morula products in order to reap the full benefits of this natural resource.

There are several techniques that can be used to screen potential feed ingredients and characterise them to determine their feed value for various types of livestock.

2.17. Techniques for estimation of feed value

Feed evaluation traditionally entailed profiling of the nutrient composition of feed ingredients in terms of energy, protein, vitamins and minerals (Jha & Tiwari, 2016). However, according to NRC (1981), the nutritive value of feed ingredients is expressed based on chemical composition, digestibility and nutrient consumption rate. In this regard, nutritive value in ruminants is dependent on voluntary feed intake and feed utilisation (Susmel & Filacorda, 1996). The role of microbial activity in the rumen also entails that fermentative processes should be considered during evaluation and characterisation of feeds for ruminants. Therefore, a chemical analysis, though essential at the beginning, does not account for processes that take place in the rumen, hence concepts of nutrient digestibility, degradability and outflow rates. These methods used in feed evaluation in ruminants are outlined as follows:

- Proximate composition: feed ingredient is subjected to determination of moisture, CP, EE, CF, NFE, ash and cell wall constituents (NDF, ADF and ADL) (Aganga

&Nsinamwa, 1997; Ribeiro & Moreira, 1998; McDonald et al., 2011). Proximate composition is very important as it shows the main chemical constituents present in a feed ingredient (Ribeiro & Moreira, 1998). It is a rapid and economical method for partially characterising a feed ingredient or feed. It indicates the potential of the feed to supply those nutrients but in reality, the feed may contain some anti-nutritional compounds that reduce its digestibility, hence reducing supply of the desired nutrients. Although chemical analysis is essential for understanding the nutritional potential of a new plant species, it is not sufficient and digestion studies would help (El hassan et al., 2000).

- *In vitro* dry matter digestibility (IVDMD): Boila et al. (1980) stated that IVDMD was developed by Tilley and Terry (1963) and can be used for ranking of forage samples or feed ingredients based on their dry matter digestibility. The *in vitro* technique was actually developed 58 years ago but has evolved over time to include the use of automated incubators (Ankom, 1997) and/or use of enzymes (Ribeiro & Moreira, 1998) and has been applied to test rumen fermentation dynamics in vitro (Madrid et al., 2002) and to measure degradation using waste gases as a proxy for rumen fermentation - *in vitro* gas production (Menke & Steingass, 1988). According to Tilley and Terry (1963), the technique involves the weighing of 0.5g of feed which is digested in buffered rumen liquor at a temperature of 38^{o}C in a water bath for 48 h. Thereafter, the next phase entails acid-pepsin digestion of the substrate for 48 h and in this study, this actually gave *in vitro* digestibility values similar to those found *in vivo* from using sheep (Tilley & Terry, 1963). Boila et al. (1980) noted that sources of variability in IVDMD values can be due to the weight of the substrate, the source of inoculum, the ratio of volume of inoculum to buffer and length of time for the first stage of the technique. Additionally, the method was standardised by using feed samples of low and high digestibility. To date, the IVDMD has been modified and it is used in several laboratories globally because of its simplicity and usefulness of data generated. However, degradation in the rumen is a time dependent process which is not adequately represented by chemical analysis and therefore the *in situ* technique (Elhassan et al., 2000) best describes time bound fermentation dynamics in the rumen.
- *In situ* or nylon bag digestibility: Osuji et al. (1993) described the nylon bag technique as a method that can be used to rank feed ingredients or feed according to the rate and extent of degradation of dry matter, nitrogen or other nutritional parameters. According

to Orskov et al. (1980), the *in situ* bag (Dacron bags, nylon bags, or rumen bag), when used to incubate feed, must be of known size, porosity and bag weight. Osuji et al. (1993) indicated that the bags' pore size should be large enough for rumen microbes to enter and access feed. Also, the bag should not allow undigested feed to escape but should allow escape of accumulated gases. Orskov et al. (1979) stated that incubated samples must be processed if the study compares the "degradation of protein in the protein supplements" to eliminate differences in particle size but a comparative study on "degradation of protein supplements" sample processing may be omitted. Hristov et al. (2019) stated that the variability of *in situ* digestibility values from different studies may be affected by host animal species, diet, feed intake, sample processing, particle size/form/fine particle losses, ratio of sample size to bag surface area, bag pore size, data modelling, microbial nitrogen contamination of bag residues and incubation sequence. The nylon bag technique method is very useful as it can provide end-point digestibility or kinetic data through incubation at different lengths of time. Nylon bag kinetic data is analysed by fitting the exponential equation developed by Orskov and McDonald, 1979; $Y=a+b(1-e^{-ct})$ or a similar equation with a lag time by McDonald (1981); $Y=a+b(1-e^{-c(t-L)})$, where Y=degradability at time t, a=intercept (degradation at time 0; thus, material washed from the bag), b=potentially degradable fraction, c=rate constant for degradation of b, L=lag time (from time 0 to the time maximum rate of degradation begins). Other different regression equations were tried after finding that the Orskov equation had been inadequate (Nasri et al., 2006; Bannink et al., 2016). The *in situ* or nylon bag technique needs more handling of animals during either insertion or withdrawal of bags and this can be tedious, especially if these activities coincide with shorter day length or the facilities lack artificial lighting for easier handling of the animals. Therefore, more user-friendly techniques such as in vitro gas production, that equally estimate kinetic data, are available where animals are handled once but most of the work is done indoors in the laboratory.

- Menke *in vitro* gas production technique: Niderkorn and Baumont (2009) described the technique as the incubation of ground substrates in a mixture of buffered mineral solution with rumen fluid to simulate animal fermentation in the rumen. Basically, it is a modification of the Tilley and Terry (1963) method on digestibility but it emphasises the waste of fermentation which are the gases. According to Osuji et al. (1993), the amount of gas produced during incubation of feed using rumen fluid is directly

proportional to the digestibility of the feed. The gas produced can be recorded at a series of time points such as 6, 12, 24, 48, 72 and 96 h. Ribeiro and Moreira (1998) and Susmel and Filacorda (1996) stated that the variability of experimental results is dependent on the composition and activity of the rumen fluid and type of diet fed to donor animals. With the gas production technique, there is no loss of fine feed particles during fermentation when compared to the *in situ* bag technique (Ribeiro & Moreira, 1998). Furthermore, the technique is easy to use, inexpensive and requires small quantities of feed (Nederkon & Baumont, 2009).

- *In vitro* ruminal degradability: The Ankom Daisy^{II} (Ankom Technology, Macedon, NY, USA) technique can be used to estimate the degradation of feed nutrients. The procedure uses substrate (diet), rumen fluid, buffer solution, digestion vessels and the Daisy incubator. Amin et al. (2021) used the Daisy incubator technique to test the susceptibility of essential oils *in vitro* at the incubation times of 0, 2, 6, 12, 24 and 48 h. In another experiment, Cudjoe and Mlambo (2014) also used the Daisy incubator technique to determine rumen degradability of nitrogen (N) *in vitro*. The researchers determined *in vitro* ruminal N degradability of leaves from four tree species. The samples were *in vitro* incubated sequentially at times of 2, 4, 6, 8, 12, 24, 36, 48 and 72 h.
- N-balance: protein is very important in the diets of growing animals and high producing adults (Pond et al., 1995) as well as in maintaining or bolstering the immune function. According to Church (1988), protein supplements are evaluated using nitrogen digestibility or N-balance. Nitrogen balance is nitrogen intake minus faecal and urinary nitrogen. The challenge with this technique (N-balance) is that Faecal-N can also contain endogenous nitrogen from enzymes or cellular material from the lining of the gut (Pond et al., 1995). However, N-balance can be adjusted for endogenous tissue and dermal nitrogen losses by using factors 0.35 and 0.018 in metabolic weight respectively. Therefore, basal endogenous nitrogen (BEN) is calculated by BEN (g/day) equals $(0.018 + 0.35) \times W^{0.75}$ (AFRC, 1993, Lima et al., 2018). Nitrogen balance indicates the usage of feed nitrogen by the animals or its loss to the environment (Okah et al., 2012). The amount of nitrogen retained in ruminant animals, ranges from 10% to 30% of nitrogen intake. In addition, the theoretical maximum possible nitrogen efficiency is estimated at 50% (Rotz, 2004). In earlier report Church (1988) stated that protein

accretion in tissues is determined by the stage of growth, rate of weight gain, type of breed and mature weight of an animal.

Therefore, a search for information on the nutritive value of MKC in both *in situ* degradability studies and animal studies in literature to identify gaps was undertaken.

2.18. *In situ* degradability studies

- Mlambo et al. (2011b) studied *in sacco* DM and nitrogen (N) degradability of MKC using castrated cannulated Matebele goats. The results from *in sacco* degradability study showed that MKC does not readily supply highly soluble N ("a" fraction) to rumen microbes. Dry matter and N potential degradability (PD) for MKC was 72.3% and 84.4% respectively. The low degradability was attributed to the presence of high residual lipids in the cake. The $ED_{0.05}$ for DM of MKC was 37.4% and $ED_{0.05}$ for N was 42%.
- Nkosi et al. (2019) compared the ruminal degradation of DM and CP from MKC, macadamia (integrifolia) and baobab (*Adansonia digitata*) seed cakes using Holstein cows. In MKC, "a" fraction of DM and CP was more soluble compared to the other two seed cakes. Effective degradability(5%/h) of MKC dry matter was higher than that of macadamia seed cake and baobab seed cake. However, effective degradability value of CP in MKC was higher than that of macadamia seed cake but similar to that of baobab seed cake. The three seed cakes had similar rumen undegradable protein values.
- Muya et al. (2020) conducted an *in situ* experiment of graded replacement of soybean meal with five levels (0%, 5%, 9%, 14% and 19 %) of MKC in complete diets contained in polyester bags. The "a" fraction of the DM increased with the increasing levels of MKC in the complete diets. However, the "b" fraction of DM was found to be high at low inclusion (5%) level of MKC in complete diets. This was further reflected by higher effective degradability (5%/h) of DM in diets with lower MKC (0%, 5% and 9%) inclusion than others. The reduced effective degradability was attributed to higher ADF and EE contents in the diets with inclusion level of 14% or 19% MKC when compared to the other diets with lower MKC inclusion (5% or 9%). In contrast, effective degradability (5%/h) of CP increased with increasing levels of MKC in the diets suggesting that more nitrogen was absorbed in the rumen. However, the researchers reported a decrease in RUP as MKC increased in the diets.

2.19. Animal studies

Information on the use of MKC in ruminants is limited; therefore, results from non-ruminants and ruminant studies were reviewed briefly in an attempt to pinpoint gaps that needed to be addressed.

2.19.1. Effects of feeding MKC in non-ruminants

- Since MKC is rich in protein (Malebana et al., 2017) and other amino acids (Malebana et al., 2017) that could be beneficial to poultry growth, Mthiyane and Mhlanga (2017) studied the effects of the replacement of soybean meal with graded (0%, 5%, 10%, 15%) rates of MKC on the performance of broiler chickens. The results showed a decline of body weight, feed intake, FCR, plucked weight, dressed weight, liver weight and head weight when MKC replaced soybean meal at varying levels. The researchers attributed the poor performance of broilers to lipid peroxidation of MKC and the presence of mycotoxins in the experimental diets.
- Mazizi et al. (2020) reported that graded inclusion of defatted MKC at 7.4%, 14.9%, 22.8% and 31.5% replacing soybean meal in complete diets (isonitrogenous and isocaloric) showed improved growth and development of Japanese Quail broilers. The body mass gain, ADG, feed intake, FCR and visceral organ mass were similar across treatments at grower phase, finisher phase and overall performance. It was concluded that defatted MKC can replace soybean meal in Japanese Quail broiler diets.
- Mabena et al. (2022) fed pigs complete diets with graded rates of MKC (0%, 5%, 10%, 15% and 20%). They reported similar dry matter intake and dry matter digestibility across the treatments. However, digestibility of crude protein, ADG, body weight gain, warm carcass weight and cold carcass weight decreased with more than 15% dietary inclusion of MKC. The low performance was attributed to increased dietary fibre and anti-nutritional factors as the content of MKC increased in the complete diets of pigs. Therefore, it was concluded that inclusion of MKC in pigs' diets must be less than 15%.
- Mazizi et al. (2022) reported that inclusion of 0%, 5.8%, 11.5%, 17.4%, or 23.3% MKC levels in the complete diets of broiler and layer Japanese quail had no negative effect on the normal functioning of the liver and kidney as per the normal range of values for the measured blood plasma surrogate markers (urea, total bilirubin, cholesterol, total protein, albumin, globulin, aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT)). The data indicates that MKC is a safe alternative protein source for use in poultry feeding. However, MKC's inclusion in poultry diets can not be

extrapolated into ruminant feeding systems since ruminants rely heavily on the function of the rumen for their nutrition.

2.19.2. Effects of feeding MKC to ruminants

- In tropical or subtropical countries, supplementation of nutritionally poor forages with other richer feed ingredients in terms of energy or protein is an important and cost-effective strategy especially during the dry season. Therefore, Mlambo et al. (2011a) investigated the response from offering supplementary MKC, soybean meal or sunflower seed cake to Nguni goats fed a basal diet of grass hay. The results showed that goats supplemented with MKC had the lowest intake of organic matter. This was attributed to high fat content in the diet. However, the total nitrogen (N) intake was highest in goats supplemented with MKC and soybean meal than in those supplemented with sunflower seed cake treatment and the unsupplemented group. Digestibility of NDF and ADF of goats fed MKC treatment decreased by 2.3% and 2.1%, respectively, when compared to the unsupplemented group. MKC and soybean meal treatment had a positive N-retention while sunflower seed cake treatment had a negative N-retention. MKC had lower urinary-N among the supplemented goats. The results of this research suggest that MKC has the potential to supplement protein in ruminants like sheep or dairy cattle.

  In a subsequent experiment where MKC was fed to cattle in feedlot compared to a commercial diet, no difference in weight gain, ADG, total feed intake and FCR was observed across the treatments. The similar growth performance from the feedlot cattle was attributed to similarity in the supply of required nutrients by the feedlot diets. However, feedlot cattle fed the commercial diet with equal amounts of MKC and urea in combination as nitrogen source had a numerically higher DMI and weight gain compared to those subjected to the other two treatments. This suggests that rumen microbes had enough soluble nitrogen from the diet that was used for their multiplication and growth. On the other hand, MKC might have provided RUP that was absorbed at the small intestine together with microbial protein contributing adequate metabolisable protein. The commercial diet with MKC resulted in 8.5% body weight increase when compared to the commercial diet with urea. The researchers concluded

that the use of MKC in complete feedlot diets had no detrimental effects on the voluntary feed intake and growth of cattle on feed.

- Mdziniso et al. (2016) conducted a feeding study using lactating dairy cows fed diets with MKC. The dietary treatments were MKC diet, soybean meal diet and concentrate mix containing equal parts of MKC and soybean meal in the same diet. Measured parameters in terms of body weight, dry matter intake, milk yield and composition were similar across the treatments. The researchers concluded that MKC can be used to replace soybean meal in dairy meals.

2.20. Oil seed cakes inclusion level in complete diets of livestock and poultry

The inclusion level of oil seed cakes or meals in complete diets of livestock and poultry is important for efficient utilisation of the protein in the offered diet to livestock. This is because the plant-protein sources may have anti-nutritional compounds like protease inhibitors, trypsin inhibitors, pepsin inhibitors, haemagglutenin, tannins and phytates, which could inhibit nutrient utilisation by the animal as well as limit their inclusion at higher level in complete diets (Sunil et al., 2015).

For instance, Chimvuramahwe et al. (2011) conducted an experiment with broilers, feeding them graded levels of baobab (*Adansonia digitata*) seedcake at 0%, 5% 10% and 15%. Broilers treated with the highest (15%) baobab seedcake recorded a high mortality rate. This was attributed to the cumulative effects of phytochemicals to toxic levels since baobab seedcake has tannins and saponins. Feed intake and feed conversion were maximised at 10% inclusion of baobab seedcake. At 15% inclusion of baobab seedcake, the fibre and fat content in the complete diet was too high for the broilers to utilise efficiently, hence a decline in feed intake from this group. It was concluded that baobab seedcake can be included in broiler diets up to 10% without a hindrance in their growth performance. McDonald et al. (2011) stated that the sunflower seed meal maximum inclusion rate is 15% and 20% in the total diet for sheep and cattle, respectively, for adequate growth performance. Lima et al. (2018) also demonstrated that inclusion of 0%, 10%, 20%, or 30% sunflower seed cake levels in lambs' complete diets resulted in a linear decrease of feed intake and nutrient intake (CP, NFC and TDN). In addition, the growth performance of the lambs showed a linear decrease and it was concluded that 30% inclusion of sunflower seed cake adversely affected animal growth. Aboul-Fotouh et al. (2015) found that inclusion of 0%, 15%, 20% or 25% olive cake levels in Ossi ram lambs' complete diets resulted in an insignificant difference in feed intake, total body weight gain and ADG. It

was concluded that 15% to 25% of olive cake can be used in sheep's complete diets. To our knowledge, no growth studies have been conducted to test graded inclusion of MKC as a sole protein source in complete diets of ruminants. However, one study (Malebana, 2018) investigated the complementary effects of MKC and soybean meal in complete diets of Dorper sheep.

Testing of new feed ingredients is incomplete without investigating the economics of such an intervention. This is because, 60% to 70% of the production cost in livestock enterprises comes from feeding as mentioned earlier (Thirumalaisamy et al., 2016) and a biological superior feed may still be economically costly.

2.21. Economics of feeding

Cost minimisation of feed in livestock production should be the goal of all enterprises. Replacement of traditional grains and conventional plant protein supplements with non-traditional feed ingredients at a cheaper cost is a welcome development (Neto et al., 2014). Muhammad et al. (2008) illustrated that inclusion of rice milling waste at 30% inclusion level in complete diets of sheep provided the best economic returns. However, the researchers indicated that it is dependent on the time and the place due to temporal variations in prices of feed ingredients. Chingala et al. (2019) showed that gross margin analysis is one of the methods that can be used to determine the financial viability of including non-traditional feed ingredients in livestock diets. Therefore, investigation of MKC should be holistic in that it should also test for its economic viability as a feed ingredient in diets of livestock.

2.22. Summary

The paucity of studies conducted on the use of MKC as a feed ingredient in ruminants encouraged an exploration of supplementary feeding for goats and the complementary effect of MKC in the complete diets of sheep, dairy cattle and finisher diets of beef cattle. Other studies characterised MKC as nutritional, although not comprehensively enough. No animal studies trialled MKC as a sole protein source in the complete diets of ruminants to ascertain its feeding value and sensory attributes of cooked meat steaks. However, one study was found using MKC as a sole protein supplement in the complete diets of pigs (Mabena et al., 2022). Additionally, livestock farmers nowadays want to adopt novel feed ingredients that are relatively cheaper and do not lower animal performance or productivity. Therefore, costing of diets incorporating MKC in ruminant feeding trials is a necessity.

In Chapter 3, the chemical characterisation of MKC is investigated to buttress the gaps that were found during the literature review. It is also very important to know the nutrient profile of ingredients before formulating balanced diets that will meet the nutrients requirements of livestock.

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CHAPTER 3

NUTRITIONAL EVALUATION OF MORULA KERNEL CAKE (SCLEROCARYA BIRREA*)* AND SUNFLOWER SEED CAKE (HELIANTHUS ANNUS) AS PROTEIN SOURCES FOR RUMINANTS

Abstract

Natural pastures in Botswana are not able to satisfy nutrient requirements of livestock throughout the year because of intermittent dry seasons which repeatedly disrupt efficient livestock production. The growing interest in the search for non-conventional supplementary feeds has triggered research aimed at characterising alternative feed resources for improvement of livestock productivity. This includes evaluating seed cakes from local plants such as Morula tree as protein source. In this experiment, composite samples of Morula (*Sclerocarya birrea*) kernel cake (MKC) or sunflower (*Helianthus annus*) seed cake (SSC) were sourced from local oil manufacturers for chemical characterisation and measurements of rumen degradation. MKC had higher ($P < 0.05$) content of gross energy, crude protein, ether extract and *in vitro* dry matter digestibility than SSC. In contrast, SSC had significantly ($P < 0.05$) higher content of cellulose, neutral detergent fibre and acid detergent lignin than MKC. Phosphorus and magnesium were higher ($P < 0.05$) in MKC samples than SSC. Additionally, MKC had very low content of potassium, sodium and calcium.

Oleic acid content was higher ($P < 0.05$) in MKC than in SSC. Sunflower seed cake had significantly ($P < 0.05$) higher content of linoleic acid than MKC. The level of unsaturated fatty acid (UFA) was similar ($P > 0.05$) in MKC and SSC. However, the polyunsaturated fatty acid (PUFA) and saturated fatty acid (SFA) ratio and UFA and SFA ratio was significantly higher in SSC than in MKC. MKC was low ($P < 0.05$) in lysine when compared to SSC. Tyrosine and cysteine were the only significantly ($P < 0.05$) higher amino acids in MKC than in SSC.

In sacco dry matter disappearance of SSC at 72 h was lower ($P < 0.05$) than that of MKC. In contrast crude protein disappearance of both cakes was similar at 72 h incubation. Dry matter of MKC had a significantly ($P < 0.05$) higher "a" fraction and potential degradability (PD) than SSC. Degradation rate (c) and effective degradability (ED; 5 %/h and 8 %/h) for crude protein were significantly ($P < 0.05$) higher in MKC than in SSC. Potential degradability of MKC and

SSC was similar ($P > 0.05$) for crude protein. However, rumen undegradable protein of MKC was significantly ($P < 0.05$) higher than that in SSC. It is thus concluded that MKC is rich in energy, crude protein and some minerals. The fact that MKC has high soluble protein as well as rumen undegradable protein (RUP) suggests its ability to balance the supply of fermentable nitrogen for microbial growth and escape protein for post-ruminal digestion. This would suggest that MKC could be a useful protein source in ruminant feeding systems with added benefits in producing healthier meat or milk products given its rich oleic acid that has a high oxidative stability.

Key words: morula kernel cake, oleic acid, *in sacco* degradability

3.1. Introduction

Livestock production in Botswana plays a very important role in the livelihood of the local people especially those living in rural areas (Statistics Botswana, 2019). There is distinct climatological partitioning of the year into two seasons, the wet and dry season, which have major influence in livestock production in Botswana. Productivity of livestock is mostly constrained during the dry season when natural pastures are limited in both quantity and its quality (Mine et al., 2002; Aganga, 2005; Kanyinji et al., 2017). The forages from natural pastures in most tropical countries are characterised by low crude protein (CP) and high neutral detergent fibre (NDF) (Solomon et al., 2008). High NDF content usually leads to low dry matter intake (DMI) in ruminants due to increased retention time and physical fill in the rumen (Aganga et al., 2007; McDonald et al., 2011). Generally, forages are a rich source of rumen degradable protein (RDP) (Klopfenstein et al., 2001) in the early part of the growing season but in the dry season RDP become deficient especially that most grasses become mature rapidly and contain a lot of lignin. When pasture quality deteriorate, improvement of livestock condition can be achieved by supplementation with RDP and/or rumen undegradable protein (RUP) that contributes to production of metabolisable protein (MP) (NRC, 2000). The result of poor nutrition in livestock holdings is reflected by frequent abortions, high mortality rates, slow growth rates and delayed slaughter weights (Kanyinji et al., 2017) as well as delay in attaining puberty in adolescent animals or resumption of rebreeding activities in adult animals (Lanyasunya et al., 2005).

During the dry season, most local farmers augment the limited feed resources by use of crop residues (Mine et al., 2002). However, crop residues may only serve as bulk forage and would not supply adequate nitrogen needed by rumen microbes to digest fibrous material. Therefore, supplementation with agro-industrial by-products (oilseed meals) is an alternative measure that is available and could be adopted by subsistence farmers to improve productivity in livestock (Solomon et al., 2008), however there are challenges for smallholder farmers in Botswana.

Oilseed cakes are residues obtained after industrial extraction of oil from the plant source such as oilseed or fruit by expelling or solvent extraction (Sunil et al., 2015). In Botswana, some farmers, especially commercial, procure supplementary protein of plant-origin like soybean meal, cotton seedcake and groundnut meal and that of animal-origin like fishmeal from neighbouring countries especially, South Africa. However, subsistence resource-limited farmers who rear their animals on natural pasture may not afford to buy conventional protein sources from abroad. Subsistence farmers used to benefit from locally produced and government subsidised blood meal and carcass meal which are now banned because of mad cow disease (MoA, 1998; Madibela et al., 2003; Madibela et al., 2013). Therefore, there is need to find cheaper and locally available alternative plant-protein supplementary feed.

MKC is a potential feed resource (Mlambo et al., 2011) that can be exploited in livestock feeding systems in Botswana. Morula tree from which the cake is derived is abundant in the southern African region and is a prolific fruit producer depending on amounts of rainfall the previous season (Botelle, 2001). Tapping on this natural resource, women cooperative groups in southern Africa collect morula fruits from which several products are derived. Morula fruits can be used for making beer or cider, jam, eaten raw and the kernel used for extracting oil which is used for cooking or cosmetics (Botelle, 2001; Hiwilepo-van Hal, 2013). Hence, MKC is obtained from extraction of oil from these kernels (nuts) of *Sclerocarya birrea* plant. Preliminary chemical characterisation of MKC from other countries shows that it is rich in protein, energy and other nutrients (Mdziniso et al., 2016; Mthiyane & Mhlanga, 2017; Malebana et al., 2017). There is no research done in Botswana to profile nutrients of locally produced MKC. However, there are some subsistence farmers who are already using MKC in animal diets without knowledge of its nutritional composition, hence the major objective of this study is to chemically characterise MKC as a potential protein feed ingredient in ruminant diets and to provide useful information required in feed formulations.

3.1.1. Specific objectives;

- To determine the proximate (dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), Ash,), *in vitro* dry matter digestibility (IVDMD), gross energy (GE), mineral content (calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), copper (Cu) and zinc (Zn), NDF and acid detergent fibre (ADF) of MKC and SSC.
- To profile the fatty acids (saturated, monounsaturated and polyunsaturated) of MKC.
- To profile the amino acids of MKC and SSC.
- To determine rumen degradation of DM and CP in MKC and SSC.

3.1.2. Hypotheses

(a) H_0: Chemical composition of MKC is similar to that of SSC.

(b) H_0: MKC CP and DM degradation in the rumen are similar to that of SSC.

3.2. Materials and methods

Three batches of each of hydraulic cold pressed SSC was obtained from Arona Pty Ltd, a processing plant for sunflower cooking oil based at Phakalane, Botswana and another batches of hydraulic cold pressed MKC was collected from DLG Naturals based in Gabane, Botswana. Thereafter, samples were kept in cool and dry place before experiments commenced.

3.2.1. Nutrient analyses

Each sample of MKC and SSC was composite from three batches and the resultant samples was ground to pass through a 2 mm screen in an electric grinder (Grinder Thomas-Wiley, laboratory mill model 4, Arthur Thomas Company, USA) and analysed in duplicates except for fatty acid determination in which samples were analysed in triplicate. All chemical analyses were repeated twice (two runs) except for *in situ* digestibility trial.

3.2.1.1. Determination of Dry matter (DM)

A porcelain crucible with 2 g of each sample was placed in the oven set at 105^{o}C overnight. Crucibles were later put in a desiccator for 40 minutes to allow cooling of the sample. The DM content was calculated as;

%DM= (dry sample mass/wet sample mass) x100 (AOAC, 1995).

3.2.1.2. Determination of ash and Organic matter (OM)

The same samples of oven-dried samples from DM procedure were incinerated in a muffle furnace (Labcon standard furnace, muffle RM4, L23767) for 4h at 550^{o}C. The furnace was allowed to cool down and crucibles with residues were placed in a desiccator for 40 minutes and weighed. Ash content was calculated as follows:

% ash= (ash mass/wet sample mass) x100 (AOAC, 1995).

% Organic matter (dry matter basis) =100- % Ash (DM basis).

3.2.1.3. Determination of Crude protein (CP)

The Kjeldahl method (AOAC, 2000) was used for CP determination. In brief, duplicate feed samples of 1.25 g were weighed in a lense tissue and placed in digestion tubes. The samples were digested with 98% sulphuric acid with selenium at temperature of 330^{o}C for 2h. The acid digest was allowed to cool at room temperature and 2 ml of hydrogen peroxide added. The mixture was digested for another 2 h. The digest was cooled at room temperature, distilled (BUCHI K-350) and standardised with sulphuric acid to determine nitrogen (N) content. The percentage of CP was then obtained as follows;

%CP= % Nx6.25

3.2.1.4. Determinations of Ether extract

A 1.5 g of sample (W_1) was weighed in filter bags and sealed with heat sealer. The sample was dried at 102^{o}C for 3 h (W_2). Fat extraction was done using Ankom XT15 using petroleum ether (500 ml) following Soxtec method (AOAC, 1995). The Ankom extractor was ran for 60 minutes with temperature of 90 ^{o}C. Samples were dried for 30 minutes in the oven at 102^{o}C. Samples were cooled at room temperature for about 30 minutes. Filter bags were re-weighed to get W_3. The total ether extract was calculated as follows:

$$\%\ Ether\ extract = \frac{(W_2 - W_3)}{W_1 \times DM} \times 100$$

Where W_1=original weight of sample, W_2=weight pre-extraction dried sample and filter bag, W_3=weight of dried sample and filter bag after extraction, DM=dry matter (AOAC, 1995).

3.2.1.5. Determination of Neutral Detergent Fibre (NDF)

Analysis of NDF was done following the modified procedures of Van Soest et al. (1991) as described in ANKOM^{220} fibre analyser manual (1997). Briefly, duplicate samples of 0.5g (W_2) were weighed into F57 filter bags (W_1) and sealed with heat sealer. The bags were placed in the bag suspender and placed in the Ankom$^{200/220}$ fibre analyser (New York, USA). A neutral detergent solution (NDS), sodium sulphite and alpha amylase were added into the Ankom fibre analyser vessel. The Ankom NDF process was run for 75 minutes. Then the bags were rinsed twice for 5 minutes with hot water and 4 ml of alpha-amylase under agitation. The third rinsing was done in water only. The filter bags were removed and water pressed out using fingers. The bags were soaked in acetone for 3 minutes and later air dried. The bags were put in the oven at 105^{o}C for 3h and thereafter cooled before weighing (W_3). The percentage NDF was calculated as follows:

$$\%\ NDF = \frac{(W_3 - (W_1 \times C_1))}{W_2 \times DM} \times 100$$

Where W_1=bag tare weight, W_2=sample weight, W_3=dried weight of bag with fibre after extraction process, C1=blank bag correction (final oven dried weight divided by the original blank bag weight), DM=dry matter.

3.2.1.6. Determination of Acid Detergent Fibre (ADF)

Analysis of ADF was done following modified procedures of Van Soest et al. (1991) as described in ANKOM^{220} fibre analyser manual (1997). Duplicate samples of 0.5 g (W_2) were weighed into F57 filter bags (W_1) and sealed in a heat sealer. The bags were placed in the bag suspender and placed in the Ankom$^{200/220}$ fibre analyser. Acid detergent solution (ADS) was poured into the Ankom fibre analyser vessel. The lid was closed and both the agitator and heat switch were turned on for 60 minutes. Thereafter, the bags were rinsed in hot water thrice (5 minutes' cycle). Excess water was removed from each filter bag by pressing with fingers. The

bags were placed in acetone and allowed to soak for 3 minutes per cycle. The bags were then removed from the beaker and air dried to remove acetone. The drying was finally done in an oven set at 105°C for 2h. The bags were cooled to room temperature and weighed (W_3).

$$\%\ ADF = \frac{(W_3 - (W_1 \times C_1))}{W_2 \times DM} \times 100$$

Where W_1=bag tare weight, W_2=sample weight, W_3=dried weight of bag with fibre after extraction process, C1=blank bag correction (final oven dried weight divided by the original blank bag weight). DM=dry matter.

3.2.1.7. Determination of crude fibre (CF)

Crude fibre was done following modified procedures of Van Soest et al. (1991) as outlined in Ankom Fibre analyser manual (1997). Duplicate samples of 1g (W_2) were weighed into F57 filter bags (W_1) and sealed with a heat sealer. Fat was extracted from all bags by soaking in petroleum ether for about 10 minutes in the beaker. After fat extraction, all bags were placed in a bag suspender and inserted in the Ankom$^{200/220}$ fibre analyser vessel. About 1900 ml of 0.3N sulphuric acid was poured into the fibre analyser vessel. The lid was closed and both the agitator and heat switch were turned on for 40 minutes. Bags were then rinsed with hot water twice. Thereafter, about 1500 ml 0.3N sodium hydroxide was poured over the sample bags inside the fibre analyser vessel. The lid was closed and both the agitator and heat switch were turned on for 40 minutes. After elapse of time, the bags were rinsed with hot water twice. Water was removed from each bag by pressing with fingers. Bags were soaked for 5 minutes in a beaker with acetone. Thereafter, the bags were air dried and oven dried for 3 h at a temperature of 105°C. After cooling in the desiccator all bags were weighed. All bags were ashed for 2 h in a muffle furnace, cooled and weighed to determine loss of weight of organic matter (W_3).

$$\%\ CF = \frac{(W_3 - (W_1 \times C_1))}{W_2 \times DM}$$

Where W_1=bag tare weight, W_2=sample weight, W_3=weight of organic matter, C_1=ash corrected blank bag factor, DM=dry matter.

3.2.1.8. Determination of Acid detergent lignin (ADL)

Procedures of Van Soest et al. (1991) were followed for ADF determination and the residue from ADF analysis was further digested in 75% sulphuric acid as described in Ankom^{220} Fibre analyser manual (1997). Samples were submerged into the sulphuric acid in the daisy jar and rotated for 3 h in the daisy incubator. After 3 h, sulphuric acid was poured out and bags were rinsed with distilled water to remove the acid. Bags were later rinsed with 250 ml of acetone for 3 minutes to remove excess water. Thereafter the bags were dried in the oven at 105^{o}C for 2-4 h (W_3).

$$\% \, ADL = \frac{(W_3 - (W_1 \times C_1))}{W_2 \times DM} \times 100$$

Where W_1 = bag tare weight, W_2 =sample weight, W_3 =dried weight of bag with fibre after extraction process, C_1 = blank bag correction (final oven dried weight divided by the original blank bag weight).

3.2.1.9. In vitro dry matter digestibility (IVDMD)

IVDMD was done following the modified procedures of Tilly and Terry (1963) as described in ANKOM^{220} fibre analyser manual (1997). Duplicate samples of 0.5 g (W_2) were weighed into F57 filter bags (W_1) and heat sealed. The buffer solutions (A and B) were pre-warmed (39^{o}C). Solution A was made of 20 g KH_2PO_4, 1.0 g $MgSO_4.7SO_4.7H_2O$, 1.0 g Nacl, 0.2 g $CaCl_2.H_20$ and 1.0 g Urea (reagent grade) in 2 litres of distilled water, Solution B was made of 15 g Na_2CO_3 and 1 g $Na_2S.9H_2O$ in 1 litre of distilled water. A 270 ml of solution B was mixed with 1330 ml of solution A. The mixture final pH was maintained at 6.8 with temperature of 39^{o}C. The mixture was added to the digestion jar with samples and placed into the Daisy incubator activated to heat and agitation switches. Rumen fluid was obtained from two rumen cannulated donor steers that were fed on a totally mixed ration for seven days. A totally mixed ration consisted of Maize grain (63.3%), Lucerne (20.8%), Wheat bran (10.1 %), Liquid molasses (3.6%), Feed lime (1.6%), Dicalcium phosphate (0.3%), Salt (0.2 %) and Vitamin and Mineral premix (0.1%) on dry matter basis. The diet was formulated to have 11.8% Crude protein, 1% Calcium and 0.4% Phosphorus. The rumen fluid was collected into two pre-warmed flasks with temperature of approximately 39^{o}C. The collected rumen fluid was prepared in the laboratory by sieving it through a 1000 µm (Star screens Test sieve) and 250 µm (Lab test sieve; endecotts, Ltd, England) screen respectively. Thereafter, a 400 ml rumen fluid was added to the digestion jar and carbon dioxide was added before closing the lid. The jar was incubated in the Daisy^{11} incubator for 48 h. After incubation jars were rinsed with cold

tap water until water was clear. The rinsed bags were placed into Ankom$^{200/220}$ fibre analyser following the procedure for determining NDF as described under section 3.2.1.5. The NDF analysis removes microbial debris and remaining soluble fractions. The bags were weighed after NDF (W_3) analysis. % IVDMD was calculated as follows;

$$\%\, IVDMD = \frac{100 - (W_3 - (W_1 \times C_1))}{W_2 \times DM} \times 100$$

Where W_1=bag tare weight, W_2=sample weight, W_3=final weight bag after *in vitro* and sequential NDF determination, C_1=blank bag correction (final oven dried weight divided by the original blank bag weight). DM = % dry matter (multiply by the decimal equivalent).

3.2.1.10. Nitrogen free extracts (NFE), Hemicellulose and cellulose

Nitrogen free extract was calculated using the formula:

$\%\, NFE = 100 - (\%CP + \%CF + \%EE + \%Ash)$ (Van Soest, 1994)

Hemicellulose (HE) was calculated as:

$\%\, HE = \%NDF - \%ADF$ (McDonald et al., 2011)

Cellulose was calculated as:

$\%\, Cellulose = \%ADF - \%ADL$ (McDonald et al., 2011)

3.2.1.11. Determination of gross Energy (GE)

Gross energy was determined using a CAL 3K-S oxygen bomb calorimeter system (Digital data system, Pty, Ltd, Randburg, South Africa). The CAL 3K-S system comprised of calorimeter, standard thread bomb vessel and oxygen filling station. In brief a sample of 0.5 g was weighed and placed in the standard thread bomb vessel for combustion. The vessel was manually filled with oxygen via an external manual oxygen filling station. The oxygen was filled to 3000kpa and 1 sample was combusted per approximately 15 minutes and gross energy was recorded in MJ/kg. All samples were analysed in duplicate.

3.2.1.12. Determination of fatty acids

Fatty acid methyl esters (FAME) were produced as described by O'Fallon et al. (2007). A 0.5 g of sample was placed into a 16 x 125 mm screw-cap Pyrex culture-tube to which 0.7 ml of 10 N sodium hydroxide (NaOH) and 5.3 ml of methanol (MeOH) were added. The tube was

incubated in a 55^{o}C water bath for 1.5 h with vigorous shaking by hand for 5 seconds every 20 minutes to properly permeate, dissolve and hydrolyse the sample. After cooling below room temperature in a cold tap water bath, 0.58 ml of 24 N sulphuric acid (H_2SO_4) in water was added. The tube was mixed by inversion and with precipitated sodium sulphate (Na_2SO_4) was incubated again in a 55^{o}C water bath for 1.5 h with shaking by hand for 5 seconds every 20 minutes. After FAME synthesis, the tube was cooled in cold water bath. Three (3) ml of hexane was added and the tube was vortex-mixed for 5 minutes. The tube was centrifuged at 3000 rpm for 5 minutes and the hexane layer containing the FAME was placed in a Gas Chromatography (GC) vial. The vial was capped and placed at -20^{o}C until GC analysis.

Determination of fatty acids profile was done at University of Botswana (UB) using Gas Chromatography (GC) with temperature ranging from 200^{o}C to 270^{o}C. The GC syringe size was 1 µl and automated. The capillary tube used was 30 m x 0.25 m x 0.25 µm (Supelco, omegawax 250). The gas chromatograph system consisted of a GC (Agilent technologies, 7890A, GC system), Mass Spectrometry (MSD GC, Agilent technologies, 5975C) and personal computer used for quantification

3.2.1.13. Determination of Amino acids

Amino acids of SSC and MKC were profiled at a New Jersey feed laboratory by the Rock River laboratory, Watertown, Wisconsin, USA. The amino acids were determined following methods of AOAC 994.12. (2005). In brief, a 200 mg sample was weighed and hydrolysed with 6 ml of 6 N hydrochloric acid for 24 h. Norleucine was added as internal standard and then an aliquot was taken and injected into High Performance Liquid Chromatography (HPLC; Agilent 1260 infinity) with Quat pump (Pickering eluents Na 270, Na 315, Na 740, RG011 used for mobile phase) coupled with pickering pinnacle PCX post column derivatisation with Ninhydrin reagent. Chromatographic analysis was done at mAu 570 and mAu 440 on molecular weight distribution (MWD). Methionine and cysteine were oxidised with performic acid prior to being hydrolysed and injected with norvaline internal standard.

3.2.1.14. Determination of mineral composition

Atomic absorption spectrophotometer (Agilent 280FSAA) was used for the determination of potassium, sodium, calcium, magnesium, copper, iron, zinc and manganese. From the digest solution obtained in section 3.2.1.3 (Chapter 3 of this thesis), an aliquot was used for determination of metal ions (potassium, sodium, calcium, magnesium, copper, iron, zinc and

manganese). The agilent hollow cathode lamps for the respective metal ion of interest were used as a light source (Cottenie et al., 1982; AOAC, 2005). UV visible spectrophotometer was used to determine phosphorus calorimetrically using sodium phenol, ammonium molybdate and ascorbic acid method (AOAC, 1996).

3.2.2. *In situ* ruminal dry matter and crude protein degradability of sunflower seed cake and morula kernel cake

The experiment was conducted at Sebele Dairy Farm Gaborone, Botswana. The farm is situated at latitude 24º 33'S and longitude 25º 57'E at altitude of 994 m above sea level (Madibela et al. 2003). Three rumen fistulated Tswana steers weighing on average 702 ± 70 kg were used in the study. The steers were kept in a pen measuring 10 m x 15 m and group-fed a totally mixed ration daily as described at section 3.2.19 at a rate of 12 kg per animal around 0800 h. Drinking water was provided daily ad *libitum*. The steers were adapted to the diet for seven days before incubation of experimental feeds.

The *in situ* bags made of nylon cloth with dimensions of 5 cm x 10 cm, 50 µm pore size (Bar Diamond, Idaho, USA) were labelled using a black permanent marker, weighed and filled with 5 g of respective feed samples. The opening of each bag was tightly secured and sealed with a fish-line string. Two bags of the same sample were bound to a very thin flexible pipe (about 6 mm diameter and 45 cm long). Therefore 6 measurements were made for each experimental feed at a given time point (3 cows x 2 duplicates x 6 time points). The bags were soaked in warm water (39 ºC) for 30 minutes before incubation (Todorov et al., 2016). Incubation of the bags in the fistulated steers was done in the morning before the animals were fed. Samples were incubated in the rumen for 0, 6, 12, 24, 48 and 72 h and withdrawn sequentially according to the incubation times. The bags were tied on 50 cm fish-line string and secured to the fistula for ease of removal as described by Madibela et al. (2003) and Todorov et al. (2016). Immediately after removal of bags from the rumen at a given time point, the bags were washed with running tap water until washing water became clear and colourless. Bags were put in the freezer until all the bags were incubated. The zero-hour (0 h) bags were immersed in a water bath (39 ºC) without agitation for 30 minutes (Betajoo & Shaver, 1998) to estimate the water-soluble material (the "a" fraction). The zero-hour bags were later washed with tap water to remove any feed particles adhering to the outside of the bags. All the 72 bags were dried at 60 ºC for 48 h, weighed and the residues used for CP analysis. The CP degradability was calculated

as the difference between concentration of CP in the original sample and in residual sample. Rumen degradable protein (RDP) was estimated as the amount of CP multiplied by its effective degradability at assumed rumen outflow rates of 0.05 per hour as described by McDonald et al. (2011). Rumen undegradable protein (RUP) was calculated as the difference between CP and RDP and expressed as % DM. Dry matter (DM) degradability was calculated as the amount of DM that disappeared after weighing the residue. Thus DMD=1- ((residue+ *in situ* bag)-*in situ* bag)/ (sample weight) *(DM))

The data were plotted against time to check for lag time using Microsoft excel. In the absence of lag time, the following equations were used for calculation of degradation kinetics constants of DM and CP in the rumen.

In situ time point degradation data for CP and DM were fitted into Orskov and McDonald (1979) equation;

$P = a + b(1 - e^{-ct})$

Where P = dry matter or crude protein degradation after time "t" ;a = is the rapidly disappearing fraction; b = is the slowly degraded fraction; c = degradation rate of fraction b. Fraction "a" and "b" are defined mathematically and not chemically. The total degradation of the diets is obtained by summation of "a" and "b" which cannot exceed 100. Therefore 100-(a + b) denotes the fraction of feed which is undegradable in the rumen (Orskov et al., 1980).

The effective degradability (ED) of DM and CP was calculated as; $ED = \frac{a+bc}{c+k}$ from Orskov, (1982), where k is the fractional outflow rate from the rumen, assumed to be 0.05/h and 0.08/h. Rumen outflow rate of 0.05/h is expected for growing animals and 0.08/h is used in high producing animals especially for lactating dairy cows as an example.

The degradation constants (a, b, c, PD, $ED_{0.05}$ and $ED_{0.08}$) of DM and CP were estimated by general non-linear models (NLIN) procedures using modified SAS program written by the Rowett Research Institute (Osuji et al., 1993) as described by Madibela et al. (2013).

3.2.1.15. Data analyses

Time series data on *in situ* DM and CP disappearance (%) of the oil cakes over time were analysed as repeated measures using the proc mixed procedure of statistical analysis system (SAS) (2002) with steer as a random factor. The model used is shown below;

$$Y_{ijk} = \mu + T_i + C_j + (T_i \times C_j) + \varepsilon_{ijk}$$ Model 1

Where μ=overall mean, T_i=effect of incubation time, i=0, 6, 12, 24, 48 and 72, C_j=effect of j^{th} cake=1 or 2, Σ_{ijk}=random error

Data on DM and CP constants (a, b, c, a + b, $ED_{0.05}$, $ED_{0.08}$), RDP and RUP were subjected to analysis of variance (ANOVA) using General Linear Model (GLM) procedures of SAS (2002) following model 2

$$Y_{ijk} = \mu + T_j + \varepsilon_{ijk}$$ Model 2

Where μ = overall mean, T_j=effect of j^{th} cake=1 or 2, Σ_{ijk}=random error

Data on proximate, energy, minerals, fatty acid and amino acids were analysed using t-test. For all statistical analysis significance was declared at $P < 0.05$.

3.3. Results

3.3.1. Proximate composition and Energy content

Nutrient composition of SSC and MKC are reported in Table 3.1. The dry matter content of SSC and MKC were not statistically different ($P>0.05$). However, organic matter content was significantly ($P< 0.05$) higher in SSC (95.4%) than in MKC. MKC had higher ($P < 0.05$) content of CP, EE, ash, GE and IVDMD than SSC. Sunflower seed cake had greater ($P < 0.05$) content of Hemicellulose, Cellulose, NDF, ADF, ADL and NFE than MKC.

Table 3.1. Chemical composition (% DM) of sunflower seed cake and morula kernel cake

Item (% DM)	Oil seedcake		RMSE	*P*-value*
	Sunflower	Morula		
Dry Matter	93.5±2.3	92.1±3.9	1.9	0.5
Organic Matter	95.4±0.2^{a}	93.1±0.7^{b}	0.3	0.0001
Crude Protein	29.3±2.3^{b}	45.3±2.0^{a}	1.2	0.0001
Ether Extract	10.3±2.6^{b}	33.9±3.1^{a}	1.7	0.0001
Neutral Detergent fibre	43.2±2.5^{a}	14.9±2.0^{b}	1.3	0.0001
Acid Detergent fibre	32.0±2.9^{a}	11.6±1.7^{b}	1.4	0.0001
Acid Detergent lignin	9.9±1.1^{a}	5.5±1.5^{b}	0.7	0.0001
Hemicellulose	11.0±1.0^{a}	3.2±0.6^{b}	0.5	0.0001
Cellulose	22.0±2.1^{a}	6.0±0.9^{b}	0.9	0.0001
Nitrogen Free Extract	27.4±1.2^{a}	3.4±0.7^{b}	0.8	0.0001
Ash	4.4±0.5^{b}	6.9±0.8^{a}	0.4	0.0001
Gross Energy(MJ/Kg)	21.0±0.6^{b}	24.5±1.6^{a}	0.8	0.0012
IVDMD	62.3±2.0^{b}	86.0±0.6^{a}	0.3	0.0001

Note: IVDMD=*in vitro* dry matter digestibility; RMSE= root mean standard error; DM=dry matter; *Means within same row with different superscripts (a, b) differ significantly (P<0.05).

3.3.2. Mineral composition

The mineral content of sunflower seed cake and MKC is shown in Table 3.2. Calcium content was significantly ($P < 0.05$) higher in SSC than MKC. However, magnesium and phosphorus content were significantly ($P < 0.05$) lower in SSC than MKC. The zinc content was significantly higher ($P < 0.05$) in SSC than MKC. However, potassium, sodium, copper, iron and manganese content in the two oilcakes were similar ($P > 0.05$).

Table 3.2. Mineral composition (% DM) of sunflower seed cake and morula kernel cake

Item (% DM)	Oil seedcake		RMSE	*P*-value*
	Sunflower	Morula		
Macro minerals				
Calcium	0.17±0.01[a]	0.13±0.02[b]	0.01	0.01
Phosphorus	0.8±0.01[b]	1.1±0.01[a]	0.01	0.0001
Potassium	0.01±0	0.01±0	0.0	0.4
Magnesium	0.6±0.001[b]	0.8±0.001[a]	0.0003	0.0001
Sodium	0.01±0.003	0.01±0.01	0.005	0.4
Micro minerals				
Copper	0.002±0.0	0.001±0.0	0.01	0.1
Iron	0.02±0.02	0.05±0.02	0.02	0.1
Zinc	0.01±0.002[a]	0.003±0.002[b]	0.001	0.03
Manganese	0.003±0.001	0.003±0.002	0.001	1.0

Note: RMSE= root mean standard error; *Means within same row with different superscripts (a, b) differ significantly (P<0.05).

3.3.3. Amino acids

Table 3.3 shows the results of the amino acid profiles of sunflower seed cake and MKC. Histidine and lysine content were higher ($P < 0.05$) in SSC than in MKC. Overall, the total essential amino acid content in SSC and MKC were within the same range (MKC; 41.6% CP vs SSC 43.7% CP). Likewise, other essential amino acids like arginine, isoleucine, leucine, phenylalanine, threonine and valine were similar ($P > 0.05$) in concentration across the oilseed cakes. On the other hand, cysteine and tyrosine were significantly higher ($P < 0.05$) in MKC than SSC. Proline was significantly higher ($P < 0.05$) in SSC than in MKC. However, alanine, aspartic acid, glutamic acid, glycine, hydroxyproline and serine were similar ($P > 0.05$) across the oilseed cakes in the current study. The predominant amino acids in both oil seedcakes were glutamic acid followed by arginine, aspartic acid and leucine.

Table 3.3. Amino acid profile (% CP) of sunflower seed cake and morula kernel cake

	Oil seedcake			
Item (% CP)	Sunflower	Morula	RMSE	*P*-value
Essential				
Arginine	11.3±3.7	12.1±3.6	3.7	0.8
Histidine	3.5±0.2[a]	2.8±0[b]	0.1	0.03
Isoleucine	4.0±0.9	4.2±0.3	0.7	0.7
Leucine	6.3±0.5	6.5±0.2	0.4	0.7
Lysine	3.3±0.1[a]	1.9±0.1[b]	0.08	0.003
Methionine	2.1±0.2	1.9±0	0.1	0.4
Phenylalanine	4.7±0.2	4.6±0.2	0.2	0.8
Threonine	3.8±0.5	2.6±0	0.3	0.07
Valine	4.7±1.1	5.0±0.4	0.8	0.8
Non-essential				
Alanine	4.1±0.3	3.4±0.1	0.2	0.08
Aspartic acid	10.2±0.1	8.9±0.7	0.5	0.12
Cysteine	1.9±0.03[b]	2.8±0.3[a]	0.2	0.04
Glutamic acid	22.4±0.4	26.6±1.7	1.2	0.08
Glycine	5.7±0.3	5.0±0.3	0.3	0.2
Hydroxyproline	0.5±0.04	0.4±0.1	0.1	0.4
Proline	4.3±0.09[a]	3.1±0.4[b]	0.3	0.04
Serine	4.9±0.1	5.0±0.3	0.2	0.5
Tyrosine	2.3±0.2[b]	3.1±0.2[a]	0.2	0.04

Note: RMSE= root mean standard error; *Means within same row with different superscripts (a, b) differ significantly (P<0.05).

3.3.4. Fatty acids

Table 3.4 shows the main fatty acids in the sunflower seed cake and MKC. Methyl palmitate content was greater (P < 0.05) in MKC than in SSC. Stearic acid content was similar (P > 0.05) across the oil seedcakes. But oleic acid content was significantly higher (P < 0.05) in MKC

than in SSC. Sunflower seed cake had a significantly higher ($P < 0.05$) linoleic acid content than MKC. 16-Octadecenoic methyl ester acid was detected in MKC only. 13-Octadecenoic acid methyl ester content was similar across the oilseed cakes. Total saturated fatty acids (SFA) and unsaturated fatty acids (UFA) content were similar ($P > 0.05$) in the oil seedcakes. Monounsaturated fatty acids (MUFA) were significantly higher ($P < 0.05$) in MKC than SSC. However, polyunsaturated fatty acids (PUFA) were significantly lower in MKC ($P < 0.05$) when compared to SSC. PUFA/SFA ratio and UFA/SFA ratio were significantly higher ($P < 0.05$) in SSC than in MKC.

Table 3.4. Fatty acid profile (% total fatty acid, TFA) of sunflower seed cake and morula kernel cake

	Oil seedcake			
Item (%TFA)	Sunflower	Morula	RMSE	*P*-value
Saturated				
Palmitic	ND	0.5±1.0	0.7	0.4
Methyl palmitate	10.2±3.4[b]	14.8±0.7[a]	2.4	0.04
Stearic acid	8.3±1.0	7.1±1.1	1.0	0.2
Monounsaturated				
Oleic acid	26.7±27.7[b]	62.0±6.6[a]	20.1	0.048
13-Octadecenoic[1]	5.6±11.1	4.4±6.5	9.1	0.9
16-Octadecenoic[2]	ND	0.4±0.5	0.3	0.1
Polyunsaturated				
Linoleic acid	49.0±25.7[a]	10.7±1.7[b]	18.2	0.02
SFA	18.6±3.9	22.4±1.2	2.9	0.1
UFA	81.2±3.7	77.6±1.2	2.7	0.1
MU	32.3±22.2[b]	66.9±0.9[a]	15.7	0.02
PUFA	49.0±25.7[a]	10.7±1.7[b]	18.2	0.02
PUFA/SFA	3.9±0.1[a]	0.6±0.1[b]	0.06	0.0001
UFA/SFA	5.3±0.1[a]	3.7±0.2[b]	1.4	0.0001

Note: ND=not detected, SFA=saturated fatty acid, UFA=unsaturated fatty acid, MU=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid, [1]13-Octadecenoic acid methyl ester, [2]16-Octadecenoic acid methyl ester, RMSE=root mean standard error *Means within same row with different superscripts (a, b) differ significantly ($P < 0.05$),

3.3.5. *In situ* dry matter (DM) and crude protein (CP) disappearance

The values for rumen disappearance of DM and CP of sunflower seed cake and MKC are shown in Table 3.5. The SSC had a significantly ($P < 0.05$) higher DM washing loss than that of MKC. However, CP washing loss was similar ($P > 0.05$) for the two oil seedcakes. When the incubation was less than 24 h, MKC had the highest DM degradability (75%) and ultimately reached 79 percentage units at the last incubation time point at 72 h. Overall, SSC had the least

DM degradability of 60 percentage units. The CP degradability of MKC reached 84 percentage units at 12 h while CP of SSC was below 70 percentage units. However, overall CP degradability of the two oil seedcakes was similar (P > 0.05) at the end of incubation.

Table 3.5. *In sacco* dry matter and crude protein disappearance (DM) of sunflower seed cake and morula kernel cake over time

	Incubation (h)						
Item	0	6	12	24	48	72	*P*-value
Dry matter degradability							
Sunflower seedcake	19.4^{aA}	49.5^{bB}	52.2^{bC}	54.0^{bD}	59.2^{bE}	60.0^{bE}	0.0001
Morula kernel cake	14.6^{bA}	67.4^{aB}	75.0^{aB}	77.5^{aC}	78.5^{aC}	79.0^{aC}	0.0001
RMSE	0.98	0.98	0.98	0.98	0.98	0.98	
P-Value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	
Crude Protein degradability							
Sunflower seedcake	18.2^{aA}	51.9^{aB}	67.4^{bB}	84.8^{aC}	92.0^{aD}	92.2^{aD}	0.0001
Morula kernel cake	14.7^{aA}	43.2^{aB}	83.7^{aC}	84.8^{aCD}	89.6^{aDE}	95.2^{aE}	0.0001
RMSE	4.1	3.2	3.2	2.9	2.9	2.9	
P-Value	0.4	0.01	0.0001	1.0	1.0	0.3	

Note: RMSE= root mean standard error; *Means within same row or column with different superscripts (a, b for columns and A, B, C for rows) differ significantly (P<0.05).

3.3.6. Dry matter degradability constants

The ruminal dynamic characteristics of DM are reported in Table 3.6. MKC had a significantly (P < 0.05) higher "a" fraction and "b" fraction value of DM than SSC. The DM degradation rate, potential degradability, effective degradability at 5% and 8% were also significantly higher (P < 0.05) in MKC than in SSC.

Table 3.6. *In sacco* dry matter (% DM) degradability parameters of sunflower seedcake and morula kernel cake

Oil seedcake				
Items (%)	Sunflower	Morula	RMSE	*P*-Value
Fraction a	8.9^{b}	12.4^{a}	1.49	0.002
Fraction b	61.1^{b}	77.0^{a}	0.63	0.0001
Degradation rate c, %/h	18^{b}	28^{a}	0.03	0.0002
Potential degradability, PD	70.0^{b}	89.4^{a}	1.71	0.0001
Effective degradability, 5%	56.6^{b}	77.7^{a}	0.92	0.0001
Effective degradability, 8%	51.2^{b}	72.3^{a}	1.04	0.0001

Note: RMSE= root mean standard error; *Means within same row with different superscripts (a, b) differ significantly (P<0.05).

3.3.7. Crude protein degradability constants and RUP

The ruminal dynamic characteristics of crude protein are shown in Table 3.7. The CP fraction "a" and potential degradability (a + b) were similar in the two oil seedcakes. The potential degradable fraction "b" was significantly (P < 0.05) higher in SSC than in MKC. The MKC had a significantly (P < 0.05) higher percentage units of effective degradability at the two outflow rates (5% and 8%). Rumen degradable protein and RUP were significantly higher (P < 0.05) in MKC than SSC.

Table 3.7. *In sacco* Crude protein degradability parameters (% DM), Rumen Degradable Protein (RDP; % DM) and Rumen Undegradable Protein (RUP; % DM)

	Oil seedcake			
Items (%)	Sunflower	Morula	RMSE	*P*-Value
Fraction a	15.7[a]	14.8[a]	2.3	0.6
Fraction b	77.0[a]	76.9[b]	0.01	0.02
Degradation rate c, %/h	9.8[b]	13[a]	0.02	0.04
Potential Degradability, PD	92.7[a]	91.8[a]	2.3	0.6
Effective Degradability,5%	66.6[b]	70.4[a]	2.1	0.04
Effective Degradability, 8%	58.0[b]	62.5[a]	2.3	0.03
Rumen Degradable Protein %	19.4[b]	35.4[a]	0.81	0.0001
Rumen Undegradable Protein %	9.8[b]	14.8[a]	0.81	0.0001

Note: RMSE= root mean standard error; *Means within same row with different superscripts (a, b) differ significantly (P<0.05).

3.4. Discussion

3.4.1. Proximate composition and Energy content

The amount of DM in MKC and SSC was similar. The two oil seedcakes have lower moisture content which might prolong their shelf-life. The main nutrients in MKC were EE and CP. Also, the summation of EE and CP content of MKC (Table 3.1) was comparable to 76.2% reported by Mdziniso et al. (2016), 80.2% reported by Malebana et al. (2017) and 81.4% reported by Mthiyane and Mhlanga (2017) for the same cakes. In contrast, summing EE and CP of SSC resulted in a lower value which was comparable to 43.9% reported by Mlambo et al. (2011). These data from literature suggests that MKC might have a higher energy density per kg dry matter when compared to SSC. Indeed, the current study observed a higher gross energy and ether extract content from MKC than SSC. Malebana et al. (2018) also reported similar findings when comparing hydraulic cold pressed MKC with soybean meal. Therefore, from an economic point of view, the use of MKC in animal diets may lower the cost of supplying energy in livestock feeds.

The MKC had a higher EE than SSC which is noted to be residual oil during processing. The residual oil from the cakes will boost energy density of the diet (Pond et al., 1995; Mlambo et al., 2011) and method of extraction (press vs solvent) determines the amount of residual oil derived. The amount of EE in MKC was similar to 29% reported by Mdziniso et al. (2016). However, Mlambo et al. (2011) and Malebana et al. (2017) reported higher EE values of 39.4% and 41.1%, respectively. The discrepancy in the amount of EE across the studies is mainly attributed to industrial processing methods used to extract oil from the seeds. The high EE in MKC has the potential to shorten the cake's shelf life as a result of oil rancidity due to lipid oxidation (Chivandi et al., 2013; Malebana et al., 2017). This calls for morula oil manufacturing companies to improve the currently used extraction procedure in order to obtain a high-quality cake with low fat content.

The lower GE of SSC compared to MKC (Table 3.1) is attributable to lower EE. Gross energy is defined as heat released when an organic substance is completely oxidised to carbon dioxide and water (NRC, 2000). The GE value of MKC in the current study was lower than 28.5 MJ/kg DM (Malebana et al., 2017), but higher than 17.3 MJ/kg DM for baobab (*Adansonia digitata*) seedcake (Mikasi, 2018). Lower energy density of feeds is a limiting factor affecting livestock productivity in sub-Saharan Africa (SSA), especially under subsistence farming operations where the use of concentrates is minimal (Nurfeta & Eik, 2014). The relatively high GE in MKC makes it a potential feed ingredient to supplement energy in both ruminant and monogastric animal diets.

The CP value of MKC (Table 3.1), is comparable to 47.0% CP reported by Mthiyane et al. (2017) and slightly higher than 39% CP value reported by Malebana et al. (2017). Soybean meal and cottonseed meal are the major proteins of plant-origin imported into Botswana from South Africa (Ministry of Agriculture, personal communication). The value of 45.3% CP in MKC in the present study is similar to 45% CP value in soybean meal but higher than 34.7% CP value in cottonseed meal reported by Kamalak et al. (2005). Interestingly, CP value of *Terminalia sericea* seed meal of 46.2% reported by Chivandi et al. (2013) is comparable to MKC crude protein concentration in the present study. Taken together, the chemical composition of the current study shows that non-conventional seedcakes like that of morula kernel have the potential to replace or partially replace conventional protein sources like SSC in animal rations to supply both protein and energy.

Neutral detergent fibre (NDF) as explained by McDonald et al. (2011) contains cellulose, hemicellulose and lignin which are all major components of plant cell wall. The rumen microbes are responsible for degradation of the cell wall and ultimately produce volatile fatty acids which are used as an energy source by the host animal. Sunflower seed cake in the current study had higher NDF content than MKC (Table 3.1). The value of 14.9% NDF in MKC in the current study is similar to 14.5% NDF value in MKC reported by Malebana et al. (2017), but lower than the 28% reported by Mlambo et al. (2011). The low NDF content in MKC compared to SSC in the present study is attributable to higher level of CP and EE in MKC (NRC, 2001). However, in the current study, it was not established which proportion of NDF was digestible and indigestible in the oilseed cakes (Lund et al., 2007; Raffrenato et al., 2018).

3.4.2. Mineral composition

According to McDonald et al. (2011), most oilseed meals are poor sources of calcium for livestock diets but are relatively good sources of phosphorus as reflected in the current study (Table 3.2) where phosphorus was high in both MKC and SSC. The phosphorus content in MKC is similar to 1.1% phosphorus content of MKC reported by Malebana et al. (2017) and demonstrates that it can be used to supplement other feed ingredients limited in phosphorus and other minerals. Phosphorus is a very important mineral in energy metabolism and is a constituent of skeletal bones in animals (McDonald et al., 2011). Phosphorus and magnesium in MKC were above the ranges of 0.16% to 0.4% and 0.1% to 0.2%, respectively recommended for beef cattle (NRC, 2000). In contrast calcium, potassium and sodium were insufficient for requirements of ruminants (NRC, 2000; Mirzaei, 2012). The recommended levels in ruminants for calcium range from 0.2 to 0.6%, sodium from 0.1 to 0.4% (Mirzaei, 2012) and potassium from 0.5 to 0.7% (NRC, 2001) while the amount of these minerals in the present study were 0.1%, 0.01% and 0.01% for calcium, sodium and potassium respectively.

Most trace minerals function as activators of enzyme systems or components of organic systems (Pond et al., 1995). They are also important in immune function such as copper and zinc in metallothioneins (MTs) which are a family of metal-binding proteins that regulate among other things; alleviation of superoxide stress and bolstering immune defences (Vignesh & Deepe Jr, 2017). Additionally, copper plays an important role in normal hair or wool pigmentation, haemoglobin formation, constituent of erythro-cuprein and boost the immune system in young animals (McDonald et al., 2011). However, overfeeding of copper should be

avoided as it causes liver damage (McDonald et al., 2011). On the other hand, zinc in the current study was adequate for ruminants as the critical dietary level is 0.003 % (Mirzaei, 2012). The concentration values for copper, iron and zinc in MKC (Table 3.2) were lower than values of 0.03%, 0.04% and 0.06% as reported by Malebana et al. (2017) respectively. The difference in mineral content between studies may be attributable to differences in soil types (Mirzaei, 2012), ecological zones and the seasons when the fruits were harvested. Copper content in MKC was adequate for ruminants as the dietary requirement for copper ranges from 0.0008 to 0.0014 % (Khan et al., 2006 cited by Mirzaei, 2012). Nutritional profiling of MKC in the current study has indeed affirmed the concept that plant protein sources provide energy and minerals in addition to protein (Church, 1988).

3.4.3. Amino acids

Dietary proteins are very important as they provide amino acids needed for the synthesis of tissue proteins and other metabolically important nitrogen-containing compounds (McDonald et al., 2011). MKC and SSC had comparable total essential amino acids (Table 3.3). Among the essential amino acids, MKC was found to be rich in arginine which plays an important role in cell division, immune function and growth (Pond et al., 1995). Malebana et al. (2017) reported a comparative amino acids profile from hydraulic cold pressed MKC. MKC as shown in Table 3.3 is rich in glutamic acid, which plays an important role in normal metabolism in the pancreases and liver (Pond et al., 1995), important in cytokine production and regulation of immune response (Wu et al., 2004). Lysine content in MKC (Table 3.3) might be limiting for proper growth of young ruminant animals and poultry (Pond et al., 1995; NRC 2001). As a result, when using MKC as a feed ingredient, lysine supplementation may be considered in non-ruminants as reported by other researchers such as Malebana et al. (2017). Research in protein nutrition in the past decade has shown that protein is critical for boosting immune function of livestock infected with nematode parasites (Donaldson et al., 2001) and specific amino acids (cysteine and glutamine; Hoskin et al., 2002; leucine; Yu et al., 2000; sulphur-amino acids; Madibela et al., 2009) may be particularly important. Therefore, MKC may play a pivotal role in supplying necessary amino acids in parasitised livestock and other diseases for activation, boosting and maintaining immune response.

3.4.4. Fatty acids

The predominant fatty acids in MKC were found to be palmitic acid (methyl palmitate; 14.8%), stearic acid (7.1%), oleic acid (62.0%) and linoleic acid (10.7%). The results are similar to

values (palmitic acid; 14.2%, Oleic acid; 65.9% and linoleic acid; 6.7%) reported by Mazizi et al. (2020) on MKC nutrient profile. Additionally, Malebana et al. (2017) also reported the main fatty acids for MKC as palmitic acid (13.5%), stearic acid (7.7%), oleic acid (74.5%) and linoleic acid (6.8%) from hydraulic cold pressed samples. According to Mariod and Abdelwahab (2012) and Mariod et al. (2017), morula oil from the seed contains 14.1% palmitic acid, 67.2% oleic acid, 5.9% linoleic acid and traces of linolenic acid. The principal fatty acid in SSC in the current study was linoleic acid and this is similar to findings of Amores et al. (2014). The differences in chemical composition and quantity of fatty acids across the studies are attributable to industrial oil extraction methods (Malebana et al., 2017), analytical laboratory methods, harvest time, rainfall patterns and morula tree genetic differences (Mariod & Abdelwahab, 2012).

Fatty acids are important functionally in ruminants as they are components of cell membranes, can be used to generate energy in the animal and some are precursors to important growth hormones (eicosanoids) (McDonald et al., 2011). Saturated fatty acids are not harmful to rumen microbes, but UFA in ruminants are biohydrogenated in the rumen since they are harmful to rumen microbes (Wood et al., 2007). However, some UFA can by-pass rumen biohydrogenation and get incorporated into animal tissues and consequently appear in animal products such as meat (Turner et al., 2012) or milk (Bezzera et al., 2016). In fact, Oliveira et al. (2012) stated that in a dietary supplementary study of African oil palm (*Elaeis guineensis*) cake and SSC to dairy cows, it resulted in milk having high lauric acid (C12: 0) and palmitoleic acid (C16:1) with respect to African oil palm cake and high oleic acid (C18:1) for milk from SSC diet, reflecting the fatty acid profiles of the respective seedcakes. Therefore, in the current study, MKC with high oleic acid content can be used in ruminant diets with spin-offs of healthy animal food products rich in oleic acid. Furthermore, oleic acid is responsible for lowering cholesterol level in the human body (Bezzerra et al., 2016) therefore, animal products (milk and meat) from such fed ruminants will benefit human health. Polyunsaturated fatty acids especially the omega-3 family (alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic (DHA)) are very important for the health of mammals as they have anti-inflammatory properties. Also PUFA (omega-3 or omega-6) are responsible for boosting phagocytic capacity and activity of monocytes or neutrophils as an example (Al-Khalaifah, 2020) during immune response.

3.4.5. *In situ* dry matter (DM) and crude protein (CP) disappearance

Chemical analysis of feeds and feed ingredients is important in giving information about their nutrient concentration, however, it run short of all components of ruminant nutrition on two counts; a) microbial action alter protein ingredient entering the rumen such that post-ruminal composition of such protein is completely different from the dietary protein; b) chemical analysis does not mimic the process and dynamics of fermentation occurring inside the rumen (El-hassan et al., 2000). Therefore, additional information about rumen degradability of feeds is needed. Dry matter and CP disappearing from bags incubated in the rumen of steers increased with increasing incubation time. This is because fermentation of dry and organic matter is a time-bound phenomenon. A similar trend of the DM and CP disappearance was observed by other researchers (Kamalak et al., 2005; Todorov et al., 2016; Lei et al., 2018).

Ruminal bacteria that digest feed in the host animal grow by binary fission following exponential growth curve (Russel, 2002; Krawielitzki et al., 2006). In the present study, MKC dry matter degradation increased before 24 h time point and flattened up to 72 h. This suggests that inoculated bacteria in the nylon bags, during incubation in the steer, had access to soluble DM (soluble carbohydrates, pectin and cellulose) from MKC which resulted in rapid bacterial growth and multiplication during the first 24 h. Additionally, Jin et al. (2018) observed that varying communities of bacteria in the rumen adhere on feed particles sequentially, reflecting various feed nutrients available and accessibility as time progresses. However, a stationary phase in bacterial growth is eventually reached when DM degradation ceases as a result of extensively lignified tissue (Church, 1988) or substrate energy depletion (Russel, 2002). In the current study, at 24 h, 77.5% DM of MKC was degraded (Table 3.5). Todorov et al. (2016) reported that at 24 h, soybean (*Glycine max*) meal and sunflower (*Helianthus annuus*) meal dry matter were degraded to 92.7% and 71.2%, respectively.

The results for the current study in Table 3.5, show that SSC dry matter degradation at 24 h was lower than that of Todorov et al. (2016), who reported a value of 71%. Lei et al. (2018) reported that at 24 h, 80.6% DM of soybean meal and 75.1% DM of rape seed (*Brassica nupus*) meal were degraded. Sehu et al. (2010) reported 75.8% DM degradability at 24 h for groundnut meal (*Arachis hypogaea*), which is similar to MKC dry matter degradation in the current study. In addition, Sehu et al. (2010) stated that high EE (30.3%) in hemp (*Cannabis sativa*) seed drastically slowed dry matter (57.7%) degradability of hemp seed after 48 h incubation. In

contrast to the current study, 77.5% DM of MKC was digested even though it had similar level of EE of 33.9% (Table 3.1) to that of hemp seed (Sehu et al., 2010). This implies that the nature or form of morula cake oil had no toxic effects on rumen microbes which might arise through reduction of cation availability or direct contact as observed earlier with other oils like linseed (*Linum usitatissimum*) oil (Church, 1988; Zubiria et al., 2019).

Crude protein disappearance in both cakes at 48 h incubation time was similar and on average was 90.8% (Table 3.5). Comparable findings on CP disappearance (93.1%) for soybean meal were reported by Lei et al. (2018) and 88.9% for groundnut meal reported by Sehu et al. (2010). The CP disappearance of MKC at 48 h incubation time was higher than 79.9% and 72.4% for rapeseed meal and distillers dried grains, respectively as reported by Lei et al. (2018). This suggests that MKC protein might have had a lot of accessible hydrolysable sites or few disulphide bonds in the protein molecules which allowed microbial degradation (Church, 1988).

3.4.6. Dry matter degradability constants

The 'a' and 'b' fractions of DM were higher in MKC than SSC (Table 3.6), indicating higher readily fermentable fraction and potentially degradable DM in MKC than SSC. The "b" fraction represents NDF content of feed (Antunes et al., 2019) and its degradable magnitude signifies that of NDF which will be degradable with time. The soluble fraction "a" of DM in MKC was comparable to the "a" fraction value for canola meal (12.18%), as reported by Sehu et al. (2010). The potentially but slowly degradable "b" fraction of MKC dry matter was also comparable to the "b" fraction of canola meal (67.3%) reported by Sehu et al. (2010). Although, the results obtained for the soluble DM fraction "a" in MKC (12.4%) was much higher than the value reported by Mlambo et al. (2011) for milled MKC (2.5 %), "b" fraction was within range at 69.8%. On the other hand, Nkosi et al. (2019) reported higher "a" fraction value (43.3%) and lower "b" fraction value (50.1%) for MKC when compared to the current findings. The differences in the "a" fraction for the three MKC studies could be attributable to differences in handling or processing of the zero-hour bag in the laboratory (Lei et al., 2018). In the study by Mlambo et al. (2011) the zero-hour bags were rinsed in warm (39 ºC) water, Nkosi et al. (2019), zero-hour bags were washed with cold water, while in the current study the zero-hour bags were placed in a water bath (39 ºC) for 30 minutes. With regard to fibre components, the current study shows that, SSC had higher lignin content (Table 3.1) which

might have slightly impeded digestion of “b” fraction even though the cake significantly had higher content of cellulose and hemicellulose than that of MKC. This is reflected by significantly higher “b” fraction in MKC than in SSC. The “b” fraction of DM in MKC in the current study was also higher than the “b” fraction in soybean meal reported by Batajoo et al. (1998) and Lei et al. (2018).

The effective degradabilities (ED) of DM in MKC at outflow rate of 0.05/h and 0.08/h were comparable (Table 3.6) to ED values of MKC reported by Nkosi et al. (2019) for MKC (81.8% and 78.1%). However, current study ED values (0.05/h and 0.08/h) were higher than ED values reported by Lei et al. (2018) for soybean meal (68.4% and 60.9%), rapeseed meal (63% and 58.2%) and distiller’s dried grains (53.9% and 50%). Similarly, Mlambo et al. (2011) also reported a lower ED value of milled MKC dry matter (37.4%) when compared to the current study findings. These differences in the results between the MKC studies might be attributable to size of the bags and pore size (Mlambo et al., 2011; bag size 6 x 12 cm and pore size of 40 µm, Nkosi et al., 2019; bag size 10 x 20 cm and pore size 50 µm vs current study; bag size 5 x 10 cm and pore size of 50 µm) used for incubation, incubation animals (Mlambo et al., 2011; Matebele goats, Nkosi et al., 2019; Friesian cows vs current study Tswana steers), handling of zero hour bags and basal diet fed to incubation animals (Lei et al., 2018). However, the PD of 89.4% for MKC (Table 3.6) suggests that there was a high rumen microbial activity or fermentation as evidenced from other earlier studies using other alternative protein sources (Kamalak et al., 2005; Sehu et al., 2010; Madibela et al., 2013). Comparing IVDMD values in the present study (86% and 62.3% for MKC and SSC, respectively; Table 3.1) they are in consonance with PD values reported in Table 3.6.

3.4.7. Crude protein degradability constants

The “a” fraction of CP is used by rumen bacteria to derive nitrogen and their multiplication to facilitate digestion of fibre and in the process produce microbial protein (Antunes et al., 2019) that will later benefit the animal in terms of production. The “a” fraction of CP in MKC and SSC were similar (Table 3.7), suggesting that both will support similar rumen environment and ideally produce similar microbial protein if all other factors are constant. The “a” fraction of CP in SSC in the current study was within range of “a” fraction of CP observed by Kamalak et al. (2005) and Todorov et al. (2016) in SSC. The “b” fraction of CP in the current study was higher in SSC than MKC (Table 3.7). The “b” fraction of CP in SSC (Table 3.7) was

comparable to findings (70.9%) of Todorov et al. (2016). In another study, Nkosi et al. (2019) reported MKC "b" fraction of CP at 54% and PD value of CP at 96.7%. However, the "b" fraction of CP in MKC (Table 3.7) was higher than "b" fraction (54%) reported by Nkosi et al. (2019) but was similar to 77.9% for rapeseed meal as reported by Todorov et al. (2016) and comparable to 83.8% for soybean meal (Lei et al., 2018). Additionally, Mlambo et al. (2011) reported high "b" fraction value of nitrogen at 82.8% and potential degradable fraction of nitrogen at 84.4% for MKC. The ED of MKC (Table 3.7) at an outflow rate of 0.05/h and 0.08/h was lower than values (82.9% and 78.6%) for MKC reported by Nkosi et al. (2019). However, Kamalak et al. (2005) and Antunes et al. (2019) reported lower ED values for cotton seedcake at outflow rate of 0.05/h when compared to the current study. Variation in degradation of protein feed ingredients might be attributed to factors like; variability in chemical composition of feed incubated, basal diet of cannulated animals and amount of sample incubated (Sehu et al., 2010). The findings from the current study suggest that the relatively high EE on MKC does not form a protective cover or layer on the feed to a degree that might impede CP degradability in the nylon bags. Additionally, the higher ED of protein in MKC imply increased protein supply to the abomasum and small intestine and will be beneficial for high productive livestock.

According to Church (1988) and NRC (2000), amino acids from microbial protein alone are not adequate to meet the needs of growing and high producing animals. Consequently, it was important to characterise MKC protein in terms of RDP and RUP to determine the amount of by-pass protein that may contribute to metabolisable protein (MP). Only one study (Nkosi et al., 2019) was found that determined RUP of MKC. In the current study, MKC had higher RDP and RUP than SSC (Table 3.7). The 14.8% DM of RUP in MKC was comparable to 17.5% DM of RUP of soybean meal (NRC, 2000). The seemly high RDP and RUP from MKC (35.4% and 14.8% respectively) than SSC (19.4% and 9.8%) imply that diets from MKC would support microbial needs for nitrogen and by-pass protein, thus the host animal benefiting from synergic provision of both microbial protein and RUP.

3.5. Conclusion

MKC as a potential feed ingredient is rich in energy, CP, phosphorus and magnesium when compared to SSC. The main fatty acids found in MKC are palmitic acid, stearic acid, oleic acid

and linoleic acid. MKC when compared to SSC had lower NDF and ADL, which are very important determinants of feed intake. Rumen undegradable protein was higher in MKC than in SSC and was comparable to RUP in soybean meal. MKC CP and DM were effectively degraded (ED; 0.05/h and 0.08/h) than in SSC. This further suggests that MKC has a potential to provide rumen microbes with soluble nitrogen and rumen undegraded protein and other nutrients needed for microbial protein production in ruminant animals.

3.6. Recommendations

- An animal study evaluating nitrogen balance (NB) when feeding MKC in complete diets of lambs should be conducted.
- An animal study evaluating graded inclusion levels of MKC in complete diets of lambs should be conducted to establish at what level animal performance would be maximised.
- Evaluating whether Morula kernel cake can be fed to supplement both protein and energy in ruminant diets is required.
- Calcium, potassium and sodium should be supplemented in ruminants when feeding MKC.

After chemical characterisation of MKC, a lamb feeding trial in Chapter 4 was conducted to understand how graded inclusion of MKC in complete diets of lambs will affect growth performance and nitrogen utilisation. It is very important to test a new feed ingredient *in vivo* as it will show true animal response under various interacting factors like climate, feeding and animal housing.

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CHAPTER 4

EFFECTS OF FEEDING MORULA KERNEL CAKE-BASED DIETS ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND NITROGEN RETENTION IN SHEEP

Abstract

Twenty (20) intact male lambs average aged seven (7) months with an average body weight of 21.6 ± 4.1 kg were used in a 76-day feeding study conducted at Department of Agricultural Research, Sebele, Gaborone. The study was conducted to evaluate feed intake, nutrient digestibility, nitrogen balance and growth performance in Tswana lambs fed MKC-based diets. The experiment was a completely randomised design (CRD) with five (5) animals per treatment. Treatments were based on inclusion levels of morula kernel cake (MKC) at 0%, 4%, 8% and 12 % in diets designated treatment A, B, C and D respectively. In treatment A urea was used as a source of nitrogen (N). Dry matter intake was significantly ($P < 0.05$) higher in MKC treatments than in the control treatment. There were no differences ($P > 0.05$) observed on final weight, weight gain, average daily gain and feed conversion ratio across these treatments. However, MKC treatments averaged together improved average daily gain and total weight gain by 43.2% and 42.7%, respectively. Inclusion of MKC linearly increased ($P < 0.05$) lambs' ether extract intake. The intake of organic matter, crude protein, ash, neutral detergent fibre and acid detergent fibre were similar ($P > 0.05$) across the treatments. With regard to nitrogen intake, absorbed-N, N-retention, retention-intake (%) and retention-absorbed (%) there were no treatment effects ($P > 0.05$) observed. But MKC treatments when averaged together had numerically higher N-retention value (7.3 g/day) than control (Treatment A; 4.3 g/day). Additionally, there was a quadratic increase (g/day) in urine-N ($P < 0.05$) with rise in the amount of MKC in diets. The inclusion rate of MKC at 4 % to 12 % in diets of lambs positively affected animal growth. Therefore, MKC can be applied in sheep diets at various level from 4 to 12% without adverse effects on nutrient digestibility, nitrogen retention, growth and hence qualifies as an alternative protein source.

Key words: **Growth, morula kernel cake, nitrogen balance, sheep**

4.1. Introduction

In most tropical and subtropical countries, the livestock sector is anchored on natural pastures in rangelands for supply of all required nutrients. However, natural pastures are characterised by seasonal biomass availability and quality of its forages which is high during the rainy season but low during the dry season. Therefore, nutrition is the major factor hampering animal productivity especially during the dry season (Mine et al., 2002; Aganga et al., 2005). In fact, feed costs account for 60-70% of the total production costs in livestock enterprises (Thirumalaisamy et al., 2016). The high cost of feed ingredients is also compounded by stiff competition between humans and animals for available grains such as maize grain that is mainly used as an energy source in animal diets. Various feed ingredients used for ruminant feeding may be classified as energy feeds, protein supplements, mineral and vitamin supplements (Aganga & Nsinamwa, 1997). Protein and energy ingredients contribute to a higher cost to livestock production (Beshir & Babiker, 2009). The major conventional plant protein supplements used in livestock feeding globally are soybean meal, canola cake, cottonseed meal and sunflower cake (Wijayanti et al., 2020). The demand for protein sources is very high especially that protein is one of the critical nutrients needed for young growing animals (Beshir & Babiker, 2009). Conversely, there is currently a high demand for animal protein food coupled with an increase in human population (Aboul-Fotouh et al., 2015). With infrastructural developments occurring in most human settlements agricultural land is being targeted for such developments thereby reducing land earmarked for fodder production and other agricultural use. Therefore, cultivation of crops to supply protein of plant origin may not be a priority in crop-livestock production system in the tropics and sub-tropics, a situation that calls for a new agenda to supply of supplementary feeds for livestock.

Non-conventional protein ingredients offer a window of opportunity as alternatives to pricy conventional protein supplements and can be used to circumvent limitations posed by climate change on livestock productivity. According to Aganga and Nsinamwa (1997), Botswana's rangelands consist of a lot of browse plants that are a potential source of livestock feed. *Sclerocarya birrea* (Morula) tree is one of the most known tree species (Moss, 1988) occurring naturally in Botswana. Morula tree produces edible fruits rich in vitamin C (Roodt, 1988). The fruits of morula tree can be eaten fresh when ripe or fermented to make alcoholic drinks. The seed kernel from the fruit can be eaten or used to extract oil. MKC is an industrial waste obtained during extraction of oil in Morula seed kernel (Mlambo et al., 2011; Mdziniso et al.,

2016). MKC is rich in crude protein (29% to 41%) as reported by Mdziniso et al. (2016) and Malebana et al. (2017). Although MKC has a good amino acid profile, lysine might be the limiting amino acid in growing monogastric animals. MKC is also rich in gross energy (28.5 MJ/Kg DM) and phosphorus (Malebana et al., 2017). According to Sunil et al. (2015), oilseed cakes are good sources of proteins even though they might have anti-nutritional compounds like protease inhibitors, trypsin inhibitors, tannins and phytates which could inhibit nutrient utilisation and limit ingredient inclusion level in complete animal diets. For instance, McDonald et al. (2011) stated that inclusion rate of sunflower seed meal in total diet of sheep is 15%, while Niger seedcake (*Guizotica abyssinica*) inclusion can go up to 57% in concentrate diets of calves, but Guar meal (*Cyamopsis tetragonoloba*) inclusion is limited at 10-15% in diets of cattle (Animal Nutrition group, 2012). Tzamoloukas et al. (2021) concluded that safe inclusion of olive by-products (olive cake silage, dried olive cake, olive cake) should be limited at 15-20% in complete diets of ruminants. To date no data is available on inclusion level of MKC as a sole protein source in complete diets of ruminants. Therefore, this study was done to evaluate the effect of feeding diets with graded levels of MKC on growth performance and nitrogen retention of Tswana sheep.

4.1.1. Specific objectives

- To evaluate the feed intake (FI), growth, average daily gain (ADG)and feed conversion ratio (FCR) of sheep fed diets with graded levels of MKC.
- To evaluate the nutrient digestibility and NB by Tswana sheep fed on MKC based diets.

4.1.2. Hypotheses

(a) H_0: There is no improvement due to graded inclusion of MKC in sheep diets on feed intake, FCR, ADG and final weight.

(b) H_0: The inclusion of graded levels of MKC in ruminant diets do not result in improvement in apparent nutrient digestibility and nitrogen balance (NB) in sheep.

4.2. Materials and Methods

4.2.1. Site and diets

A feeding trial was conducted at Department of Agricultural Research (DAR), Sebele Research Station, Gaborone. The study area is situated between latitude of 24º 33'S and longitude 25º 51'E at an altitude of 994 m above sea level (Madibela et al., 2004). Cold-pressed MKC was obtained from DLG Naturals Pty Ltd based in Gabane, Botswana. Other feed ingredients used in feed formulations were sourced from other local livestock feed suppliers. The diets of lambs were formulated using Aries software (California, USA) to meet nutrient requirements (protein=14.7% and energy=78% TDN or ME 11.8 MJ/kg) of sheep aged approximately 7 months old as outlined by National Research Council (NRC, 1985). The diets were isonitrogenous and isoenergetic and MKC was used to formulate complete diets at 0, 4, 8, 12% levels designated as A, B, C and D, respectively. The treatment ingredients and chemical composition of the diets are shown in Table 4.1. Treatment A was used as the control (without MKC and urea was used as a source of N in the diet).

Table 4.1: Ingredient and chemical composition of dietary treatments

	Treatment			
Ingredient (%)	A	B	C	D
Ground maize grain	58.3	64.2	60.4	56.6
Sorghum stover	18.7	25	24	23
Morula kernel cake	0.0	4.0	8.0	12
Wheatbran	18.7	2.9	4.3	5.6
Urea	1.6	1.6	1.0	0.5
Molasses	1.2	1.0	1.0	1.0
Feed lime	0.7	0.8	0.8	0.8
Dicalcium phosphate	0.5	0.4	0.4	0.4
Sodium chloride	0.3	0.1	0.1	0.1
Lab results[1] (%)				
Dry matter	90.1	96	96.3	96.2
Organic matter	91.8	93.5	94.2	94.9
Crude protein	14.9	16.5	14.7	15.7
Ether extract	3.1	4.5	4.6	6.3
GE (MJ/Kg)	16.9	17.3	17.5	18.3
ME (MJ/Kg)[2]	8.5	8.7	8.8	9.2
Neutral detergent fibre	44.9	36.5	42.1	44.4
Acid detergent fibre	20.1	15	17	21.6

[1]Laboratory Chemical composition results= % dry matter basis, [2]ME=Calculated metabolisable energy (ME=Gross energy * 0.5; adopted from NRC, 2007 cited by Ma et al., 2019).

4.2.2. Growth trial

Twenty (20) intact male Tswana lambs of seven (7) months old were used in a CRD experiment running for ninety-seven (97) days comprising 21 days of adaptation and 76 days of data collection. The sheep were dewormed using Ivermax (Cipla Agrimed Pty, Ltd, Durban, South Africa) administered at 1 ml subcutaneously per sheep. Animals were weighed for two consecutive days to determine their initial weights and then randomly allocated to four (4) groups balanced for weight. Thereafter, the groups were randomly allocated to treatments. There were five (5) animals per treatment and each animal was individually housed in a pen

measuring 2.5 m x 1 m. The pens had a common roof with concrete floors and an enclosure made of timber all round. This enabled the lambs to interact visually as the individual pen walls were not made of mortar and bricks. The lambs were fed their respective diets *ad libitum* (Table 4.1) in pens and offered diets adjusted daily to allow for 10 % daily refusals. Fresh water was provided daily ad *libitum* in pens before fresh feed was offered. Pens were cleaned every morning. The leftovers from the previous day for each animal were weighed using a platform electronic scale (Digital scale; model DS-530, Teraoko Seiko, Japan) to determine daily feed intake. Animals were weighed every two (2) weeks for two consecutive days in the morning before feeding using a walk-in scale (crane scale; Tal tec Pty, Ltd, South Africa). Representative diet samples and feed ingredients were collected in sampling bags for laboratory analysis. At day 76 of data collection, the growth trial was terminated and animals were weighed for two (2) consecutive days to determine final body weights.

4.2.3. Nitrogen balance (NB) and Digestibility experiment

A fourteen (14) day NB and digestibility study was conducted immediately following the growth experiment. Three (3) intact male Tswana sheep aged 10 months on average were taken from each treatment used in the growth experiment to form treatment groups with similar average weight. The lambs were individually kept in a metabolism crate designed for separate collection of urine and faeces. Faecal collection was achieved by fitting lambs with collection bags. The lambs continued to eat their respective diets from the growth experiment. At the beginning of the trial, a seven (7) day period was allowed for the lambs to adjust to the metabolism crates. Thereafter, the remaining seven (7) days were used for data and sample collection (feed, urine and faeces). The animals were given feed *ad libitum* and the amount of feed given was adjusted daily to allow for 10% daily feed refusal. The sheep complete diets had graded levels of MKC at 0% MKC, 4% MKC, 8% MKC and 12% MKC as described in the growth experiment and were designated A, B, C and D, respectively. Fresh water was provided daily to each individual sheep *ad libitum*. Each morning, before providing fresh feed, the leftovers from the previous day were weighed and used for determination of daily feed intake. The daily total faeces for each animal were collected and weighed in the morning before feeding and watering. About 10% of the faecal material was collected and oven dried at 70 ºC for 72 h and bulked for each animal to use for chemical analysis (Lakpini et al., 2015). The urine of each animal was also collected into plastic container with 25 ml of 10% H_2SO4 (Lakpini et al., 2015). Thereafter, 10% of the measured daily urine was sampled, composite for

each animal was collected in labelled plastic bottles and stored in the refrigerator at 4 ºC until analysis of nitrogen content. Daily samples of feed were also collected and composited per treatment for chemical analysis of dry matter (DM), crude protein (CP), organic matter (OM), ether extract (EE), Ash, neutral detergent fibre (NDF) and acid detergent fibre (ADF). The feed samples and faecal samples were ground using 2 mm sieve in an electric grinder (Grinder Thomas-Wiley, laboratory mill model 4, Arthur Thomas Company, USA) before being subjected to chemical analysis.

Nitrogen balance (NB) =Nitrogen Consumed-Nitrogen in excreta (faeces + urine)

Basal endogenous nitrogen (BEN) was calculated using the following equation by Agricultural and Food Research Council (AFRC) (1993):

$BEN(g/d) = (0.35 + 0.018) \times BW^{0.75}$

The corrected value for Nitrogen retention is;

$NR(g/d) = NB - BEN$

Apparent digestibility (AD) of nutrients was calculated using the following formula by Ekeocha, (2012):

$$Nutrient\ digestibility = \frac{Nutrient\ consumed - Nutrient\ in\ faeces}{Nutrient\ consumed} \times 100$$

4.2.4. Feed and faecal Analysis

The proximate components (dry matter, crude protein, ash and ether extract), NDF, ADF and ADL of the experimental feed and faecal samples were analysed as described in Chapter 3 of the thesis at DAR, Animal Nutrition Laboratory,

4.2.5. Data analysis

For time series data (fortnight body weights) proc mixed procedure of SAS (2002) was used for analysis using repeated measures as per model 1. The statistical differences between means were done using Fisher's least significance difference (LSD). The model used is as shown below;

$Y_{ijk} = \mu + T_i + C_j + (T_i \times C_j) + \varepsilon_{ijk}$ Model 1

Where Y_{ijk}= is the Kth observation of the ith treatment of the jth days, μ=is the overall mean, T_i= is the fixed effect of the ith treatment (i=1,2…4), C_j=is the (time) days (j=1, 2….6), $T_i \times C_j$= interaction between treatment and time (days), ε_{ijk}k=random residual error

Data on growth performance (Final body weight, weight gain, ADG, DMI, FCR, nutrient intake and digestibility and NB) were analysed using proc GLM of SAS (2002) using model 2. Initial weight was used as a covariate and was dropped when found not significant. The means of analysed data were presented in tables. The data on nitrogen balance and digestibility were also subjected to response surface regression analysis and evaluated for linear and quadratic effects using polynomial constrasts following the quadratic model: $y=ax^2 + bx + c$, where y= response variable; a and b are the coefficients of the quadratic equation; c is intercept; and x is dietary MKC (%) as described by Manyeula et al. (2021). Statistical significance was declared at $P < 0.05$ except for trends in which significance was declared at $P < 0.1$.

$Y_{ijk} = \mu + T_i + \varepsilon_{ijk}$……Model 2

Where Y_{ijk} = is the Kth observation of the ith treatment; μ = is the overall mean; T_i = is the fixed effect of the ith treatment (i=1,2…4); ε_{ijk} = random residual error

4.3. Results

4.3.1. Nutrient intake

The results of nutrient intake from the digestibility study of lambs fed graded levels of MKC are presented in Table 4.2. Organic matter intake (OMI), crude protein intake (CPI), ash intake, neutral detergent fibre intake (NDFI) and acid detergent fibre intake (ADFI) were not influenced by the levels of inclusion of MKC in the diets of lambs. There was an increasing linear effect of the levels of MKC on intake of ether extract (y=24.5(± 3.2) + 2.9 (± 1.3) x; R^2=0.83, P= 0.02) by lambs. Lambs in treatment D had a significantly (P < 0.05) higher intake of EE (54.7 g/day) whereas lambs in treatment A had the least intake of EE (23.7 g/day). On the other hand, there was also a tendency (P=0.1) for lambs' intake of ADF to increase quadratically as MKC increased in the diet.

Table 4.2: Nutrient intake (% DM) of intact male lambs fed graded levels of MKC in complete diets

	Treatment effects						Polynomial effects	
Item, g/day	A	B	C	D	RMSE	*P*-value	Linear	Quadratic
OM	705	795.7	880.3	826	106.8	0.7	0.5	0.8
CP	114.3	140.3	137.0	137.0	17.9	0.5	0.2	0.7
EE	23.7^{c}	38.0^{b}	42.7^{b}	54.7^{a}	5.4	0.02	0.02	0.7
Ash	66.3	55.3	54.0	44.3	46.4	0.08	0.2	0.9
NDF	344.7	310.3	393.3	386.7	46.4	0.5	0.3	0.6
ADF	154	128	158.3	188.3	20.4	0.2	0.1	0.1

OM= organic matter, CP= crude protein, EE= ether extract, NDF= neutral detergent fibre, ADF= acid detergent fibre, A= 0% MKC, B= 4% MKC, C= 8% MKC, D= 12% MKC, RMSE= root mean standard error, ^{abc}Means with different superscripts within the same row are significantly different (P < 0.05).

4.3.2. Digestible nutrients intake and apparent digestibility

Apparent digestible nutrient intake and nutrient digestibility of intact male lambs are shown in Table 4.3. Lambs intake of digestible organic matter (DOM), digestible crude protein (DCP), digestible neutral detergent fibre (DNDF), digestible acid detergent fibre (DADF) and

digestible ash were similar across the treatments (P > 0.05). Intake of digestible ether extract (DEE) was higher (P < 0.05) in lambs from treatment D (49.8 g/day) while lambs from treatment A had the lowest (P < 0.05) intake of DEE (21.1 g/day). A linear increasing effect (y=21.8 (±3.0) + 2.4 (± 1.2) x; R^2=0.83, P= 0.03) was observed for DEE with increasing levels of MKC in the diets of lambs (Table 4.3). Digestible dry matter intake (DDMI), DOM intake, DNDF intake, DADF intake and digestible ash intake were not significant (P > 0.05) for either linear or quadratic effects. No differences were observed across these treatments for apparent digestibility coefficients of DM, OM, CP, EE, NDF, ADF and ash. However, DCP tended to increase linearly (P=0.07) as MKC increased in the diet. In addition, lambs' intake of DADF tended to show a quadratic decrease (P=0.06) as MKC increased in the diet.

Table 4.3 : Digestible nutrient intake (g/day) and apparent nutrient digestibility (%) of intact male lambs fed on graded levels of MKC in complete diets

	Treatment effects						Polynomial effects	
Item	A	B	C	D	RMSE	*P*-value	Linear	Quadratic
Digestible nutrients intake								
OM	548.5	674.5	730.9	710.6	93.9	0.5	0.2	0.9
CP	88.1	123	116.8	121.3	17.1	0.3	0.07	0.98
EE	21.1^{c}	33.6^{b}	38.1^{b}	49.8^{a}	4.7	0.02	0.03	0.9
Ash	31.8	37.5	35.9	37.5	10.3	0.8	0.6	0.6
NDF	240.4	231.3	286.5	310.9	43.2	0.6	0.7	0.3
ADF	96.9	82.5	96.7	143.3	20.9	0.1	0.4	0.06
Apparent digestibility								
DM	75.5	83.4	82.6	85.5	6.6	0.5	0.2	0.4
OM	77.4	84.5	83.5	86.3	6.1	0.5	0.2	0.4
CP	76.5	87.4	85.7	90	6.1	0.2	0.07	0.3
EE	88.5	88.2	89.5	91.3	4.2	1.0	0.9	0.7
Ash	49.3	67.0	67.7	69.3	14.9	0.4	0.2	0.3
NDF	69.3	73.8	73.6	80.8	9.3	0.7	0.6	0.4
ADF	62.4	63.7	62.2	76.6	12.6	0.6	0.9	0.3

A= 0% MKC; B = 4% MKC; C = 8% MKC; D = 12% MKC; RMSE = root mean standard error; ^{abc}Means with different superscripts within the same row are significantly different ($P < 0.05$). OM= organic matter; DM= dry matter; CP= crude protein; EE= ether extract; NDF= neutral detergent fibre; ADF= acid detergent fibre.

4.3.3. Nitrogen balance

The NB of intact male lambs fed varying inclusion levels of MKC is shown in Table 4.4. Nitrogen intake by lambs did not differ ($P > 0.05$) between the treatments. However, lambs in treatment A had numerically lower nitrogen (N) intake of 18.3 g/day. The amount of Urine-N excreted by the intact male lambs was higher ($P < 0.05$) for treatment B (7.7g/day), intermediate for treatment C and D (6.2 g/day and 6.6 g/day respectively) and lower for treatment A (5.2 g/day). Additionally, Urine-N excretion by lambs tended to increase ($P < 0.1$)

quadratically with graded incorporation of MKC in the diets of lambs. The amount of Faecal-N excreted across the treatments was similar (Table 4.4). Faecal-N content ranged from 2.5 g/day to 4.2 g/day. Urinary-N ranged from 5.2 g/day to 7.7 g/day. The amount of nitrogen excreted, absorbed-N, BEN, N-retention, %retention-intake and %retention-absorbed were not different among the treatments ($P > 0.05$). However, absorbed-N tended to increase quadratically ($P=0.1$) as MKC increased in the diet. Percentage (%) retention-I and % retention-A tended to increase linearly ($P=0.1$) as MKC increased in the diet.

Table 4.4 : Nitrogen balance of intact male lambs fed on graded inclusion levels of MKC in complete diets

	Treatments effects						Polynomial effects	
Item (g/day)	A	B	C	D	RMSE	*P*-value	Linear	Quadratic
N-intake	18.3	22.5	22.0	21.9	2.8	0.5	0.6	0.2
Urine-N	5.2^{b}	7.7^{a}	6.6^{ab}	6.2^{ab}	0.9	0.03	0.7	0.1
Faecal-N	4.2	2.8	3.2	2.5	1.2	0.4	0.3	0.4
Excretion	9.4	10.6	9.8	8.7	1.9	0.2	0.4	0.4
Absorbed-N	14.1	19.6	18.7	19.4	2.7	0.3	0.3	0.1
Retention								
BEN	4.5	5.4	5.6	5.9	1.0	0.3	0.1	0.7
N-retention	4.3	7.2	7.0	7.8	2.1	0.4	0.2	0.4
Retention-I (%)	22.9	31.7	32.3	35.7	8.4	0.3	0.1	0.6
Retention-A (%)	29.0	36.2	37.6	40.2	8.3	0.2	0.1	0.7

N=nitrogen, BEN= basal endogenous nitrogen, Retention-I= retention intake, Retention-A= retention absorbed, B= 4% MKC, C= 8% MKC, D= 12% MKC, RMSE= root mean standard error. ^{abc}Means with different superscripts within the same row are significantly different ($P < 0.05$).

4.3.4 Growth performance

The growth performance of intact male Tswana lambs fed on graded levels of MKC is shown in Table 4.5. The increasing levels of MKC in the diets of lambs did not affect ($P > 0.05$) final weight, weight gain, ADG and FCR (Table 4.5). However, lambs on the control diet had numerically lower final weight (28.5 kg) and total weight gain (8.4 kg) while MKC treatments averaged together had numerically higher final weight (33.5 kg) and total weight gain (12 kg). Lambs on the control diet (treatment A=0 % MKC) and MKC treatment diets had similar ADG and FCR. But lambs fed treatment A had numerically the lowest ADG (110.9 g/day) while ADG in MKC treatments averaged together was 158.8 g/day. Dry matter intake (DMI) was significantly lower ($P < 0.05$) for lambs fed on treatment A (667.3 g/day) compared to the averaged DMI of all lambs fed on MKC diets (867.7 g/day). The DMI showed a linear increase ($y= 671(\pm 41.5) + 48.2 (\pm 15.6) x$; $R^2=0.61$; $P=0.03$) response ($P < 0.05$) with treatment D

having higher value of 885.5 g/day. However, DMI was similar among the MKC treatments. Treatment had no effect (P > 0.05) on FCR although lambs fed on treatment A had a numerically poor FCR (6.6). In addition, there was a tendency for total weight gain (P=0.1) and average daily gain (P=0.09) to increase linearly as MKC increased in the diet. Final weight, total weight gain, ADG and FCR were not significant for linear or quadratic effects (P > 0.05).

Table 4.5 : Effect of graded dietary inclusion level of MKC on growth performance of intact male Tswana lambs

	Treatments effects						Polynomial effects	
Item	A	B	C	D	RMSE	*P*-value	Linear	Quadratic
IWT (kg)	20.1	23.4	21.9	22.1	2.6	0.9	-	-
FWT (kg)	28.5	34	33.4	33.1	2.5	0.5	0.2	0.8
WTG (kg)	8.4	11.7	12.3	12.0	2.7	0.4	0.1	0.6
ADG (g)	110.9	154.3	164.2	157.9	34.5	0.3	0.09	0.6
DMI (g)	667.3[b]	833.0[a]	884.5[a]	885.5[a]	65.2	0.01	0.03	0.46
FCR	6.6	5.6	5.5	5.8	1.5	0.8	0.4	0.7

Note: IWT=initial body weight, FWT, final body weight, WTG= body weight gain, ADG= Average daily gain, DMI= dry matter intake/day, FCR= feed conversion ratio (DMI/ADG), A= 0% MKC, B= 4% MKC, C= 8% MKC, D= 12% MKC, RMSE=root mean standard error

The mean fortnightly body weights of intact male lambs fed on graded dietary inclusion of MKC in complete diets are shown in Figure 4.1. In Figure 4.1, the mean fortnightly body weights were similar across the treatments (P > 0.05) throughout the duration of the experiment. Although the line graph showed that fortnightly body weights of MKC treatments were numerically superior to control treatment throughout the growth study.

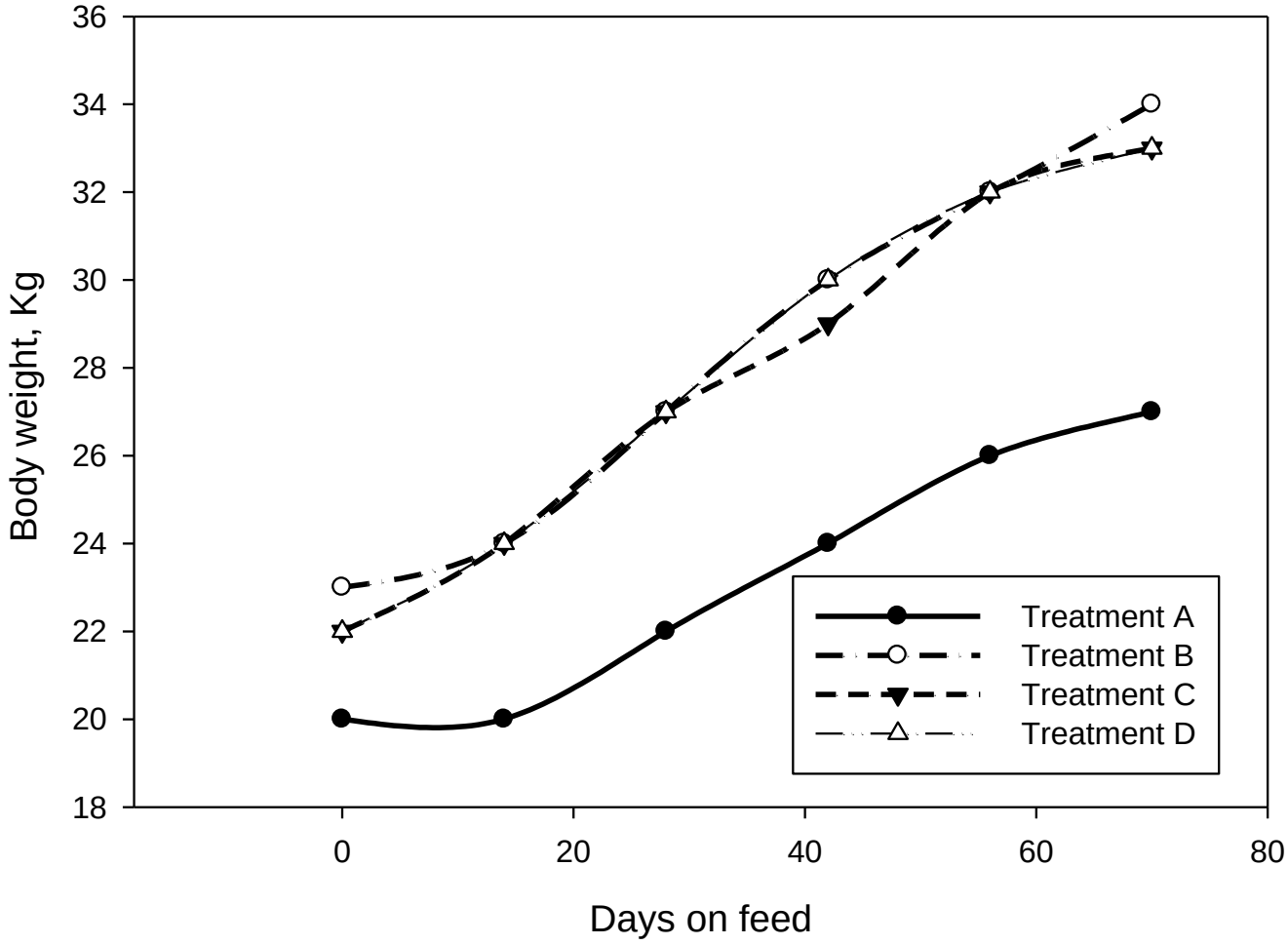

Figure 4.1 : Fortnightly body weights of intact male lambs fed on graded dietary inclusion of MKC in complete diets of lambs**.** A=0% MKC, B=4% MKC, C= 8% MKC, D=12% MKC

4.4. Discussion

4.4.1. Dry matter and nutrients intake

Dry matter intake (DMI) is optimised when feed eaten by growing animal provides all the nutrients required by rumen microbes and tissues of the host animal. In the current study, DMI was influenced by inclusion of MKC and averaged 867.7 g/day for all MKC treatments. The lambs from treatment A (without MKC) diet had a lower DMI (667.3 g/day) which is associated to numerically lower dry matter and organic matter digestibility coefficients (Table 4.5). However, DMI as a percent of body weight across the treatments was within the range of

2-5% recommended by NRC (1985) report for small ruminants. In contrast, Malebana (2018) reported an average DMI of 938.8 g/day by intact male Dorper lambs when levels of 0%, 5%, 9%, 14% or 19% MKC substituted for soybean meal in complete sheep diets. The discrepancies in DMI between the two studies are attributed to differences in diet composition, breed and animal management; (for example, inclusion of soyabean meal and use of Dorper instead of local breed). Organic matter intake was similar among the treatments and according to Castro et al. (2020), would have a direct effect on intake of other nutrients evaluated as reflected in Table 4.2. Intake of CP by lambs in the current study averaged 138 g/day for MKC treatments and it was similar to intake of CP (138 g/day) by intact male Dorper lambs fed by Malebana (2018) on complete diet including 19% MKC. Therefore, this suggests that adequate nitrogen was supplied to rumen microbes for lambs that were fed on MKC diets. In the process, rumen microbes grew, multiplied and efficiently digested the ingested feed culminating in increased DMI when compared to treatment A. However, in general, daily intake of CP in the current study seemed to have met protein requirements of seven months old Tswana lambs.

Lambs from treatment D had higher intake of EE (54.7 g/day) than other treatments. This is attributed to treatment D diet having inherently higher EE (Table 4.1; 6.3%). In contrast to diets from the current study, Malebana (2018) reported much higher EE intake ranging from 110.7 g/day (0% MKC diet) to 123.4 g/day (19% MKC diet) in a feeding trial of intact male Dorper sheep fed on complete diets with levels of 0%, 5%, 9%, 14% or 19% of MKC replacing soybean meal. The amount of EE (6.3%) in diet D in the current study did not negatively affect DMI by the sheep. However, Alves et al. (2016) reported lower DMI by lambs fed on complete diets with sunflower seed or cake. The authors pointed out that the high EE level (7.9%) was responsible for reduced DMI when compared to the complete diet with soybean meal (ether extract=3.4%).

The use of graded inclusion of MKC in the current study resulted in partial replacement of wheatbran (Table 4.1) without compromising energy density of the diet. The diets were formulated isoenergetic and as such had metabolisable energy within the same range (Table 4.1). According to Wang et al. (2019), energy intake levels have a direct effect on animal growth or average daily gains. Therefore, this suggests that lambs from treatment A with numerically lower intake of organic matter, ether extract and crude protein may have a lower productive performance when compared to MKC treatments. The diets in the current study had

a comparable intake of digestible NDF and ADF as shown in Table 4.3. Neutral detergent fibre is considered as a major feed attribute that regulates DMI (McDonald et al., 2011). In the current study, it seems NDF from MKC treatments was more digestible as evidenced by numerically higher values of apparent NDF digestibility when compared to treatment A. This partly explains the significantly higher DMI in MKC treatments.

4.4.2. Digestible nutrient intake and apparent nutrient digestibility

Digestibility is the proportion of feed eaten that is digested and absorbed into the body of the animal (McDonald et al., 2011). Digestible nutrients are used for animal growth, development (Wang et al., 2019) and other productive purposes (milk production, pregnancy and immune function). There were no differences recorded across the treatment for the intake of DDM, DOM, DCP, DNDF and DADF. However, treatment D had a numerically superior values on intake of all digestible nutrients evaluated (Table 4.3) except for intake of DOM. There was a tendency (P=0.07) for linear increase in intake of DCP due to treatment and this result is attributed to numerically high digestibility coefficient of crude protein and other nutrients provided to rumen microbes from MKC. There was a linear increase on intake of DEE with graded inclusion of MKC in the diet. Digestible ether extract (DEE) had an increase of 2.4 g/day with the addition of each percentage unit of MKC to the diet. This is attributed to high digestibility of EE (Table 4.3). In addition, digestibility coefficient of OM, CP, EE, NDF and ADF were similar across treatments. This implies that the conditions for rumen fermentation were adequate and rumen microbes were able to use the available carbohydrates and degradable nitrogen to make microbial protein (McDonald et al., 2011; Muhammad et al., 2016).

Therefore, MKC in the current study did not interfere with the digestibility of other nutrients and produced high total tract digestibility of the measured nutrients. In contrast, Castro et al. (2020) reported lower CP digestibility values (61%, 57%, 66% and 71%) and NDF digestibility values (57%, 57%, 52% and 49%) for complete sheep diets with inclusion levels of 0%, 8%, 16% and 24% for delinted cottonseed, respectively. The authors attributed the reduced apparent digestibility of the measured nutrients to high EE level which ranged from 5% (0% delinted cottonseed) to 6.4% (24% delinted cottonseed). Though EE content of diets used by Castro et al. (2020) were in same range as the current study, the differences between the studies could be due to other adverse compounds not measured by the two studies, absent in the current study

but present in Castro et al. (2020) diets. In the current study, lambs from treatment D numerically had higher apparent digestibility coefficients for the measured parameters.

4.4.3. Nitrogen balance

The digestion of protein to peptides and amino acids in the rumen is done by bacterial proteases and peptidases (Wang & MacAllister, 2002). Dietary protein is degraded to ammonia by rumen bacteria and for some feeds, another protein fraction may escape rumen digestion to get digested by the animal's enzymes in the lower tract. McDonald et al. (2011) stated that rumen ammonia plays an important role as a source of nitrogen used by rumen microbes to degrade feed and produce microbial protein. Although the amount of intake of nitrogen (N) by the lambs was similar across treatments, the nitrogen (N) retained in lambs fed diets with MKC averaged together was numerically higher (7.3 g/day) than in Treatment A (control; 4.3 g/day). Therefore, MKC treatments improved nitrogen retention (NR) by 71% compared to the control. It is likely that MKC provided additional bypass protein to the host animal over and above microbial protein from the rumen digestion. MKC contain rumen undegradable protein (RUP=14.8% DM; Chapter 3 of thesis) even though it can be classified as rumen degradable protein (RDP) source when compared to other natural protein sources like blood meal or carcass meal. In the current study, retained nitrogen from MKC treatments under practical conditions may result in heavier animals than those from the control thereby fetching a good price for the farmer since at the market animals are bought on weight basis. In another study, Castro et al. (2020) reported comparable NR values for delinted cottonseed treatments ranging from 5.9 g/day (8% delinted cottonseed) to 7.1 g/day (24% delinted cottonseed) in complete sheep diets with graded inclusion of delinted cotton seed (DCS) at 0%, 8%, 16% and 24%. The NR was similar across the treatments in the current study mainly because the CP content of the experimental diets was isonitrogenous and nitrogen intake was at par.

Cirne et al. (2015) stated that NB reflects nitrogen used in tissue accretion, new enzymatic system or repair of old tissue. On the other hand, animal feed with high RDP result in high ammonia production in the rumen in excess of rumen microbes needs. Therefore, ammonia-nitrogen (Ammonia-N) not utilised by the rumen microbes is converted to urea in the liver of the host animal and excreted in urine (McDonald et al., 2011). In the current study, the amount of urine-N excretion tended to increase quadratically with treatment B having higher urine-N than treatment A. The higher urine-N in treatment B may be attributed to numerically higher

nitrogen intake (Table 4.4) or perhaps higher utilisation of protein as a source of energy. In the current study urinary-N ranged from 5.2 g/day to 6.2 g/day and Faecal-N ranged from 2.2 g/day to 4.4 g/day. Faecal-N, though not statistically different, was lower for MKC diets and it arises from indigestible ingesta and endogenous losses. Additionally, in MKC treatments, the excretion of urinary and Faecal-N as a proportion of N intake in the present study was on average 31% and 12.8%, respectively. In a "supplementary feeding goat study" of Mlambo et al. (2011), urinary and faecal-N as a proportion of N intake was on average 49.3% and 12.3%, respectively, for MKC treatment. This data from the two studies shows that MKC is rapidly degraded in the rumen resulting in production of a lot of ammonia which will ultimately be excreted in urine. However, this can be minimised by synchronising supply of both soluble feed nitrogen and fermentable carbohydrates in the diet (Costa et al., 2021). There was also a tendency for linear increase (P=0.1) of %retention-intake and %retention-absorbed.

4.4.4. Growth performance

Growth in livestock is achieved by increase in body size and weight. In intensively managed animals for example, growth follows a sigmoid curve due to consistent supply of adequate nutrients (McDonald et al., 2011). The growth results in Figure 4.1 show that lambs across the dietary treatments followed a similar trend of growth and fortnightly weights were similar across treatments throughout the duration of the experiment. This suggests that the diets had enough nutrients needed for rumen microbial multiplication and subsequent digestion of ingested dietary organic matter. Similar performance among treatments is also attributable to similar intake of CP and energy. A similar trend in growth was observed by Malebana (2018), for intact male Dorper lambs fed diets with graded levels of MKC substituting soybean meal especially that dietary treatments were also formulated to have similar CP as in the current study.

In the current study, final weight, ADG and FCR did not differ across the graded inclusion levels of MKC in lamb diets. Similarly, Malebana (2018) reported no difference across the treatments in final weight, ADG and FCR of sheep fed on graded dietary substitution of soybean meal with MKC inclusion levels of 0%, 5%, 9%, 14% and 19% in sheep complete diets. Our findings showed that all the treatments used in the current study satisfied minimum energy and protein requirements needed for lamb growth but MKC diets were much superior (110 vs 154, 164 and 158 g/d for diet A, vs B, C and D respectively) thus supporting more than

150 g/day of growth. Thus, mean values of MKC treatments grouped together was 43.2% and 42.7% greater than the control (treatment A) for ADG and weight gain, respectively. The discrepancy observed is attributable to the MKC providing, RDP, RUP, minerals and carbohydrates while with treatment A urea provided the major RDP to the diet. Mean ADG (159g) for lambs fed MKC diets is comparable to 171g of lambs fed complete diets containing sunflower cake as source of protein (Alves et al., 2016). This was not surprising as amino acid profile of sunflower seed cake and MKC are almost similar (Chapter 3 of this thesis). In addition, Muhammad et al. (2016) reported significant differences in final weight, weight gain and ADG in the diets with varying levels of *Sclerocarya birrea* nut meals in yearling male Uda sheep diets. The diet with five (5%) percentage units of *Sclerocarya birrea* nut meals performed better than other treatment groups (10 and 15% Morula nut meal) and sheep growth performance tended to decline with increase *of Sclerocarya birrea* nut meals from 0% to 15%. The researchers reported that increased intake of EE by the Uda sheep affected DMI and digestibility, a fact that was not observed in the current study.

In fact, dietary fat plays an important role in livestock nutrition by increasing caloric value. However, fat supplementation reduces fibre digestion and lowers DMI at levels greater than seven (7%) percentage units of diet total dry matter (Wanapart et al., 2015; Muhammad et al., 2016). In contrast, in the current study, all lambs from MKC dietary treatments performed similarly in all growth parameters evaluated (Table 4.5) with EE (fat) in the current study, ranging from 4.5% to 6.3% (Table 4.1) and seems to have promoted conducive rumen environment as evidenced by high fibre digestibility (Table 4.3). This suggests that EE level in MKC diets was not harmful to the rumen microbes. For instance, dietary particles in the rumen were not coated by EE, rendering them inaccessible to rumen microbes and rumen microbes were also not deprived of the much-needed cations as a result of salt formation in the rumen (Enjalbert et al., 2017).

4.5. Conclusion

Feeding MKC supported lambs' growth and development as per the measured weight and nutrient utilisation. Inclusion of MKC in the lambs' diet from 4% to 12% of total diet dry matter did not interfere with nutrient intake and digestibility. Digestibility of NDF and ADF improved with graded inclusion of MKC. Though statistical differences were not observed between the

diets, MKC elicited superior growth performance on lambs offered MKC-based diets. Also, MKC was highly degradable in the rumen which led to high nitrogen being excreted through urine. However, numerically, more nitrogen was retained in MKC treatments than in treatment A (control). Therefore, MKC can be used in sheep diets as an alternative protein source especially when offered low quality diets when forage protein and energy content are low in natural pastures. To counteract high degradability of MKC in the rumen, treatment of MKC is needed to prevent waste of nitrogen and also to increase supply of high quality protein and other nutrients to the small intestine.

4.6. Recommendation

- Treatment D inclusion level can be used in feeding strategies aimed at maximising sheep growth especially when finishing sheep for markets.
- Treatment B inclusion level can be used to feed replacement sheep that require moderate growth.
- A follow-up experiment should be conducted using similar diets in the current study to verify whether MKC is safe and does not compromise the welfare of animals.

In chapter 5, a follow-up experiment was done to examine whether graded inclusion of MKC in complete diets of lambs is really safe for animal feeding by examining blood metabolites and animal growth holistically.

4.7. References

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CHAPTER 5

EFFECTS OF FEEDING MORULA KERNEL CAKE-BASED DIETS ON BLOOD METABOLITES, BLOOD PROFILE AND GROWTH PERFORMANCE OF FEMALE SHEEP

Abstract

Twenty Tswana female lambs with an average age of seven (7) months and an average body weight of 18 ± 2.3 kg were used in the study conducted at the Department of Agricultural Research, Sebele, Gaborone. A 91-day feeding study was conducted to evaluate feed intake, blood metabolites and profile and growth performance of the lambs. The lambs were fed graded levels of MKC (*S. birrea*) incorporated in complete sheep diets. The experiment was a completely randomised design (CRD) with five (5) animals per treatment. Treatments were, according to inclusion levels of MKC, at 0%, 4%, 8% and 12% in the diets of the lambs' designated treatments A, B, C and D, respectively. Treatment A (0% MKC) had urea as major source of N in the diet. The treatments did not differ ($P > 0.05$) in dry matter intake, final weight, weight gain, average daily gain and feed conversion ratio. However, among MKC treatments, treatment D had numerically higher final weight, weight gain and average daily gain than treatment B and C. The morphometric measurements (body length, heart girth, paunch girth, height at rump and height at withers) were not affected ($P > 0.05$) by the diet. The lambs' serum values of total protein and bilirubin were similar ($P>0.05$) across the treatments. Serum urea levels increased linearly ($P < 0.05$) with graded inclusion of MKC (mmol/L). The blood parameters (white blood cells, red blood cells, haemoglobin and packed cell volume) were not affected ($P > 0.05$) by dietary treatments. The inclusion rate of MKC at 4 % to 12 % in the diets of lambs affected ($P<0.05$) growth, blood metabolites and blood cells positively. Therefore, MKC can be used as an alternative protein source in the complete diets of ruminants.

Key words: **Growth, morula kernel cake, blood metabolites, sheep**

5.1. Introduction

The primary feed resources for ruminants in most African countries are natural pastures and crop residues. The pastures are usually characterised by low nutritive value, especially during

the dry season. The problem is compounded by either insufficient supply or lack of protein feed ingredients, especially in semi-arid countries like Botswana. In this context, according to Soren et al. (2017), a shortage of protein-rich feedstuffs combined with escalating costs of conventional feedstuffs in developing countries has prompted animal nutritionists to search for alternative protein feed supplements from the agro-forest based industrial by-products.

Oilseed cakes provide an alternative to protein or energy supplementation in livestock diets. Naturally, oilseed cakes are known to contain anti-nutritional factors (Sunil et al., 2015). For instance, the utilisation of Mahua (*Bassia latifolia*) seed cake is very limited in animal complete diets due to its extremely bitter taste and presence of saponins and tannins (Patial et al., 2013). Even so, saponins and tannins at low levels in animal diets may have a positive effect on rumen fermentation (Patil et al., 2013). Generally, when condensed tannins in animal diets exceed 5% of the total diet dry matter, feed intake and digestibility would decrease. Tannins actually affect digestibility of nutrients either by binding digestive enzymes or by binding feed nutrients (Kushwaha et al., 2011). In the process, some ingested feed nutrients like nitrogen might leave the animal gastrointestinal tract undigested, resulting in high faecal nitrogen. Hence, feed ingredients with high anti-nutritional factors are usually incorporated in animal complete diets at low levels. Aruwayo and Maigandi (2013) also stated that Neem (*Azadirachta indica*) seed cake has anti-nutritional factors (like azadirachtin, meliacin, salanin and valassin) that result in pungent odour and bitter taste. Therefore, feeding neem seed cake without treatment has been shown to have a negative effect on nutrient digestibility and performance of ruminants. Nonetheless, feeding treated neem seed cake to lambs up to 20% level in the total diet dry matter had no significant effect on the haematological and biochemical characteristics (Aruwayo & Maigandi, 2013). On the other hand, oilseed cakes like sunflower cake may not be fully exploited in ruminant feeding because of their higher ether extract content that may cause digestive disturbance and reduced feed intake (De Goes et al., 2019). Obeidat (2020) demonstrated that black cumin (*Nigella sativa*) meal at a level of 15% total diet dry matter enhanced growth performance of growing Awassi lambs. The improved lambs' growth was attributed to improved gastrointestinal tract health, antimicrobial effect on the pathogenic microorganisms in the digestive system and enhanced palatability due to incorporation of black cumin meal. Furthermore, Prusty et al. (2019) stated that feeding of karanj cake resulted in deleterious effects on nutrient utilisation, blood biochemistry profile, rumen fermentation pattern and pathological changes in vital organs in sheep. The above information indicates that

caution should be exercised when contemplating to use non-conventional oil seed cake in livestock diets.

MKC is one of the alternative non-traditional feed ingredients that has recently emerged in the SADC region and can be considered in ruminant feeding. MKC is a by-product of oil producing plants obtained after extraction of oil from Morula seed (Mlambo et al., 2011a). MKC has a protein content of 29% (Mdziniso et al., 2016) to 41.1% (Malebana et al., 2017), depending on the efficiency of oil extraction. Ether extract in MKC can be as high as 41.1% and the amino acid profile is good, except that lysine may be limiting in growing monogastric animals (Malebana et al., 2017). MKC has a calcium content of 0.1% and phosphorus is at 1.1% on DM basis (Mthiyane et al., 2017). Preliminary studies on the possibility of using MKC in ruminant diets have recently been explored by other researchers (Mlambo et al., 2011a, Mlambo et al., 2011b and Malebana, 2018). Inclusion of oilseed cakes in complete diets for ruminants can have an influence on rumen fermentation characteristics and blood profile, depending on the level and type of oilseed cake used. Therefore, the aim of this study was to assess the growth parameters, blood composition indicators and blood biochemistry of lambs fed a graded inclusion of MKC in complete diets.

5.1.1. Specific objectives

- To evaluate the feed intake (FI), growth, morphometric measurements, average daily gain (ADG) and feed conversion ratio (FCR) of lambs fed graded inclusion of MKC.
- To measure the blood metabolites and profile of Tswana lambs fed MKC based diets.

5.1.2. Hypotheses

(a) H_0: There are no differences in FI, ADG, morphometric measurements, FCR and final weight of lambs fed diets with graded inclusion of MKC.

(b) H_0: There is no difference in blood metabolites and profile in lambs fed diets with graded inclusion of MKC.

5.2. Materials and methods

5.2.1. Site and diets

A feeding trial was conducted at the Department of Agricultural Research (DAR), Sebele Research Station (Gaborone) using the same location and diets as described in Chapter 4 under section 4.2.1.

The treatments' ingredients and chemical composition of the diets are shown in Table 5.1. Treatment A was used as the control (without MKC).

Table 5.1: Ingredient and chemical composition of treatment diets.

	Treatments			
Ingredient (%)	A	B	C	D
Ground maize grain	58.3	64.2	60.4	56.6
Sorghum stover	18.7	25	24	23
Morula kernel cake	0.0	4.0	8.0	12
Wheat bran	18.7	2.9	4.3	5.6
Urea	1.6	1.6	1.0	0.5
Molasses	1.2	1.0	1.0	1.0
Feed lime	0.7	0.8	0.8	0.8
Dicalcium phosphate	0.5	0.4	0.4	0.4
Sodium chloride	0.3	0.1	0.1	0.1
Lab Analysis[1] (%)				
Dry matter	99	99	91	99
Organic matter	93.3	93.6	84.4	93.6
Crude protein	14	14.6	14.2	14
Ether extract	4.8	6.6	6.7	7.8
Neutral detergent fibre	39.1	36.4	38.1	28.9
Acid detergent fibre	24.2	23.8	26.9	24.4

[1]Laboratory Chemical composition results= % dry matter basis,

5.2.2. Experimental animals and management

Twenty (20) Tswana female lambs of seven (7) months old were used in a CRD experiment running for ninety-one (91) days, including 7 days of adaptation. The lambs were dewormed using Ivermax (Cipla Agrimed Pty, Ltd, South Africa) at 1 ml subcutaneously per lamb and weighed for two consecutive days to determine initial weights. The lambs were divided into four groups with five replicates balanced for weight. Each animal was housed in a pen measuring 2.5 m x 1 m. The pens have a common roof with concrete floors and an enclosure made of timber all round. The lambs were fed on respective diets (Table 5.1) in pens *ad libitum* and feed offered was adjusted daily to allow for 10% daily refusals. Drinking water was available *ad libitum* and provided daily in pens before fresh feed was offered. Pens were cleaned every morning. The feed offered and refused for each animal were weighed using a platform electronic scale (Digital scale; model DS-530, Teraoko Seiko, Japan) and recorded daily to determine dry matter intake (DMI). Animals were weighed every two (2) weeks for two consecutive days in the morning before feeding using a walk-in scale (crane scale; Tal tec Pty, Ltd, South Africa). Feed conversion ratio was calculated as proportion of DMI to corresponding ADG. Representative diet samples were collected in sampling bags for laboratory analysis. At day 84, the feeding trial was terminated and animals were weighed for two (2) consecutive days to determine final body weight.

5.2.3. Morphometric determination

Body morphological measurements for each female lamb were measured in their respective pens while standing on a concrete floor using a flexible measuring tape as described by Sigh et al. (2005) and Kumar et al. (2017). The body measurements of body length (BL), height at withers (HW), heart girth (HG), paunch girth (PG) and height at rump (HR) were taken every two weeks and recorded in centimetres. Body length was measured as the distance between the point of the shoulder and pin bone. Heart girth was measured as the circumference taken behind the forelegs. Height at rump was the vertical distance from the top of the hip to the base of the hoof on the floor. Paunch girth was determined as the circumference measurement at the abdomen area just before the hind legs. Height at withers was a vertical distance taken from the top of the shoulder to the base of the hoof on the floor. Body condition score (BCS) was carried out by two (2) technicians and their scores were averaged for each sheep evaluated. Body condition score was rated from the scale of 1-emaciated to 5-obesity following the procedures of Kenyon et al. (2014).

5.2.4. Blood Biochemistry

At the completion of the feeding trial, blood samples from each lamb used in the growth experiment (20 lambs in total) were drawn from the jugular vein into a 4 ml vacuette-tube coated with ethylene diamine tetra-acetic acid (EDTA) and another blood sample was poured into a 5 ml vacuette-tube without EDTA for serum collection. All blood samples were sent to a private medical laboratory named "Diagnofirm" based in Gaborone, Botswana for analysis. The serum samples were centrifuged for 5 minutes at 3500 rpm using a Heraus megafuge-8-benchtop centrifuge (Thermo Scientific, Massachusetts, USA). However, the full blood count samples were processed as whole blood.

Total protein, total bilirubin and urea were run on the "Abbott Alinity ci" while a full blood count was run on the Abbott Celldyn Emerald 22AL (Abbott Laboratories, Illinois, USA).
Total protein: the Biuret method was used for the analysis of the total proteins (Abbott Alinity, 2017). In brief, polypeptides containing at least two peptide bonds react with the Biuret reagent. In an alkaline solution, cupric ions form a coordination complex with protein nitrogen with very little difference between albumin and globulin on a protein-nitrogen basis.
For the total bilirubin, the Diazonium salt method was used (Abbott Alinity, 2017). In brief, total bilirubin couples with a Diazo reagent in the presence of a surfactant to form azobilirubin. The diazo reaction is accelerated by the addition of a surfactant as a solubilising agent. The increase in absorbance at 458 nm due to azobilirubin is directly proportional to the total bilirubin concentration.

To determine urea, the urease method was used (Abbott Alinity, 2017). In brief, in the initial reaction, urea in the sample is hydrolysed by urease to ammonia and carbon dioxide. The second reaction glutamate dehydrogenase converts ammonia and α-ketoglutarate to glutamate and water with the concurrent oxidation of nicotinamide adenine dinucleotide hydrogen (NADH) to nicotinamide adenine dinucleotide (NAD). Two moles of NADH were oxidised for each mole of urea present. The initial rate of decrease in absorbance at 340 nm was proportional to the urea concentration in the sample.

For full blood count, three types of measurement (electrical impedance, UNI-FLOW technology and Absorption Spectrophotometry) were used to count, size and classify blood cells and to measure haemoglobin. Electrical impedance counting was used for counting white

blood cells (WBC) and red blood cells (RBC). The pulses of various amplitudes were sorted into various size channels according to their amplitude (Abbott Emerald, 2018). The UNI-FLOW technology was used for counting and for haemoglobin measurement (Abbott Emerald, 2018).

Absorption Spectrophotometry was used to measure haemoglobin using a chromogen formed using lyse reagent. The chromogen was measured by means of absorption spectrophotometry using a 555 nm LED source. The LED shone through the WBC counting chamber after the WBC count had been completed. The haemoglobin concentration was directly proportional to the absorbance of the sample (Abbott Emerald, 2018).

5.2.5. Feed Analysis

The proximate components (DM, CP, Ash and EE) and fibre (ADF and NDF) of experimental feed were determined as outlined in Chapter 3.

5.2.6. Data analysis

For time series data (body weights), proc mixed of SAS (2002) was used for analysis using repeated measures as per model 1. The statistical differences between means were determined using Fisher's least significance difference (LSD).

$Y_{ijk} = \mu + T_i + F_j + (T_i \times F_j) + \varepsilon_{ijk}$..... Model 1

Where Y_{ijk}= is the Kth observation of the ith treatment of the jth days, μ = is the overall mean, T_i= is the fixed effect of the ith treatment (i=1,2…4), F_j = is the (time) days (j=1, 2….6), ($T_i \times F_j$) = interaction between treatment and time (days) and ε_{ijk} = random residual error

Data on growth performance (Final body weight, weight gain, ADG, body physical measurements, DMI, blood metabolites and blood profile) were analysed using proc GLM of SAS (2002) as per Model 2. Initial weight was used as a covariate and was dropped when insignificant. The means of analysed data are presented in tables. Polynomial contrasts were used to determine the effect of MKC level in diets of lambs as described in chapter 4 of this thesis.

$Y_{ijk} = \mu + T_i + \varepsilon_{ijk}$..... Model 2

Where Y_{ij} = is the kth observation of the ith treatment, μ = is the overall mean, T_i = is the fixed effect of the ith treatment (i=1,2…4) and ε_{ijk} = random residual error

The level of significance was set P < 0.05.

5.3. Results

5.3.1. Growth

The growth performance of female lambs fed on graded levels of MKC is shown in Table 5.2. The measured growth parameters were not significant (P > 0.05) for both linear and quadratic effects. The increasing levels of MKC in female lambs' diet did not affect (P > 0.05) final weight, weight gain, ADG and FCR (Table 5.2). Lambs on a control diet (Treatment A; 0% MKC) and MKC treatments had similar ADG and FCR. The ADG ranged from 120.8 g/day to 138.9 g/day. Treatments had no effect (P > 0.05) on FCR of lambs and it ranged from 6.0 to 7.1. The dry matter intake by lambs was similar across treatments (P > 0.05) and averaged 814 g/day for all the dietary treatment.

Table 5.2: Effect of graded dietary inclusion level of MKC on growth performance of female lambs

	Treatments					
Item	A	B	C	D	RMSE	*P*-value
Initial weight (kg)	18.3	17.9	17.9	17.5	2.3	1.0
Final weight (kg)	29.5	28.0	28.3	29.3	1.9	0.5
Weight gain (kg)	11.7	10.2	10.5	11.5	1.9	0.5
[1]ADG (g/day)	138.9	120.8	124.3	136.2	22.9	0.5
[2]DMI (g/day)	818.0	824.8	747.9	866.0	80.8	0.2
[3]FCR	6.2	7.1	6.0	6.4	1.4	0.6

ADG[1]= Average daily gain, DMI[2]= dry matter intake, FCR[3]= feed conversion ratio (DMI/ADG), A= 0% MKC, B= 4% MKC, C= 8% MKC, D= 12% MKC, RMSE=root mean standard error

The mean fortnightly body weights of female lambs fed on graded dietary inclusion of MKC in complete sheep diets are shown in Figure 5.1. Graphical illustration in Figure 5.1 shows that

the mean fortnightly sheep body weights across treatments were similar (P > 0.05) from day 14 up to day 84 and body weight increased with time across the treatments.

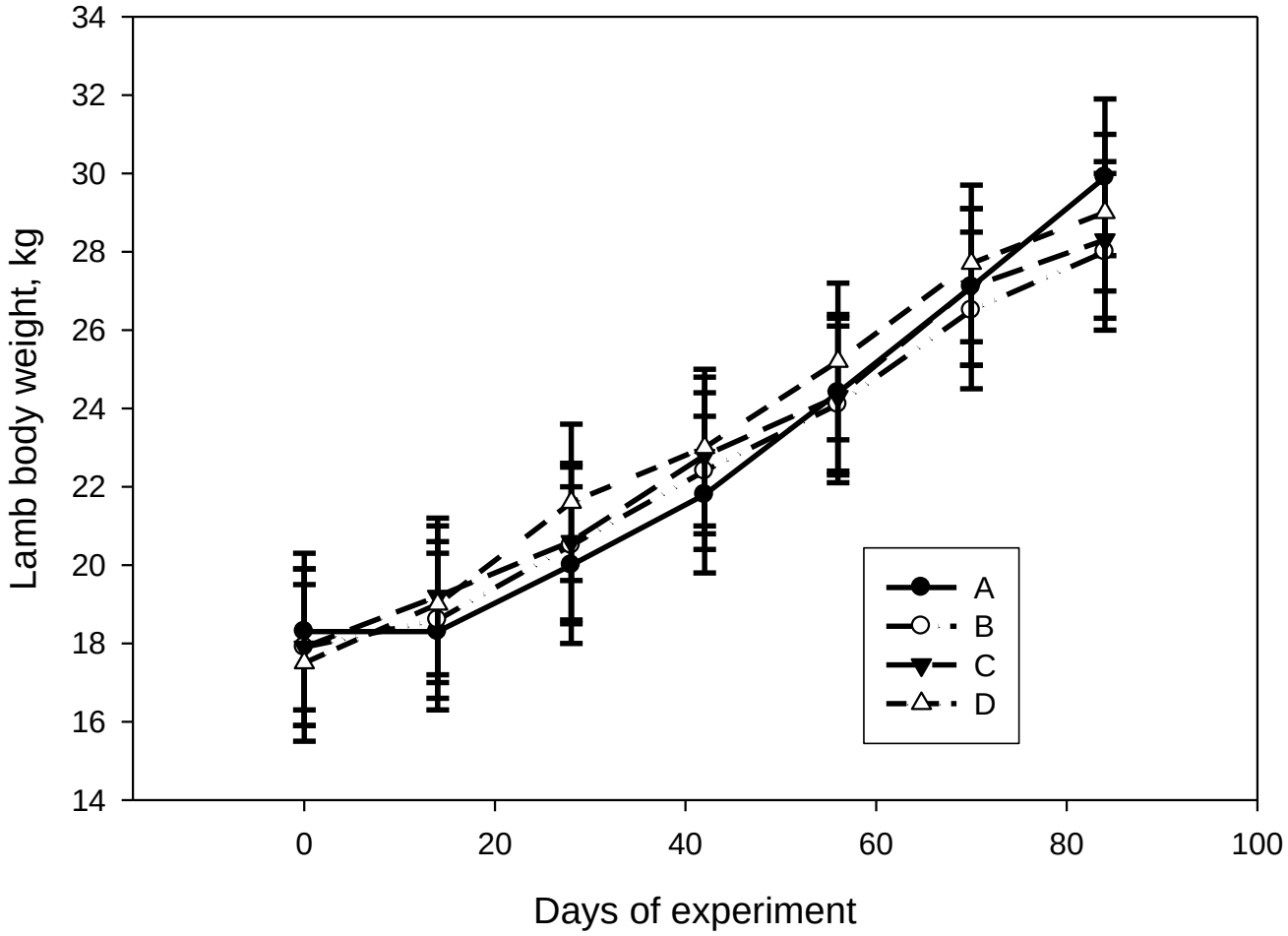

Figure 5.1 : Effect of graded inclusion of morula kernel cake on mean fortnightly body weight of female lambs (A= 0% MKC, B= 4% MKC, C= 8% MKC and D= 12% MKC)

5.3.2. Body physical measurements

The effect of graded inclusion of MKC on body physical measurements is shown in Table 5.3. The measured body physical parameters were not significant (P > 0.05) for either linear or quadratic effects. Increasing levels of MKC had no effect (P > 0.05) on lambs' final body length (BL) and BL ranged from 65 cm to 67 cm (Table 5.3). The body length gains of lambs were similar (P > 0.05) across the treatments and averaged 7.1 cm. No differences (P > 0.05) were

observed in female lambs' final height at withers (HW) and HW ranged from 64.8 cm to 65.7 cm. The lambs' height at withers (HW) gain was not affected ($P > 0.05$) by the MKC levels of the diets and it averaged 11.3 cm. The final heights at rump (HR) were similar ($P > 0.05$) across the treatments and ranged from 66.4 cm to 68.2 cm. The HR gain was not affected by increasing levels of MKC in the lambs' diets and it averaged 10 cm. Additionally, the final heart girth (HG) of lambs was also not affected ($P > 0.05$) by treatments and ranged from 75.4 cm to 77.2 cm. The HG gain averaged 7.7 cm. No differences ($P > 0.05$) were observed on final paunch girth (PG) that ranged from 79.8 cm to 81.9 cm. The paunch girth gain had an averaged value of 5.9 cm.

Table 5.3: Effect of graded inclusion of MKC on body physical measurements of female lambs

	Treatment					
Item (cm)	A	B	C	D	RMSE	*P*-value
Initial BL	59.1	57.4	59.6	59.3	1.7	0.2
Final BL	67.0	65.0	66.0	65.6	3.5	0.8
BL gain	7.9	7.6	6.4	6.5	3.5	0.9
Initial HW	54.0	53.2	54.0	54.6	1.4	0.5
Final HW	65.7	65.0	64.8	65.5	2.7	0.9
HW gain	11.7	11.8	10.8	10.9	2.8	0.9
Initial HG	69.4	67.4	69.4	68.6	2.1	0.4
Final HG	76.8	75.4	77.2	76.2	3.1	0.8
HG gain	7.4	8.0	7.8	7.6	3.1	1.0
Initial PG	76.4	75.0	75.8	75.5	1.9	0.7
Final PG	81.9	79.8	83.0	81.4	3.6	0.6
PG gain	5.5	4.8	7.2	5.9	3.6	0.8
Initial HR	57.3	56.2	56.6	58.5	1.8	0.2
Final HR	67.0	66.4	67.0	68.2	2.0	0.6
HR gain	9.7	10.2	10.4	9.7	1.8	0.9
Initial BCS	3.0	3.0	3.0	2.8	0.2	0.5
Final BCS	4.0	4.0	4.2	4.0	0.4	0.8
BCS gain	1.1	1.1	1.1	1.5	0.3	0.2

BL=body length, HW= height at withers, HG= heart girth, PG= paunch girth, HR= height at rump, BCS=body condition score; visual appraisal (no units), A= 0 % MKC, B= 4% MKC, C= 8% MKC, D=12% MKC, RMSE= root mean standard error.

5.3.3. Blood chemistry

The effect of graded inclusion of MKC on blood chemistry (total protein, urea and bilirubin) and haematological profile (packed cell volume, haemoglobin, RBC and white blood cells) of female lambs is shown in Table 5.4. Dietary graded inclusion of MKC in lamb diets did not influence ($P > 0.05$) lambs' serum mean values of total protein and bilirubin. A linear increase ($y=13.8(\pm1.2) + 0.5 (\pm 0.5) x$; $R^2= 0.31$; $P= 0.01$) effect on blood urea of female lambs was observed, with values of 5 mmol/L with 0% MKC and 6.4 mmol/L with 12% MKC. The mean values for blood haematological (packed cell volume, haemoglobin, red blood cell, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils and basophils) evaluation were the same ($P > 0.05$) among the treatments. However, monocytes showed a quadratic decrease ($y= 1.2 (\pm0.2) – 0.2(\pm 0.09) x + 0.02(\pm 0.01) x^2$; $R^2= 0.30$; $P=0.03$) as MKC increased in the diet. White blood cells and haemoglobin tended to show linear increase ($P = 0.1$) as MKC increased in the diets. On the other hand, eosinophils tended to exhibit linear decrease as MKC increased in the diets.

Table 5.4 : Blood metabolites and blood profile of female lambs fed graded inclusion levels of morula kernel cake in complete diets of lambs

	Treatment effects						Polynomial effects	
Item	A	B	C	D	RMSE	*P*-value	Linear	Quadratic
Metabolites								
Total Protein (g/L)	63	64	63	65	0.5	0.9	0.6	1.0
Urea (mmol/L)	5	5.2	6.5	6.4	2.8	0.05	0.01	0.8
Bilirubin (µmol/L)	0.3	0.3	0.3	0.3	0.01	0.5	0.2	0.4
Blood profile								
WBC($x10^9$/L)	7.5	7.2	9.0	8.9	1.9	0.4	0.1	0.9
RBC ($x10^{12}$/L)	7.6	7.9	7.7	8.7	1.2	0.5	0.2	0.5
Neutrophils (%)	23.7	29.6	24.4	31.0	7.9	0.4	0.3	0.9
Lymphocytes (%)	70.6	65.1	71.6	62.3	7.9	0.2	0.3	0.6
Monocytes (%)	1.2	0.9	0.8	1.6	0.5	0.1	0.3	0.03
Eosinophils (%)	1.4	1.0	0.5	0.7	0.8	0.4	0.1	0.5
Basophils (%)	3.1	3.5	2.6	4.5	1.0	0.06	0.1	0.1
Haem[1] (x10g/L)	11.4	12.0	11.4	12.9	1.1	0.2	0.1	0.4
PCV ($x10^{-2}$ L/L)	24.4	25.5	24.6	28.2	4.0	0.4	0.2	0.5

WBC= white blood cells, RBC= red blood cells, PCV= packed cell volume, A= 0% MKC, B= 4% MKC, C= 8% MKC D= 12% MKC, RMSE= root mean standard error. Haem[1]=haemoglobin

5.4. Discussion

5.4.1. Growth

Dry matter intake (DMI) is one of the factors that influences weight gain (Chen et al., 2020). Feeds with low digestibility negatively affect DMI as they take longer in the rumen to be digested and have a slow passage rate, especially high fibre containing feed (NRC, 2001). On the other hand, with high concentrate feeds, DMI is mainly regulated by the amount of energy absorbed into the blood stream in terms of volatile fatty acids (Pond et.al., 1995). In fact,

propionic acid concentration in the blood stream is the major factor regulating feed intake (Pond et al., 1995). In the current study, DMI was similar across the treatments and it ranged from 747.9 g/day to 866 g/day. The dietary treatments as shown in Table 5.1 had similar ADF, which is the predictor of digestibility (McDonald et al., 2011). This further suggests that the treatment diets were digested in a similar fashion resulting in having normal ruminal pH, rumen motility and extensive digestibility, especially that fibre content was almost similar in all the diets. The DMI as percentage of bodyweight ranged from 2.6% to 3% body weight and satisfied national research council (NRC, 1985) requirements for small ruminants. DMI is usually maximised if the feed provides all the nutrients required by rumen microbes and the animal tissues (Tarekegn et al., 2021).

MKC is rich in EE (Mlambo et al., 2011a); therefore, with graded inclusion of MKC in treatment diets, the amount of EE was higher for treatment D since EE is higher in MKC compared with other ingredients in the experimental diets. Ether extract (EE) ranged from 4.8% to 7.8% (Table 5.1). Budel et al. (2017) observed that diets with high EE level (15% DM) resulted in the longest microbial colonisation time of feed due to a delay in the adhesion of microbes to dietary fibre. Additionally, a high EE level in the diet affects bacteria either by physical impediment or interference with the activity of microbial enzymes (Budel et al., 2017). Therefore, under practical feeding conditions, this might manifest into lower DMI in animals. In the current study, the increment of the EE level seemed not to have affected DMI on MKC treatments. Treatment D specifically had a numerically higher DMI (866 g/day). This finding implies that MKC's EE level was not toxic to fibre digesting bacteria. Attkinson et al. (2006) stated that an EE level not exceeding 9.4% of DM in high concentrate diets has no detrimental effects on ruminal microbes. Similar to the current study's findings, Castro et al. (2020) reported no treatment effect on DMI in finishing sheep fed graded inclusion (0%, 8%, 16% and 24% DM) of delinted cottonseed cake that replaced soybean meal in formulated isonitrogenous complete diets. The researchers reported DMI ranging from 733.7 g/day to 902.2 g/day and the diets had EE ranging from 5% to 6.4%. In contrast, Sponchiado et al. (2019) reported a linear decrease of DMI in the lambs fed isonitrogenous complete diets with graded (0%, 5%, 10% and 15% DM) soybean cake. The researchers attributed the decrease in DMI to an increase in EE content as it increased with progressing inclusion of soybean cake. Interestingly, the EE level ranged from 2.3% to 4.6% DM which was within the maximum tolerable limit for ruminants. It therefore suggests that it is perhaps the type of fatty acids in oilcakes that

determines the level at which fibre digestion will be impeded and not necessarily all fatty acids. Indeed, Palmquist and Jenkins (2017) noted that DMI depression in ruminants is caused by fatty acid with a long chain length and a high degree of unsaturation (number of double bonds). In addition, unsaturated fatty acids are found to be more toxic to the rumen bacteria than saturated fatty acids (Russel, 2002). In other studies, (Soren et al., 2017), DMI was depressed in some oil seedcakes like detoxified karanj (*Pongamia glabra*) seed cake. The low DMI was ascribed to the presence of anti-nutrients like karanj, pongamol and glabrin that make feed to taste bitter and have a pungent smell. Depression of DMI would certainly impinge on body weight gain and that could come from other factors in the cake than fatty acids.

Body weight is one of the economic indices used to value livestock, especially the fact that it is directly related to hot and cold carcass weight. In the current study, final weight, ADG and FCR were similar across the treatments. The lack of differences in measured growth parameters of the lambs is attributable to similarity of supply of required nutrients by the diets used in the study (Table 5.1). The growth results in Figure 5.1 also show that the lambs across the dietary treatments followed a similar trend of growth. Fortnightly, weights were similar throughout the duration of the experiment, suggesting that the diets had enough nutrients needed for rumen microbes multiplication and the subsequent digestion of ingested dietary organic matter. Surprisingly, in the last fortnight of the experiment (Figure 5.1), lambs from treatment D had a slowed growth that was triggered by reduced dry matter intake. This might be the result of poor palatability of the diet due to possible rancidity of fats as the feed was stored for almost three months. Nevertheless, the average DMI (Table 5.2) was similar across the treatments implying that the lambs had similar nutrient intake and nutrient digestibility. The ADG for all the female lambs across the treatments was 130 g/day and was 13% lower than the ADG of their male counterparts of similar age and breed given similar diets (Chapter 4 of this thesis). This is attributable to male hormones (androgens) that stimulate growth in muscles by promoting protein accretion while oestrogen in females promotes fat deposition (Alemneh & Getabalew, 2019). Additionally, Silva et al. (2016) also reported no significant differences in body weight, ADG and total weight gain in non-castrated male lambs fed on cottonseed cake replacing soybean meal at a rate of 0%, 33%, 66% and 100% DM in complete diets. In contrast, Sponchiado et al. (2019) reported a linear decrease in ADG and slaughter weight of lambs fed on increasing levels of soybean cake (0%, 5%, 10% and 15% DM). The increase of EE with addition of soybean cake in complete diets was implicated in the decline of ADG (from 285

g/day to 226.7 g/day). Although EE plays an important role in increasing the energy density of the diet, sometimes even if it is within the recommended level, it can negatively affect animal growth performance by interfering with fibre digestion or poisoning fibre digesting bacteria (Sponchiado et al., 2019; Costa et al., 2021). However, in the current study, treatment D had a numerically higher final weight, weight gain and ADG when compared to other MKC treatments, even though it had a higher EE content.

5.4.2. Body physical measurements

Body physical measurements in livestock can be used to evaluate the growth of both skeletal parts and body tissues as documented in several publications (Ravimurugan et al., 2013; Kumar et al., 2016; Temoso et al., 2017; Elsaid & Elnahas, 2019 and Ibrahim et al., 2020). Body physical measurements like HG, HW and PG had been proven to have a positive correlation with animal weight (Ravimurugan et al., 2013). Therefore, morphometric measurements can be used to estimate body weight which is mostly used to judge the value of livestock (Ashour et al., 2020). This suggests that body physical measurements in the absence of weighing scales as stated by other researchers (Temoso et al., 2017) can be used to appraise livestock visually to sell them or to take breeding decisions. Recently, Temoso et al. (2017) have shown that HG can be used to estimate the body weight of Tswana sheep using a regression equation. In the current study, several body measurements (heart girth, paunch girth, height at rump, height at shoulders and body length) were taken to determine skeletal growth (body length, height at shoulders and height at rump) and body tissue growth (heart girth and paunch girth) in response to graded inclusion of MKC in complete diets of lambs (Table 5.3). All the body physical measurements taken from the female lambs were similar across the dietary treatments affirming that lamb diets provided similar nutrients needed for growth. Ravimurugan et al. (2013) stated that both body measurements and weight increase simultaneously during the growth of the animal as they have a positive correlation. It was actually confirmed in the current study to a certain extent as there were no treatment effects on both body weight and body physical measurements. Similarly, Singh et al. (2005) reported no treatment effects on ADG, final body weight, HW, chest girth and PG of male Corriedale lambs fed on complete diets with inclusion levels of 0, 16, 24 and 32 % of mustard (*Brassica napus*) oil cake replacing groundnut (*Arachis hypogaea*) cake. In another study, Silva et al. (2016) also reported no effect of treatments on morphometric measurements of the lamb carcasses (carcass length, leg length, leg width, leg depth and breast depth) of sheep that were fed on cottonseed (*Gossypium* spp) cake replacing

soybean (*Glycine max*) meal (0%, 33%, 66% and 100% DM) in complete diets. The lack of treatment effect on carcass morphometric measurements was attributed to completed growth of bone tissue in the lambs before commencement of the study. In contrast, Lima et al. (2018) reported a linear reduction in morphometric measurements of HW, HR and BL when intact male Santa Ines lambs were fed on complete diets with graded inclusion of sunflower seed cake at 0%, 10%, 20% and 30% DM. The researchers attributed the decline in growth performance to reduced nutrient digestibility, low intake of crude protein and non-fibrous carbohydrate as sunflower seed cake increased in the complete diets. Therefore, this culminated in increase of fiber and ether extract content in diets.

Body condition score (BCS) is used to assess the fatness of an animal (Kenyon et al., 2014). There is a strong link between nutrient intake and BCS. According to Lanyasunya et al. (2005), low BCS can result in low pregnancy rates. Determination of BCS is easy and requires no specialised equipment (Ndlovu et al., 2007 and Kenyon et al., 2014). Kenyon et al. (2014) stated that liveweight and BCS have a positive relationship. This was demonstrated in the current study as BCS and final liveweight were similar across the treatments indicating that the plane of nutrition was similar. Sanston et al. (1993), as cited by Kenyon et al. (2014), stated that BCS is a better estimate of energy reserves than liveweight.

5.4.3. Blood chemistry

Oil seedcakes have anti-nutritional factors that might either interfere with nutrients utilisation by the animal or cause gross pathological lesions in organs when ingested in toxic amounts. In fact, Pati et al. (2013) noted that saponins have a general reaction on lipid membranes and cause lysis of RBC in vivo and in vitro at toxic amounts in animal diets. Soren et al. (2017) reported reduced liver weight suggestive of hepatic changes in lambs fed on detoxified karanj seedcake at an inclusion level of 22.5% in complete sheep diets. According to Malebana (2018), MKC has oxalate, phytate-phosphate, saponins and tannins. For this reason, blood sampling in the current study was used to confirm the health status, welfare and nutritional status of lambs fed on graded inclusion of MKC in complete diets. No published animal studies, except one doctoral thesis study (Malebana, 2018) was found that examined the blood profile of sheep fed on complete diets having MKC. Blood metabolites concentrations and blood profiles are indices that can be used to determine the adequacy of nutrient supply, utilisation (Ndlovu et al., 2007) and health (Soch et al., 2011). White blood cells' (leukocytes) count value

increases when an animal is infected by various kinds of diseases (Tortora et al., 1995) or stressed (Amuda & Okunlola, 2018). The WBC count value in the current study ranged from 7.2 (x 10^9/L) to 8.9 (x 10^9/L) and these values were within the reference range values for a healthy sheep (Aiello & Mays, 1998). White blood cells in animals fight foreign bodies in the blood stream by phagocytosis and antibody production (Tortora et al., 1995). The neutrophils, lymphocytes, monocytes, eosinophils and basophils values (Table 5.4) across the treatments were within the reference range values (10 % – 50 %, 40 % -75 %, 0 % – 6 %, 0 % – 10 % and 0 % – 3 % for neutrophils, lymphocytes, monocytes, eosinophils and basophils respectively) for clinically healthy sheep (Aiello & Mays, 1998). The lymphocytes values across the treatments in the current study were higher in number than neutrophils as earlier stated by Tortora et al. (1995). The high lymphocytes count could be attributed to the physiological adaptive strategy inherited from the parental animals to resist prevalent diseases common in a given ecological zone (Amuda & Okunlola, 2018). The RBC (erythrocytes) are filled with haemoglobin and are responsible for transporting oxygen and carbon dioxide (Nelson & Cox, 2005). The RBC value in the current study ranged from 7.6 (x 10^{12}/L) to 8.7 (x 10^{12}/L) and the values were slightly under the lower limit of the normal range value (Aiello & Mays, 1998; Varlyakov et al., 2013). The haemoglobin values ranged from 11.4 (x 10 g/L) to 12.9 (x 10 g/L). The values in the current study were within the range of 9 (x 10g/L) to 15 (x 10g/L) for a normal sheep, as stated by Aiello and Mays (1998). The haemoglobin values in the current study showed that diets were capable of supporting high oxygen carrying capacity in animals (Amuda & Okunlola, 2018). The packed cell volume (PCV) across these treatments (Table 5.4) ranged from 24.4 (x10^{-2} L/L) to 28.2 (x10^{-2} L/L) and were within the reference values (22.5 (x10^{-2}L/L) to 30.3 (x10^{-2}L/L)) for a normal healthy sheep, as reported by Amuda and Okunlola (2018) and Aiello and Mays (1998). Given the normal range of values for blood chemistry, it can be confirmed that the animals in this experiment were not negatively affected by dietary treatments.

Wang et al. (2019) stated that blood biochemical indices or constituents play a very important role in showing the nutritional status of the animal. In the current study, the total protein and blood urea-N were measured to determine the protein status in sheep. There is specifically no single metabolite measured that can fully reflect the protein status (Ndlovu et al., 2007). The total serum protein represents all blood protein without contribution from blood cells and fibrinogen (Varlyakov et al., 2013). In the current study, the total serum protein ranged from

63 g/L to 65 g/L and the values were within the reference values (60 g/L to 80 g/L), as stated by Varlyakov et al. (2013). The total serum protein was similar across the treatments (Table 5.4) and this was attributable to the diets which were formulated to be isonitrogenous. The current findings agree with Shahen et al. (2004), as cited by Abdel-Ghani et al. (2011), who stated that there was a positive correlation between dietary protein and plasma protein levels. Ndlovu et al. (2007) stated that the total serum protein reflected the availability of protein and their concentration declined in the event of protein deficiency which was not the case in the present study as reflected by ADG of 130 g (Table 5.2). Blood serum urea is an indicator of protein metabolism *in vivo* (Wang et al., 2019). Serum urea values in the current study ranged from 5 mmol/L to 6.5 mmol/L (Table 5.4). The blood urea values were within the reference range values of 3.7 mmol/L to 9.3 mmol/L for a healthy sheep (Aiello & Mays, 1998). Additionally, serum urea increased linearly (P = 0.01) with graded inclusion of MKC. Therefore, treatment C and D had higher values (6.5 mmol/L) of serum urea than other treatments. This might have been due to more ruminal ammonia that was produced from rumen degradable protein resulting in greater quantities of ammonia being detoxified in the liver to form urea (Hatfield et al., 1998). In this regard, providing adequate protein and energy in animal diets may increase the animal's capacity to retain nutrients in the body and avoid nitrogen waste to the environment (Costa et al., 2021). Therefore, it would be worthy research to investigate the nitrogen retention of sheep given diets with MKC included at these levels.

Bilirubin is produced in the spleen from haemoglobin released from dying RBC (Nelson & Cox, 2005). Haeme is converted to biliverdin and then bilirubin (Nelson & Cox, 2005). The amount of bilirubin in the current study averaged 0.3 µmol/L and was within the reference range values of 0 µmol/L to 4.5 µmol/L (Desco et al., 1989). Therefore, the liver and bile duct of lambs in the current study were not impaired (Nelson & Cox, 2005) as a result of dietary treatments used. Other researchers (Elangovan et al., 2013) reported gross pathological changes in the heart and liver of lambs fed on jatropha (*Jatropha curcas*) seed cake suggesting negative pathological events in the liver. Although tannins and saponins or other deleterious compounds were not measured in the diets used in the present study, it can be inferred that the levels of tannins and saponins were below 5% as no deleterious effects were observed in measured metabolites (Patil et al., 2013).

5.5. Conclusion

Feeding MKC supported lambs' growth and development as per the measured weight and body physical measurements. The inclusion of MKC in the sheep's diet from 4 % to 12 % of the total DM did not interfere negatively with their growth, blood metabolites and profile. The measured blood metabolites and blood profile parameters were within the reference ranges. Therefore, MKC can be used in sheep diets as an alternative protein source in extension areas where it is readily available.

5.6. Recommendation

- Morula kernel cake can be used in complete sheep diets at levels of 12% or below since it has no deleterious effects on animal growth.
- Treatment D should be evaluated further with other conventional protein sources in sheep diets to establish how it affects growth performance, carcass characteristics, meat quality and sensory attributes.

In chapter 6, the best diet from Chapter 5 was compared with a lamb commercial finisher diet and another complete diet with sunflower seed cake as a protein source. The three diets are compared with regard to growth performance, nitrogen balance, meat quality, sensory analysis and gross margin analysis.

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CHAPTER 6

NUTRIENT DIGESTIBILITY, GROWTH AND CARCASS CHARACTERISTICS OF TSWANA SHEEP FED DIETS CONTAINING MORULA KERNEL CAKE AND SUNFLOWER SEED CAKE AS PROTEIN SOURCES

Abstract

A growing-finishing trial evaluated lambs' performance, metabolism and carcass characteristics when fed a complete diets containing different protein sources. Eighteen (16.9 ± 0.7 kg of BW and 8 months old) castrated male Tswana lambs were used in a CRD trial and fed on complete diets containing Lucerne (CD; control diet) or Morula kernel cake (MKD) or Sunflower seed cake (SCD) as protein sources over a 103-day experiment. Each treatment had six (6) replicates and was balanced for weight. No significant differences ($P > 0.05$) were observed in the dry matter intake, final body weight, average daily gain and feed conversion ratio. This is attributable to all the diets providing an equal supply of nutrients to the sheep. However, lambs fed MKD had a higher ($P < 0.05$) intake of digestible ether extract than lambs on CD. The apparent digestibility of crude protein was higher ($P < 0.05$) in lambs fed CD and similar in lambs fed MKD and SCD. The digestibility of dry matter, organic matter, ether extract, neutral detergent fibre (NDF) and acid detergent fibre (ADF) was similar ($P > 0.05$) across the treatments.

Urinary-nitrogen output and faecal-nitrogen output were similar ($P > 0.05$) across the dietary treatments. However, N-retention was greater ($P < 0.05$) in lambs on CD than in SCD and intermediate in lambs on MKD. Meat quality attributes (pH, shear force and colour) and proximate composition (moisture, protein, fat and ash) were the same ($P > 0.05$) among the treatments. *Longissimus dorsi* muscle had a high level of palmitic acid across the treatments. *Longissimus dorsi* muscle of lambs fed on MKD had a significantly higher ($P < 0.05$) oleic acid content than *Longissimus dorsi* muscle of lambs fed on CD while *Longissimus dorsi* muscle of lambs fed on SCD had no oleic acid detected. The polyunsaturated fatty acid (PUFA) and saturated fatty acid (SFA) ratio in *Longissimus dorsi* muscle were similar ($P > 0.05$) across treatments. Offal weights were also similar ($P > 0.05$) across the treatments. *Longissimus dorsi* muscle's organoleptic quality did not differ ($P > 0.05$) across the treatments. However, MKD

resulted in numerically higher sensory ranking in all the attributes evaluated (appearance, taste, flavour, juiciness, tenderness and overall impression). The gross margin analysis was significantly greater ($P < 0.05$) when feeding SCD than on CD and was intermediate on MKD. Commercial diet had significantly ($P < 0.05$) higher variable costs.

Feeding MKC may be beneficial to lambs' growth and development. This was reflected by the normal range of nutrient intake, nutrient digestibility, positive nitrogen retention and average daily gain observed in the current study and would replace sunflower seed cake where it is available. *Longissimus dorsi* muscle from lambs fed on MKD had higher oleic acid which may provide health benefits upon human consumption. Morula kernel cake can be used for fattening lambs when common protein sources are either not available or expensive.
Key words: *Longissimus dorsi* muscle, morula kernel cake, nitrogen retention, organoleptic attributes, lamb

6.1. Introduction

In most sub-Saharan countries like Botswana, agriculture is the main economic activity for most people living in rural areas. For instance, livestock production provides people with animal protein products (milk and meat) and employment opportunities. Recently, Statistics Botswana (2019) reported that livestock production is performing below par because of periodic droughts and chronic endemic diseases. The production indices for sheep for instance were reported as 32.8%, 14.1% and 4.5% for birth rate, death rate and off-take, respectively (Statistics Botswana, 2019). Nutrition is the major problem affecting livestock production in Botswana (Aganga, 2005). On the other hand, consumers nowadays are increasingly demanding for safe and quality food in animal products, especially when it comes to meat. Meat is a good source of proteins, fat, vitamin B and minerals for human diets (Martins et al., 2018). However, there has been a concern about saturated fatty acids from meat that predispose cardiovascular disease (Martins et al., 2018) in humans. Chiofalo et al. (2020) stated that rumen biohydrogenation of dietary unsaturated fatty acids by ruminal microbes results in the production of saturated fatty acids that are absorbed and deposited in animal tissues. According to Martins et al. (2018), the deposition of fat in tissues is determined by the animal's diet and sex. Additionally, Junkuszew et al. (2020) stated that dietitians recommend meat low in fat and rich in n-3 and n-6 fatty acids to avoid heart-related diseases in humans. This therefore calls

for ruminant nutritionists to look for novel feed ingredients that will promote the accumulation of healthy fat in animal tissues and also to meet holistic market requirements for finished animals.

The main challenge faced by livestock farmers to produce a consistent supply of quality animal products is the inadequate supply of required nutrients due to seasonal fluctuation of forages from natural pastures. As a result, some farmers, as stated by Yagoubi et al. (2018), end up feeding their animals with low quality forages or allow them to graze on degraded natural pastures, especially during the dry season. Obviously, such animals will grow slowly and take a longer period to reach market weights. Feeding strategies aimed at providing supplementary nitrogen or energy to rumen microbes are very important to improve the intake of poor quality roughages by ruminants. Dietary proteins that reach the small intestine of ruminants are made of microbial protein and rumen undegradable protein, which are in summation regarded as metabolisable protein (NRC, 2001). Ruminants usually require quality protein sources that contain the right profile of amino acids for the growth and development of young animals and that escape rumen degradation. According to Ruzic-Muslic et al. (2014), microbial protein is not adequate to supply amino acids required for optimum animal growth as such protein sources with high rumen undegradable protein result in better growth performance. Most conventional plant protein sources used to supplement protein and energy in ruminants are very expensive resources for poor farmers. Unconventional oilseed cakes like MKC can be explored further for use in ruminant diets to lower the cost of livestock production. MKC is a residue remaining after oil extraction from Morula nut (Mlambo et al., 2011) and Malebana et al. (2017) have shown that MKC is a good source of protein, fibre, fatty acids and minerals. Earlier researchers demonstrated that MKC can be used in diets of goats (Mlambo et al., 2011) and sheep (Malebana, 2018). However, information on its effects on meat quality is lacking. Consumer decision on meat quality is based on meat palatability components such as flavour and tenderness (Tshabalala et al., 2003; Ngambu et al., 2012; Machete et al., 2016). Additionally, age (Simela, 2005) and diets (Arsenos et al., 2009) also affect meat palatability. This study was therefore designed to assess the effects of an MKC-based diet on feed intake, weight gain, carcass characteristics, meat quality, sensory traits and gross margin (GM) analysis when compared to a sunflower seed cake-based diet.

6.1.1. Objectives

The objective of the study was to assess the effects of MKC based diets compared to SSC diets on growth performance in terms of feed intake, nitrogen balance, weight gain, average daily gain, final body weight, feed conversion ratio, meat quality, meat sensory attributes and gross margins.

6.1.2. Hypotheses

(a) H_o: The use of MKC or sunflower seed cake (SSC) in complete diets of lambs elicits a similar influence on feed intake (FI), growth performance (Final weight, DMI, FCR, ADG), meat quality (DM, fat, protein and ash), sensory attributes and gross margins.

(b) H_o: Dietary inclusion of MKC or SSC in complete diets of lambs elicits similar nitrogen retention.

6.2. Materials and methods

6.2.1. Experimental site and climate

A feeding trial was conducted at the Department of Agricultural Research (DAR), smallstock pens in Sebele, Gaborone and South Eastern Botswana. The study area is situated between latitude of 24º 33'S and longitude 25º 51'E at an altitude of 994m above sea level (Madibela et al., 2004).

6.2.2. Experimental diets

Two treatment diets were formulated and prepared using MKC and SSC as protein sources to meet the nutrient requirements (protein=14.7% and energy=78% TDN or ME 11.8 MJ/kg) of sheep aged approximately 7 months old as outlined by National Research Council (NRC, 1985). A commercial diet (CD) for sheep with Lucerne (*Medicago sativa*) as protein source was used as a control diet. Other ingredients in the commercial diet were yellow maize, maize bran, wheat bran, feed lime, ammonium chloride, liquid molasses, salt and vitamin premix (Feed dealer; personal communication). The diets were isonitrogenous and isoenergetic. The ingredients for MKC diet and sunflower seed cake (SSC) diet were prepared as shown in Table 6.1.

Table 6.1: Ingredients and chemical composition of treatment diets (% dry matter).

	Treatments		
Ingredients (%)	MKD	SCD	CD*
Ground white maize grain	59	61	-
Sorghum stover	24	21	-
Sunflower seed cake	-	10	-
Morula kernel cake	12	-	-
wheat bran	2.4	5	-
Urea	0.5	1.2	-
Liquid molasses	1.2	1.4	-
Feed lime	0.5	0.7	-
Dicalcium phosphate	0.5	0.5	-
Salt	0.5	0.1	-
Analysed composition[1] (%)			
Dry matter	94.7	95.1	95.5
Organic matter	93.0	92.7	92.7
Crude protein	15.9	14.1	14.1
Ether extract	9.4	2.9	4.8
Ash	7.0	7.3	7.3
Neutral detergent fibre	41.7	39.4	33.5
Acid detergent fibre	18.4	18.1	9.2
GE(MJ/Kg)	16.5	17.6	16.8
ME(MJ/Kg)[2]	8.3	8.8	8.4

* - ingredient proportions in a complete diet unknown but has Lucerne (*Medicago sativa*) as protein source. MKD=morula kernel cake diet; SCD=sunflower seed cake diet; CD=commercial diet; [2]ME=GE x 0.5 adopted from NRC, 2007 cited by Ma et al. (2019); [1–] Laboratory results reported on % dry matter basis.

6.2.3. Selection of experimental animals, feeding and growth

Eighteen (18) castrated male Tswana lambs aged 8 months on average were obtained from Botswana University of Agriculture and Natural Resources (BUAN) farm. These lambs were each weighed for two consecutive days to determine their initial weights. Each lamb was injected subcutaneously with 1ml of Ivermax (Copal Grimed Pty, Ltd, South Africa) before the experiment began to treat for internal parasites. These lambs were divided into three (3) groups balanced for weight (16.9 ± 0.7) and the groups were randomly distributed accordingly to the three dietary treatments of commercial diet (CD), Morula kernel cake diet (MCD) and sunflower seed cake diet (SCD). There were 6 animals per treatment and each animal was housed in a pen measuring 2.5m x 1m in a CRD. These lambs were adapted to the diets and pens for seven (7) days. Each lamb was given daily feed *ad libitum* with 15% allowance of leftovers in their individual pens for 103 days. Feed leftovers and feed given were weighed daily using a platform scale (Digital scale; model DS-530, Teraoko Seiko, Japan) and recorded. Weighing of lambs was done before morning feeding for two (2) consecutive days every 2 weeks to determine fortnightly weight using a mobile walk-in scale (Crane scale: Tal tec Pty, Ltd, South Africa). The pens were cleaned daily before providing fresh feed and water.

6.2.4. Digestibility experiment

After 90 days of the feeding experiment, four lambs from each treatment were randomly selected and transferred to metabolic crates for a digestibility experiment. The same treatments from the growth experiment were used accordingly. The lambs were kept individually in a metabolism crate designed for the collection of faeces and urine in separate containers. The lambs were adjusted to metabolic crates and faecal bags for 7 days. A period of 6 days was used for data and sample collection. Fresh water was provided daily for each sheep *ad libitum*. Each morning, before providing fresh feed, the leftovers from the previous day were collected and weighed to determine daily feed intake. Daily samples of feed were collected and composited per treatment for chemical analysis.

Daily total faeces for each animal were collected and weighed in the morning before feeding and watering. About 10% of the faecal material was collected, oven dried at 70^{o}C for 72h and bulked for each animal to use for chemical analysis (Lakpini et al., 2015). The feed samples and faecal samples were ground using an electric grinder (Grinder Thomas-Wiley, laboratory mill model 4, Arthur Thomas Company, USA) to pass through a 2 mm sieve. The urine of

each animal was collected in a plastic container with 25ml of 10% sulphuric acid (H_2SO_4) (Lakpini et al., 2015). Thereafter, 10% of the measured daily urine was sampled and a composite sample for each animal was poured in a labelled screw-capped plastic bottle, stored in the refrigerator at 4ºC pending analysis of nitrogen. Apparent digestibility (AD), nitrogen balance (NB), basal endogenous nitrogen (BEN) and nitrogen retention (NR) were calculated as described in Chapter 4 under section 4.2.3.

6.2.5. Slaughter procedure

After 103 days of feeding, all lambs from the experiment including those used in digestibility experiment were humanely slaughtered at a local abattoir in the morning after a night fast. The lambs were electrically stunned and immediately bled by cutting the jugular vein, carotid arteries, trachea and oesophagus using a sharp knife. All blood from the severed veins was collected and weighed for each animal. After bleeding each sheep, it was hung by both hind legs on the rail. The carcass was then skinned and eviscerated. The head and feet were removed and thereafter weighed. The front and hind feet were cut at the proximal metatarsal and metacarpal joints, respectively; the head was disjointed at the occipito-atlantal articulation and weighed. The visceral organs were also separated and weighed. The fat depot (abdominal, kidney area and heart area) was also weighed. The rumen and intestines were weighed with contents and later without contents. The carcass was then weighed to obtain hot carcass weight using a spring balance. The empty body weight (EBW) was obtained by subtracting the rumen and intestinal contents weight from the final weight (NRC, 2001). After carcass weighing, *Longissimus dorsi* muscle was sampled from the left and right side of each carcass for use in sensory analysis and meat quality determination. Dressing out percentage (DOP) was calculated as a proportion of hot carcass weight (HCW) to empty body weight, according to the following formula:

$$DOP = \frac{HCW}{EBW} \times 100$$

6.2.6. Meat quality measurements

6.2.6.1. Meat pH

Approximately 300g of the left *Longissimus dorsi* muscle from each lamb was used for ultimate pH (24h) determination using a pH meter (model HI99163, USA). The pH meter with a sharp probe was used to pierce through the muscle tissue and readings were taken from three

locations and averaged to obtain mean value as described by Machete et al. (2016). Six replications (lambs) for each treatment were recorded.

6.2.6.2. Meat colour

The colour of 300g left *Longissimus dorsi* muscle on the external surface was measured using precision meat colorimeter (Miniscan, model NR20XE, 3NH Technology Co, Ltd, Shenshen, China) after 24h storage in the cold room. The device was standardised using a black tile and white tile as described by Machete et al. (2016). Readings were taken from three random locations on each meat sample and the average readings for lightness (L*), redness (a*) and yellowness (b*) were recorded for six meat samples in each treatment. Therefore, six replications (lambs) for each treatment were recorded.

6.2.6.3. Tenderness

Firmness of 200g right *Longissimus dorsi* muscle was determined using Digital firmness tester (Model Agro R15, France). The muscles were cooked after 48h of storage at 4^{o}C in the refrigerator. Meat samples from six lambs per treatment were cooked at medium heat (electric stove) for 1 h 20 minutes. Salt (7.5g) was added after 15 minutes of cooking time (personal experience). The meat was cooked until water dried up and shallow frying was done for approximately 2-3 minutes. The meat samples were cooled and allowed to equilibrate to room temperature. Three cores of approximately 2cm diameter were removed from each cooked meat sample with the blade of firmness tester. Readings were taken from three intact random locations and an average value was recorded for each muscle tissue according to the methodology described by Machete et al. (2016).

6.2.6.4. Meat sensory analysis

A meat sensory evaluation was performed at Food Nutrition and Technology Laboratory, BUAN, using portions from the right *Longissimus dorsi* muscle. An untrained taste panel of twenty-two (22) persons of mixed gender, aged 26 to 55 years was used in the sensory evaluation. Meat samples had been refrigerated (4^{o}C) for 48h after slaughter and composite samples from six (6) lambs for each treatment weighing 1.1 kg were used. The meat samples were prepared by washing with 2 litres of tap water at room temperature. Samples were then diced into approximately 1cm^3 and put into stainless steel pots filled with 500ml cold tap water. The meat samples were cooked as described under section 6.2.6.3. (chapter 6) before presentation to the panellists. The taste panel evaluated the meat on the following organoleptic parameters; appearance, taste, flavour, tenderness, juiciness and overall impression. The

panellists were inducted on how to evaluate the meat samples before carrying out the exercise. The panellists were requested to rinse their mouth with potable water after each sample tasting to limit residual effect of the treatments as described by Ngambu et al. (2012). A meat sensory characteristic Evaluation Form containing a nine-point rating scale of meat characteristics was used, comprising; 1. Dislike extremely, 2. Dislike very much, 3. Dislike moderately, 4. Dislike slightly, 5. Neither like nor dislike, 6. Like slightly, 7. Like moderately, 8. Like very much and 9. like extremely according to Morlein (2019).

6.2.6.5. Proximate analysis of *Longissimus dorsi* muscle

Meat samples of *Longissimus dorsi* muscle obtained from the left side of each carcass were sent to National Food Technology Research Centre (NFTRC), Kanye, Botswana, for chemical analysis. Six samples (300g/sample) per treatment were analysed for moisture, protein, fat and ash.

6.2.6.6. Meat fatty acid analysis

Six samples of the left *Longissimus dorsi* muscle (200 g each) from each treatment were ground using a food processor (Mini food chopper, RES004;2.9L, Robot Couple Manufacturer, Germany) for extraction of fatty acids methyl esters (FAME) following procedures of O'Fallon et al. (2007) as described in Chapter 3 under section 3.2.1.12.

6.2.7. Chemical analysis of feed and faecal samples

Feed and faecal analysis was determined at DAR, Animal Nutrition Laboratory, Sebele, Gaborone. Analytical methods are fully described in Chapter 3 of the thesis.

6.2.8. Gross Margin (GM) analysis

The amount of feed consumed by each lamb over a period of 103 days of feeding was quantified and feed costs for each treatment were calculated. The variable costs were; labour (looking after trial animals), drugs, feeds, abattoir costs, transportation of animals to abattoir and purchase cost of weaners. The price of slaughter for each lamb on weight basis and price for various offals was obtained from four local meat retailers and an average price for each was used in GM calculations. The carcass value and edible offals value for each treatment were summed up to estimate revenue for each treatment. Gross income was calculated from sale of each animal carcass and edible offals. Therefore, GM= Gross Income (GI) – Variable Costs (VC). Percentage (%) return on investment was calculated as (gross margin/total variable cost) x 100.

6.2.9. Data Analysis

Final body weight, ADG, DMI and FCR data were subjected to one-way Analysis of Variance (ANOVA) using GLM procedures of Statistical Analysis System (SAS, 2002) to test the effect of diet treatment using Model 1;

$Y_{ijk} = \mu + T_i + b(X_{ij} - \sum x/n) + e_{ijk}$ Model 1

Where Y_{ij} =response, μ=population mean, T_i = treatment effect i=1, 2, 3. b= covariate analysis of initial body weight of an animal on subsequent performance, X_{ij} = initial body weight of individual sheep, Σx/n=mean of initial body weight in the experiment, e_{ijk}= random error

For time series data (fortnightly bodyweight), the proc mixed procedure of SAS (2002) was used for analysis using repeated measures model and animal was used as a random factor. The statistical differences between means were determined using Fisher's Least Significance Difference (LSD) model as shown below:

$Y_{ijk} = \mu + T_i + F_j + (T_i \times F_j) + \varepsilon_{ijk}$ Model 2

Where Y_{ijk}= is the K^{th} observation of the i^{th} treatment of the j^{th} days, μ=is the overall mean, T_i= is the fixed effect of the i^{th} treatment (i=1,2,3), F_j=is the (time) days (j=1, 2….84), $T_i \times F_j$= interaction between treatment and time (days), ε_{ijk} = random residual error

Data on sensory evaluation, NB and meat quality were analysed as CRD using GLM of SAS (2002) and are represented by Model 3 below:

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$ Model 3

Where Y_{ij}= is the K^{th} observation of the i^{th} treatment, μ=is the overall mean, T_i= is the fixed effect of the i^{th} treatment (i=1,2,3), ε_{ij} = random residual error

Level of significance was declared at $P < 0.05$.

6.3. Results

6.3.1. Nutrient intake

The intake of nutrients by the lambs fed on dietary treatments is presented in Table 6.2. A treatment effect was observed for intake of EE ($P<0.05$) by the lambs. The intake of EE was greatest ($P<0.05$) in lambs fed on MKD (83.7g/day), intermediate for CD (48.4g/day) and least for SCD (27.9g/day). Intake of ADF was significantly higher ($P<0.05$) and similar in lambs fed

MKD and SCD while the lambs fed CD had the least intake of ADF. The intake of GE, OM, CP and NDF by the lambs were similar ($P>0.05$) across the treatments. Intake of lignin was significantly lower ($P<0.05$) for lambs given CD and MKD compared to those on SCD.

Table 6.2 : Dry matter intake and nutrient intake from digestibility experiment (dry matter basis) of lambs

	Treatments				
Item (g/day)	CD	MKD	SCD	RMSE	P-value
DM intake	1008.0	890.6	954.0	129.7	0.2
OM intake	934.4	828.3	884.3	120.3	0.1
GE intake, MJ	16.9	15	16	2.2	0.2
CP intake	142.1	141.6	134.5	18.7	0.5
Ether extract intake	48.4^{b}	83.7^{a}	27.9^{c}	7.8	0.0002
NDF intake	367.4	371.4	375.4	55	0.5
ADF intake	92.7^{b}	163.9^{a}	172.7^{a}	18.7	0.004
Lignin intake	41.3^{b}	39.2^{b}	53.4^{a}	6.1	0.02
Ash intake	73.6	62.4	69.6	3.4	0.1

RMSE=root mean standard error; CD=commercial diet; MKD=Morula kernel cake diet; SCD=sunflower seedcake diet; ^{abs} - Means in same row with different superscript are statistically different at $P < 0.05$.

6.3.2. Digestible nutrient intake and apparent digestibility

The intake of digestible nutrients and apparent digestibility of the treatments are given in Table 6.3 The intake of digestible EE by lambs from MKD (69.6 g/day) was significantly greatest ($P<0.05$), intermediate for CD (42.9 g/day) and least for lambs fed SCD (19.4 g/day). However, the intake of digestible DM, OM, CP and NDF by the lambs was similar across the treatments ($P > 0.05$). On the other hand, intake of digestible ADF was lower ($P < 0.05$) in lambs fed CD but higher and similar in the lambs fed SCD and MKD. Digestible ash intake was higher ($P < 0.05$) in lambs fed CD, intermediate in lambs fed SCD and lower in lambs fed MKD. The digestion coefficients of DM, OM, NDF and ADF were similar ($P>0.05$) across the treatments. However, CP digestibility was significantly higher ($P<0.05$) in lambs fed CD compared to the other treatments. Lambs fed MKD and SCD treatments had similar apparent digestion coefficients of CP. Apparent digestibility of ash was higher in CD than on MKD and SCD that were similar.

Table 6.3 : Digestible nutrient intake (g/day) and apparent nutrient digestibility (%) (on-dry matter basis) by the lambs.

	Treatments				
Item	CD	MKD	SCD	RMSE	*P*-value
Digestible Nutrients Intake					
Dry matter	937.2	711.8	790.8	122.7	0.08
Organic matter	870.7	670.6	715.5	124.3	0.1
Crude protein	131.6	118.8	110.8	17.6	0.2
Ether extract	42.9^{b}	69.6^{a}	19.4^{c}	6.9	0.0001
NDF	313.0	252.3	244.0	49.6	0.2
ADF	72.0^{b}	100.0^{a}	116.8^{a}	14.8	0.02
Ash	66.5^{a}	41.3^{b}	51.1^{ab}	8.9	0.03
Apparent Digestibility					
Dry matter	92.7	81.3	83	4.1	0.08
Organic matter	92.9	82.3	80.6	5.1	0.07
Crude protein	92.3^{a}	84.8^{b}	82.5^{b}	2.8	0.01
Ether extract	89.1	84.9	72.5	8.8	0.1
NDF	84.7	70.0	65.6	7.4	0.2
ADF	76.7	62.0	68	7.4	0.2
Ash	90.0^{a}	68.3^{b}	73.7^{b}	5.3	0.02

RMSE=root mean standard error; CD=Commercial diet; MKD=Morula kernel cake diet; SCD=sunflower seedcake diet; ^{abc}Means in same row with different superscript are statistically different at $P < 0.05$.

6.3.3. Nitrogen (N) balance

Nitrogen balance of lambs fed the dietary treatments is shown in Table 6.4. Intake of nitrogen by the lambs was similar across the treatments. There were no statistically significant differences (P>0.05) in faecal-N, absorbed-N, urinary-N and total nitrogen excretion among the dietary treatments. Nitrogen retention was significantly higher (P<0.05) in lambs from CD than in SCD. Lambs from MKD and SCD had similar NR. Nitrogen retention as a percent of nitrogen intake was highest (P < 0.05) in lambs from CD, intermediate for MKD and lower for SCD. Nitrogen retention as a percent of absorbed-N was statistically similar (P > 0.05) across the treatments but numerically lower in SCD fed lambs.

Table 6.4 : Nitrogen balance of lambs fed the different diets

	Treatments				
Item	CD	MKD	SCD	RMSE	*P*-value
N-Intake(g/d)	21.5	20.5	20.4	3.1	0.5
Excretion(g/d)					
Urinary-N	8.5	8.7	9.8	2.3	0.8
Faecal-N	1.5	2.6	3.8	0.8	0.06
Total	10.1	11.3	13.6	2.6	0.4
% UN-Excretion	84.1	77.6	72.1	5.9	0.08
Absorbed-N	20.0	18.0	16.6	2.8	0.2
%UN-AN	42.7	48.0	59.0	9.1	0.08
N-Retention					
N-retention, g/day[1]	8.7[a]	6.4[ab]	3.9[b]	1.9	0.03
Retention-I (%)	39.9[a]	31.6[ab]	19.1[b]	7.7	0.03
Retention-A (%)	43.1	35.8	23.6	8.9	0.07

RMSE=root mean standard error; CD=commercial diet; MKD=Morula kernel cake diet; SCD=sunflower seed cake diet; N=nitrogen, I=intake; A=absorbed, U=urinary, [abc]Means in same row with different superscript statistically vary at P < 0.05.[1]corrected for metabolic loss

6.3.4. Growth performance

Table 6.5 shows the effect of treatments on the final body weights, weight gain, ADG and carcass traits of lambs. There were no significant differences (P>0.05) observed for final body

weight, DMI, ADG and FCR among these treatments. However, lambs fed on MKD had numerically lower DMI (867.8g). Commercial diet and SCD had the highest but insignificant (P>0.05) ADG at 176.3g and 174.3g, respectively. The hot carcass weight, empty body weight and dressing out percentage (%) were not significantly different (P>0.05) across the treatments. Dressing out percentage (%) ranged from 58.6% to 60.3% but was similar (P>0.05) between treatments.

Table 6.5 : Growth performance and carcass traits of lambs given the treatments

	Treatments				
Item	CD	MKD	SCD	RMSE	*P*-value
Performance					
Initial weight(kg)	16.2	16.7	17.9	0.5	0.9
Final weight(kg)	35.3	34.0	35.1	2.3	0.6
DMI(g)	890.4	867.8	905.9	71.5	0.7
ADG(g)	176.3	163.7	174.3	22.8	0.6
FCR	5.7	6.2	5.9	0.8	0.6
Carcass traits					
Empty body wt (kg)	29.1	28.5	30.3	2.0	0.3
Hot carcass wt (kg)	17.1	16.9	17.1	1.0	0.2
Dressing (%)	58.6	59.2	60.3	1.8	0.4

CD=commercial diet; MKD=Morula kernel cake diet; SCD=sunflower seed cake diet; RMSE=root mean standard error; DMI=dry matter intake; ADG=average daily gain; FCR=feed conversion ratio; wt=weight

Fortnightly body weight

There were no significant differences (P>0.05) in the fortnightly mean body weights of the sheep during the 84-day growth period (Figure 6.1).

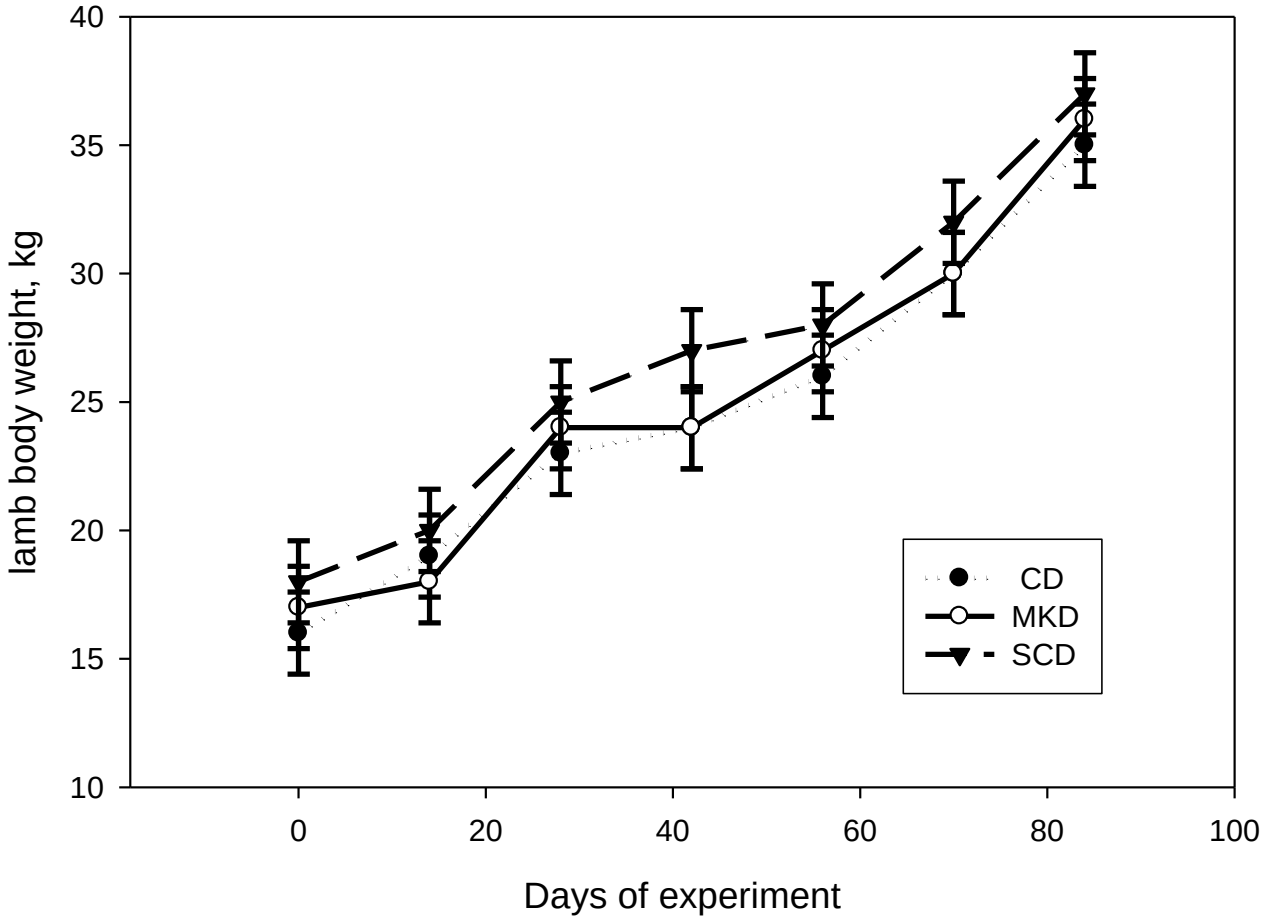

Figure 6.1 : Effect of different protein sources (treatment) on mean fortnightly body weights of lambs. CD=commercial diet; MKD=Morula kernel cake diet; SCD=sunflower seed cake diet.

6.3.5. Organ mass

The effect of treatments on body organs or viscera of sheep is shown in Table 6.6. The weight of visceral organs was statistically similar (P>0.05) across the treatments.

Table 6.6 : Edible and non-edible carcass offals of lambs fed different treatment diets

	Treatments				
Item (kg)	CD	MKD	SCD	RMSE	*P*-value
Edible offals					
Head	1.8	1.9	1.8	0.1	0.8
Heart	0.1	0.1	0.1	0.1	0.8
Lungs	0.4	0.4	0.3	0.1	0.6
Liver	0.4	0.4	0.4	0.07	0.8
Kidneys	0.1	0.1	0.1	0.01	0.3
Visceral fat	1.1	1.1	1.1	0.3	1.0
Front feet	0.4	0.4	0.4	0.05	0.6
Hind feet	0.4	0.4	0.4	0.05	0.4
Spleen	0.02	0.03	0.02	0.01	0.5
Stomach	0.9	0.8	1.0	0.2	0.4
Intestine	1.6	1.5	1.6	0.2	0.6
Non-edible offals					
Blood	1.1	1.1	1.1	0.1	0.9
Skin	3.0	3.1	3.1	0.3	1.0
Trachea	0.05	0.05	0.05	0.03	1.0

RMSE=root mean standard error; CD=commercial diet; MKD=Morula kernel cake diet; SCD=sunflower seed cake diet.

6.3.6. Meat quality

6.3.6.1. Physico-chemical and proximate composition

Proximate composition and meat quality attributes are shown in Table 6.7. No differences (P>0.05) were observed for moisture, crude protein, ash and fat on *Longissimus dorsi* muscle of the lambs across treatments. Also, colour, pH and shear force were not significantly influenced (P>0.05) by the dietary treatments.

Table 6.7 : Physico-chemical attributes and proximate composition of *Longissimus dorsi* muscle (from left side of carcass) from lambs

	Treatments				
Items	CD	MKD	SCD	RMSE	*P*-value
Physico-chemical					
Shear force (N)	22.9	20.1	24.0	4.3	0.3
pH_{24h}	5.0	4.8	5.0	0.4	0.8
L*	39.4	40.9	43.7	7.3	0.6
a*	20.7	21.6	22.3	2.4	0.6
b*	7.6	8.9	8.3	1.4	0.3
Proximate composition					
Moisture (%)	73.5	72.5	72.5	2.2	0.7
Protein (%)	28.3	27.5	26.9	3.6	0.8
Fat (%)	4.0	5.2	6.9	3.0	0.3
Ash (%)	1.2	1.3	1.2	0.3	0.8

RMSE=root mean standard error; L*=lightness; a*=red content; b*=yellow content; CD=commercial diet; MCD=Morula kernel cake diet; SCD=sunflower seed cake diet.

6.3.6.2. Fatty acid profile of *Longissimus dorsi* (LD) muscle

Table 6.8 shows the fatty acid profile of *Longissimus dorsi (LD)*muscle. The major fatty acids in the lamb's *LD* muscle from all treatments were palmitic acid (Palmitic methyl ester), stearic acid (Stearic methyl ester) and myristic acid (Myristic methyl ester). Oleic acid was significantly high ($P < 0.05$) in LD muscle of lambs fed MKD and was not detected in LD muscles of lambs offered SCD. The proportion of palmitic acid was significantly ($P < 0.05$) higher in LD muscles of lambs fed SCD but similar ($P > 0.05$) in LD muscles of lambs given CD and MKD. Methyl isostearate was detected only in LD of lambs on CD and SCD. 7, 10-Octadecadienoic methyl ester and 8, 11-Octadecadienoic acid methyl ester were detected in LD muscle of lambs from SCD. Trans-vaccenic methyl ester, cis-13-Octadecenoic acid methyl ester, trans, cis-Octadecadienoate acids were detected only from LD muscle of lambs from CD. No statistical differences ($P > 0.05$) were observed across treatments for saturated fatty acids

(SFA), unsaturated fatty acid (UFA), polyunsaturated fatty acids (PUFA): saturated fatty acid (SFA) ratio and UFA: SFA ratio in LD muscle of lambs. Monounsaturated fatty acids were significantly higher ($P < 0.05$) in LD muscle of lambs fed MKD, intermediate in CD and lower in lambs fed SCD. Polyunsaturated fatty acids (PUFA) were significantly higher ($P< 0.05$) in LD muscles of lambs fed CD but similar in LD muscles of MKD and CD fed lambs.

Table 6.8 : Fatty acid profile (% of total identified fatty acids) of *Longissimus dorsi* muscle of lambs fed CD, MKD and SCD

	Treatments				
Item	CD	MKD	SCD	RMSE	*P*-value
Saturated					
Myristic, methyl ester	2.3	2.2	2.9	0.3	0.1
Palmitic, methyl ester	27.9[b]	27.7[b]	30.7[a]	0.6	0.005
Stearic, methyl ester	6.5	20.8	10.9	9.9	0.3
Methyl isostearate	14.0	ND	9.9	9.9	0.3
Monounsaturated					
Methyl 10-Octadecenoate	ND	ND	21.6	13.7	0.2
Oleic acid	14.3[b]	46.1[a]	ND	15.7	0.049
Cis-13-Octadecenoic acid[1]	14.9	ND	ND	16.3	0.5
Methyl, 8-Octadecenoate	ND	ND	20.9	13.2	0.2
Trans-vaccenic, methyl ester	15.0	ND	ND	16.4	0.5
Polyunsaturated					
Methyl linoleate	1.8	ND	1.8	2.3	0.6
Methyl 9-cis, 11-trans-Oct[2]	ND	0.9	ND	1.0	0.5
7,10-Octadecadienoic[3]	ND	ND	1.5	0.9	0.2
Trans,cis-Octadecadienoate	1.9	ND	ND	2.1	0.5
8,11-Octadecadienoic acid[4]	1.6	0.9	ND	2.0	0.7
Saturated fatty acid	50.5	50.9	54.3	1.3	0.06
Unsaturated fatty acid	49.5	49.0	45.7	1.3	0.06
Monounsaturated fatty acid	44.2[ab]	46.1[a]	42.5[b]	1.0	0.02
Polyunsaturated fatty acid	5.3[a]	2.9[b]	3.2[b]	0.4	0.02
Polyunsaturated: Saturated	0.1	0.06	0.4	0.2	0.3
Unsaturated: Saturated	0.97	0.67	0.85	0.3	0.5

RMSE=root mean standard error; CD=commercial diet; MKD=Morula kernel cake diet; SCD=sunflower seed cake diet; [1]Cis-13-Octadecenoic acid methyl ester; [2]Methyl 9-cis, 11-

trans-Octadecadienoate; 7, 10-Octadecadienoic methyl ester[3]; 8, 11-Octadecadienoic acid methyl ester[4]; [abc]Means in same row with different superscript statistically vary at $P < 0.05$; ND=not detected.

6.3.6.3. Sensory analysis

Table 6.9. shows the panel rating of cooked *Longissimus dorsi* muscle from lambs. The dietary treatments did not result in any significant ($P>0.05$) difference in the parameters measured (appearance, taste, flavour, tenderness, juiciness and overall impression).

Table 6.9 : Sensory evaluation of *longissimus dorsi* muscle (from right side of carcass) from the lambs

	Treatments				
Item	CD	MKD	SCD	RMSE	*P*-value
Appearance	6.5	7.2	6.9	1.8	0.5
Taste	7.1	7.7	7.0	1.4	0.8
Flavour	7.4	7.5	7.3	1.6	0.3
Tenderness	7.2	7.7	7.0	1.6	0.3
Juiciness	6.8	7.6	7.2	1.6	0.3
Overall impression	7.5	8.0	7.3	1.0	0.09

RMSE=root mean standard error; CD=commercial diet; MCD=Morula kernel cake diet; SCD=sunflower seed cake diet; rating scale, 1=dislike extremely to 9=like extremely.

6.3.7. Gross margin analysis

Table 6.10 presents the gross margin analysis of the treatments from the current study. SCD had a significantly ($P < 0.05$) higher gross margin value of BWP212.7 per lamb, MKD was intermediate (BWP 117.7) and CD had the lowest GM value of BWP 14.0. The CD had significantly higher ($P < 0.05$) variable costs of BWP 1390.50 and MKD and SCD had similar variable costs (BWP 1277.30 vs BWP 1292.7 respectively). Percentage return on investment was higher on SCD, intermediate on MKD and lower on CD. However, total output value was similar ($P > 0.05$) across the treatments.

Table 6.10 : Gross margin analysis of the three treatments per lamb

	Treatments				
Item (BWP)	CD	MKD	SCD	RMSE	*P*-value
Hot carcass value	1337.22	1321.58	1431.06	140.5	0.3
Edible offal value	55.61	53.39	58.73	6.3	0.7
Head and feet value	34.80	36.40	34.80	3.0	0.6
Total output (BWP)	1404.60	1389.10	1505.40	146.2	0.4
Variable costs (BWP)					
Feed	420.40	242.10	244.70	-	
Weaner	800.00	800.00	800.00	-	
Abattoir costs	98.90	98.90	98.90	-	
Drugs	4.40	4.40	4.40	-	
Labour	55.60	55.60	55.60	-	
Transport	13.90	13.90	13.90	-	
Total variable costs	1390.50^{a}	1277.30^{b}	1292.70^{b}	35.0	0.0001
GM (BWP)	14.00^{b}	111.70^{ab}	212.70^{a}	129.7	0.05
% ROI	1.0^{b}	8.7^{ab}	16.2^{a}	9.7	0.04

RMSE=root mean standard error; GM=gross margin; 1USD=10BWP; CD=commercial diet; MKD=Morula kernel cake diet; SCD=sunflower seed cake diet; %ROI=return on investment; ^{abc}Means in same row with different superscript statistically vary at $P < 0.05$.

6.4. Discussion

6.4.1. Nutrient intake

According to Zhao et al. (2016), efficient livestock production is dependent on precise knowledge of their energy and nutrient requirements. Dry matter intake (g/day) in the current

study was similar across the treatments and total dry matter intake as a percent body weight was within the recommended range of 2 to 6% for sheep (ARC, 1980). Similar dry matter intake across the treatments in the current study is attributed to similar intake of CP across the treatments (Table 6.2). This suggests that nitrogen supplied by the diet in the rumen resulted in increased microbial population that digested the ingested feed thereby culminating in the current observed DMI. Additionally, current study treatments were formulated iso-energetic and iso-nitrogenous which explains the similarity in DM and CP intake as earlier observed by Yagoubi et al. (2021), implying perhaps presence of anti-nutritional factors in each of these diets at negligible amounts. Similarly, Ahmed and Abdalla et al. (2003) reported non-significant DMI by desert rams fed on complete diets with different nitrogen sources (cotton seed cake, sesame seed cake, groundnut cake and sunflower seed cake). In contrast, Alves et al. (2016) reported significant differences of DMI by lambs fed on complete diets with different protein sources (Soybean meal, castor bean cake, sunflower cake and sunflower seed). The lower DMI for lambs fed on sunflower cake-based diet was explained by Alves et al. (2016) to have been caused by higher fat content than in soybean-based diet. Nutrient intake is a function of digestibility of feed ingested and diets with high digestibility have high intake (McDonald et al., 2011) of nutrients. Mlambo et al. (2011) reported that an inclusion of MKC (*Sclerocarya birrea*) in the diets of Nguni goats significantly reduced total organic matter intake. The reason advanced by Mlambo et al. (2011) for low total organic matter intake was high fat content in the MKC (*Sclerocarya birrea*) diet. Also, Muhammad et al. (2016) reported that increased EE in *Sclerocarya birrea* nut meal diets of Uda sheep decreased intake of nutrients and digestibility. The diets used in their (Muhammad et al., 2016) study had EE ranging from 5% to 8%. In contrast, EE in MKD, in the current study was higher at 9.4%, with 84g/d EE intake, however the nutrient intake (crude protein, neutral detergent fibre and acid detergent fibre) was not depressed. This suggests that fibre digesting rumen microbes (cellulolytic or fibrolytic bacteria) in the current study were not impeded by EE level. Malebana (2018) also reported improved average daily gains of 194g/day in Dorper sheep fed on MKC complete diet with exceedingly higher EE content of 13.3% on DM basis. The present study together with that of Malebana (2018), suggests that residual oil from MKC (*Sclerocarya birrea*) can be used to boost the energy density of the diet without negatively affecting nutrient intake and utilisation. However, conflicting results across the studies may be attributed to differences in diet composition, genotype of experimental animals and trial management. According to McDonald et al. (2011) caution should be exercised when incorporating fat in ruminant diets since high

fat (EE) content tends to impair fermentation and digestion of plant cell wall constituents in the rumen. Therefore, future research should interrogate fermentation characteristics of animals fed similar diets as was in the present study, to reveal reasons for lack of detrimental effects of high EE in MKD diets.

6.4.2. Digestible nutrient intake and apparent digestibility

Intake of digestible crude protein (DCP) by the lambs was similar across the treatments (Table 6.3). The lambs' intake of DCP ranged from 111g/day to 132g/day. Basing on Pond et al. (1995) categorisation of sheep and goats, the Tswana sheep can be classified as a small-sized breed with CP requirements of either 121g/day or 145g/day with potential ADG of 150g/day and 200g/day, respectively. Recently, Rakobe (2019) was able to register daily gain of 150g/day after having fed the growing Tswana sheep with complete diets with freshly sun-dried maize husk. Intake of DCP by the lambs in the present study is comparable to findings of Muhammad et al. (2016), who reported intake of DCP ranging from 108g/day to 156.1g/day by Uda sheep fed on diets with graded levels of *Sclerocarya birrea* kernel meal.

The lambs from CD had higher but non-significant digestible intake of DM, OM, CP and NDF. This is attributable to high digestibility of CP (92.3%) in CD, which supplied rumen microbes with readily degraded nitrogen which increased their growth and led to higher degradation rate of the diet (Kanyinji et al., 2017). Morula kernel cake diet and SCD had lower digestibility of CP. This may be explained by higher intake of ADF or possible presence of anti-nutritional factors from MKC and sunflower seed cake. According to Malebana (2018) MKC contains anti-nutrients such as saponins, tannins and phytate. On the other hand, anti-nutrients such as chlorogenic acid, caffeic acid and phytate are found in sunflower seed cake (Vasudha and Sarla, 2021). However, when compared to the current study, digestibility of crude protein recorded by Muhammad et al. (2016) was lower and ranged from 67% to 79.4% for *Sclerocarya birrea* kernel meal in Uda sheep diets. The researchers (Muhammad et al., 2016) attributed lower CP digestibility to high fat content in the diet. Digestibility of ash was significantly higher in CD fed lambs (90%) and lower for lambs consuming either MKD or SCD. The CD had a mineral premix and this suggests that the amount of all important minerals were adequately available thereby supporting proliferation of rumen microbes and normal fermentation function. However, mineral premix was not incorporated in MKD and SCD but animals fed these diets still performed similar to CD. This implies that MKC and sunflower seed cake also supplied

enough minerals needed by rumen microbes for degradation of dry matter. Most smallholder farmers in developing countries like Botswana cannot afford buying mineral and vitamin premixes, hence, in the experimental diets mineral premixes were purposely not included in order to mimic smallholder farm setting.

6.4.3. Nitrogen balance (NB)

Monitoring of NB is an important and accurate tool of evaluating the value of protein sources in ruminants (Kanyinji et al., 2017). Intake of nitrogen by these lambs were similar across treatments. This is attributed to similar protein content and supply by the dietary treatments. According to Van Soest (1994), increased intake of nitrogen lead to high urea production in the liver and ultimately of its release in urine. However, a decreased intake of nitrogen results in reduced urea excretion in urine to maintain a homeostatic balance in the animal's body. Emission of nitrogen from livestock intensification is a major concern to all stakeholders in the livestock industry. According to Vasconcelos et al. (2007), nitrogen (N) is excreted in the animal waste in the form of urea and other organic N forms (ammonia and nitrous oxide). Therefore, some of the nitrogen loss in the form of nitrous oxide and ammonia are an environmental concern as they contribute to global warming (Rotz, 2004). Cowley et al. (2019) stated that about 5 to 20% of N consumed during feeding period is retained in the carcass with the balance being excreted in urine and faeces. In the current study %urine-N excretion (Table 6.4) was similar across the treatments and most of the consumed N was excreted in urine suggesting that the treatment diets were highly degradable in the rumen. Cowley et al. (2019) explained that N in urine is mostly from ingested feed with the remaining urine-N being derived from catabolism of endogenous proteins. Loss of N from the animal's body is unavoidable and Rotz (2004) pointed out that nitrogen use efficiency by ruminants can be improved by either matching the animal's protein requirements with protein quality fed or through improvement of animal productivity.

In the current study, the fact that nitrogen digestibility and NB were lower in SCD than in CD suggests that nitrogen from the Lucerne was readily available to rumen microbes and extra nitrogen from the diet was used by the host animal for tissue development and other bodily functions. Nitrogen digestibility and NB of sheep fed MKD and SCD were similar. However, sheep fed MKD had a higher but non-significant nitrogen retention (6.4g/day compared to

3.9g/day for SCD animals). This can be explained by MKC providing RUP of 14.8% to the diet while sunflower seed cake provided RUP of 9.8% to the diet (Chapter 3 of this thesis). Abubakar et al. (2010) also reported comparable NR value of 5.9g/day for sheep fed on cottonseed cake in complete diet. Mlambo et al. (2011) reported significantly higher NR (2.8g/day) in Nguni goats supplemented with MKC compared to sunflower seed cake (1.1g/day). Their (Mlambo et al., 2011) results were both lower than values obtained in the current study. The differences in the results between the studies could be due to experimental animals used, diet composition and management. The positive NB in all these diets in the present study shows that intake of nitrogen was enough to supply rumen microbes with required nitrogen amount needed for DM degradation and to ensure sufficient positive balance as observed earlier by Abubakar et al. (2010).

6.4.4. Growth performance

The study reveals that under intensive feeding system in conditions similar to those of the current study, Tswana sheep are able to attain daily weight gain of 171g. This is regardless of difference in sources of protein in the different diets. The reason being that the diets were formulated to be isonitrogenous and isoenergetic, hence the lambs grew in a similar fashion by having similar ADG across treatments. This suggests that all treatments supplied comparable nutrients (Bonanno et al., 2012; Nurfeta et al., 2013) needed for maintenance and growth by the lambs. The similarity in nutrient supply is further shown by similar intake of both DDM and DCP (Table 6.3). Yagoubi et al. (2021) also reported non-significant differences in body weight gain of lambs fed different nitrogen sources (soybean meal and faba bean). In contrast, Ruzic-Muslic et al. (2014) reported significant differences on ADG of lambs fed on complete diets with different protein sources (sunflower meal, soybean meal and fish meal). In the study by Ruzic-Muslic et al. (2014) both soybean meal and fish meal complete diets had superior ADG than sunflower meal diet suggesting that high content of RUP made the difference among the three protein sources. The ADG in the present study was higher than that of 9 months male Nellore lambs fed different diets based either in groundnut cake (75.3g/d), sunflower cake diet (82.7g/d) or karanja seed cake diet (*Pongamia glabra*) (28.3g/d) by Nagalakshmi et al. (2011). However, Malebana (2018) reported comparable ADG of 194g/day when 19% MKC in total diet was fed to Dorper sheep. Growth in animals is measured by the increase in body size, weight and development of organs (McDonald et al., 2011). The treatments in the current study supported normal growth of sheep from day zero (0) of the experiment to day 84 as shown in

Figure 6.1. The pattern of growth showed that the lambs consistently gained weight over time. In line with these findings, Bonanno et al. (2012) evaluating four concentrates with different legume grains (*Cicer arietinum* (Chickpea), *Vicia faba* (Faba bean), *Pisum sativum* (Pea) and *Glycia max* (Soybean meal) found that Comisana lambs' growth was similar among the four protein treatment diets.

Live weight at slaughter (final weight) is an important variable as it determines both hot carcass weight and cold carcass weight. In the current study, final weight was comparable among all the treatments indicating that the diets supplied similar nutrient profile and quantities to animals for growth.

Assan (2020) stated that nutrition plays a pivotal role in promoting ideal carcass characteristics and meat quality attributes. Therefore, provision of supplementary feeds to livestock usually results in high dressing percentage. In the current study hot carcass weight (HCW), empty body weight (EBW) and hot carcass yield (HCY) were similar across the treatments. The similarity of these traits (HCW, EBW and HCY) is probably due to similarities in the intake of both gross energy and protein and their utilisation. Therefore, the lambs had similar amounts or quality of nutrients availed for absorption and assimilation.

6.4.5. Organ mass

The kind of offals that are edible and non-edible differ from one country to the next based on culture and preference. Edible offals in Botswana are sold to consumers in bulk at retail outlets. Non-edible offals are used in the pet industry as a source of protein and minerals. Zhao et al. (2016) demonstrated that restricted feeding in lambs decreased mass of liver, heart, kidney, gastrointestinal tract and rumen in comparison to *ad libitum* feed intake. On the other hand, Fluharty et al. (1999) reported that grazing of Lucerne (*Medicago sativa*) by lambs increased weight of liver, omasum, abomasum and intestines in comparison to concentrate feeding. Organ mass was measured in the current study as an index of organ development especially that different body components grow at different patterns or rates (Yildirim et al., 2014) based on nutritional supply. According to Hashemi et al. (2014), poor diets that reduce visceral organ mass appears to contribute to lowering maintenance energy requirements especially in the liver and gastrointestinal tract. Sun et al. (1994) reported that liver mass is affected by digestible organic matter intake. The mass of edible and non-edible offals in the current study was similar

across treatments and within the range of previously published research (Suliman & Babiker, 2007; Nagalakshmi et al., 2011 and Maulid, 2015). This indicates that the diets in the current study supplied similar nutrients for internal organ development. Similarly, Nagalakshmi et al. (2011) reported no differences in organ weights from male Nellore lambs (aged 9 months) fed on different complete diets of groundnut cake diet, sunflower cake diet and karanja seed cake diet. In contrast to the current findings, Suliman and Babiker et al. (2007) in their work with intact male sheep (aged 9 months) of Sudan desert breed reported significantly lighter organ weights (skin and liver) in sunflower seed cake-fed group in comparison to other protein sources (ground nut cake, sesame cake and cottonseed cake). Likewise, research by Rao and Kumar (2015) observed significant decrease in liver weight for male sheep (aged 6 months) fed on graded Karanja seedcake (*Pongamia glabra*) in complete sheep diets. The discrepancy in the results of the studies could be attributed to different diet ingredients, anti-nutritional factors, animal age, breed and animal management practices in the respective studies.

6.4.6. Meat quality attributes and proximate composition

The normal adult mammalian muscle consists of 75%, 19%, 2.5% and 0.65% for moisture, protein, fat and ash, respectively (Toplu et al., 2013). The protein content of the *Longissimus dorsi* muscle in the current study ranged from 26.9% to 28.3% while fat content range was 4% to 6.9%, without any significant differences across diets. According to Caneque et al. (2003), high energy intake from the diet may contribute to high intramuscular fat. The protein, fat and ash values from the present study were not influenced by treatments and are slightly higher than results reported by Kotsampasi et al. (2017) on *Longissimus thoracis et lumborum* muscle of Greek breed Florina sheep fed graded levels of olive cake in complete diets. From the current study, the content of moisture was similar to results of previous studies (Bezerra et al., 2016; Chai et al., 2018). The high protein content across diets suggest that sheep meat could be an alternative source of essential amino acids (Polidori et al., 2011) for human diet, especially in Botswana where consumers tend to prefer beef and chicken over lamb/mutton. As a result, educating Batswana about the health benefits of sheep meat is a necessary paradigm shift. This will contribute to diversification of the livestock industry from beef to lamb production and support of female farmers who are in most cases custodian of smallstock.

The type of diet consumed by livestock affects meat quality attributes such as colour, pH, and tenderness. Chai et al. (2018) stated that the changes in muscle lightness (L*) and yellowness

(b*) show the dietary effects of pre-slaughter glycogen and marbling levels. In the current study, colour parameters and pH were determined at 24h post-mortem on the *Longissimus dorsi* muscle after storage at 4^{o}C. The results on lightness (L*), redness index (a*) and yellowness (b*) across treatments were not different and are comparable to findings of Bezerra et al. (2016) on *Longissimus lumborum* from intact male crossbred lambs (aged 5 months) fed graded levels of peanut cake in complete diets. Chai et al. (2018) stated that meat from lamb is preferred by consumers when it is bright red. In fact, the results on lightness (L*), redness index (a*) and yellowness (b*) in the current study were within the accepted range of 30.03 to 49.47, 8.24 to 23.53 and 3.38 to 11.10, respectively, for lamb meat (Warris, 2003, as cited by Bezerra et al., 2016). The lack of effect of diet on the colour of meat has also been reported earlier by Facciolongo et al. (2018) when comparing linseed with soybean in complete diets.

The pH in a muscle of a live animal is about 7.1 (Maltin et al., 2003). An animal has a certain amount of energy stored in the muscle as glycogen. Immediately after slaughter, muscle glycogen is converted to lactic acid through anaerobic glycolysis thereby reducing pH to the range of 5.4 to 5.7 after 24h post-mortem (Maltin et al., 2003). The ultimate pH is used to determine the quality of meat under commercial production of meat. Depletion of muscular glycogen reserves before slaughter results in poor quality meat with a high ultimate pH (more than 5.7) and renders meat susceptible to immediate bacterial spoilage (Silva et al., 1999). In the current study, the ultimate pH across treatments was not significantly different as observed by Chiofalo et al. (2020) and was within the acceptable range of 4.8 to 5.0, assuming that ultimate pH greater than 5.7 is classified as undesirable (Maltin et al., 2003).

6.4.7. *Longissimus dorsi* (LD) muscle fatty acid profile and human health

Nutritional properties of meat are also determined by fatty acid composition among other nutritional attributes (Smeti et al., 2018). Saturated fatty acids from consumption of red meat by humans are linked to cancers and coronary heart disease. However, recently research has started to demystify negative image of meat especially that meat consumption provides humans with an essential nutrient (Pereira & Vicente, 2013) especially for growing youth and the elderly (Leroy et al., 2022). In the current study, palmitic acid was the predominant (27.7% to 30.7%) saturated fatty acid across the treatments (Table 6.8). Palmitic acid and myristic acid are considered as being hypercholesteraemic because they have a negative influence on the blood plasma low density lipoproteins (LDL) and high-density lipoproteins (HDL) content

(Oliveira et al., 2011). Low density lipoproteins are linked to cardiovascular risk in humans. The amount of palmitic acid in the current study was comparable to palmitic acid values reported by Bezerra et al. (2016) in the *Longissimus lumborum* (LL) muscle of lambs (aged 8 months) fed graded levels of peanut cake in complete diets. On the other hand, *LD* muscle of lambs fed MKD had a significantly ($P < 0.05$) higher oleic acid content than *LD* muscle of lambs offered CD while *LD* muscle of lamb fed SCD had no oleic acid detected. Oleic acid is known for reducing the risk of cholesterol accumulation (Mazizi et al., 2020) in humans by increasing HDL-cholesterol in blood. The high oleic acid content in *LD* muscle of lambs fed MKD is attributed to some oleic acid from the diet escaping rumen biohydrogenation and being absorbed in the small intestine for tissue assimilation. Additionally, *LD* muscle of lambs fed MKD had higher though insignificant stearic fatty acid content compared to that of other diets, therefore Δ-9 desaturation elongation process of stearic acid was greatly reduced due to possible higher oleic acid content in MKD (Baldi et al., 2019). Stearic fatty acid is regarded as a neutral fatty acid not contributing to cardiovascular diseases in humans and normally ranges from 10 to 20% of fats produced by ruminants (Bezerra et al., 2016). According to Bas et al. (2007), polyunsaturated fatty acid (PUFA) in sheep ranges from 4.6% to 12.5% and in the current study, PUFA content ranged from 2.9% to 5.3% with *LD* muscle of lambs fed CD having relatively higher levels of PUFA, though not significantly different from other treatments. This, therefore, suggests that most PUFA were either biohydrogenated in the rumen or were not available in large quantities from the diets of the current study. The polyunsaturated fatty acid and saturated fatty acid ratio (P/S ratio) is one of the indices used to evaluate the nutritional value of meat for human consumption. The minimum value of P/S ratio recommended for human diet is 0.45 (Herdmann et al., 2010). Subsequently, index with a higher value is more desirable. In the current study, all the treatments had P/S ratio value lower than the recommended 0.45. However, the P/S ratio in meat is mostly 0.1 and nutritional manipulation in most cases does not increase the P/S ratio above 0.06 to 0.15 due to rumen biohydrogenation (Bezerra et al., 2016). Moreover "stearic acid plus oleic acid" to "palmitic acid" ratio describes possible beneficial effects of different lipids found in red meat with acceptable range of 2.1 to 2.8 for sheep meat (Bansklieva et al., 2000 cited by Kotsampasi et al., 2017). In the current study, the ratio was satisfactory (2.4) for *LD* muscle from lambs fed MKD only.

6.4.8. Sensory analysis

The findings from the current study revealed that dietary treatments did not cause differences in the sensory parameters evaluated; being appearance, taste, flavour, tenderness, juiciness and overall impression. Consequently, the panellists' score for all evaluated sensory traits were higher in meat from sheep fed MKD, but not significantly different from other treatments. Interestingly, sensory tenderness and instrumental tenderness were scored better (numerically) in meat from sheep fed MKD which also had numerically lower pH. According to Sen et al. (2011), tenderness is the most important textural characteristics of meat and is the main determinant of consumer acceptance of meat. In most cases older animals tend to have tough meat and younger animals more tender meat. This is attributed to development and increase of connective tissues in muscles of animals as they age (Sen et al., 2011). In the current study, all animals were contemporaries (aged 12 months at slaughter), hence there was no differences observed in tenderness as a result of age or diet (Wood et al., 1999). The sensory attribute of juiciness in meat steaks is determined by water retention, lipid content, flavour and texture. Panellists rated the flavour of cooked meat steaks as 7.4 (like moderately) and similar across treatments. In accordance with Xu et al. (2020) the variety and content of amino acids are responsible for flavour determination in muscles especially umami amino acids like alanine, aspartic acid, glutamic acid and glycine. However, in the current research the content of umami amino acids was found to be similar (Chapter 3 of this thesis; Table 3.3) in the cakes used in the current experimental diets, hence, it may partly explain the non-significant difference of flavour in the meat steaks. In general, the sensory meat steak ratings of the evaluated parameters from the cooked meat steaks were non-significant and this could also be explained by non-significant physico-chemical parameters. Therefore, meat evaluated in the current study is placed at a rating score of "like moderately".

6.4.9. Gross-margin analysis

Livestock production entails feeding of animals with various feedstuffs to attain product output at a projected period of time. Feed costs account for 60% to 70% of variable costs on intensively raised livestock (Salami et al., 2019). In the current study, the feed costs on average for oilseed cakes (MKD and SCD) accounted for 58% of fattening sheep when total variable costs excluded the cost of buying weaners. Partial or complete replacement of maize grain or conventional protein sources in animal feeding may reduce pressure on conventional energy and protein feed ingredients (Okendo et al., 2006). The current study compared MKD with CD

and SCD. Therefore, MKD and SCD had similar gross margins while commercial diet (CD) was pricy due to inclusion of vitamin-mineral premix. Similarly, Chingala et al. (2019) reported lower gross margins from steers fed on soybean meal complete diets than in steers fattened by Baobab (*Adansonia digitata*) complete diet and white thorn tree (*Vachelia polyacantha*) leaf meal diet, respectively. The use of MKC in complete animal diets is a viable option to use in Botswana especially during the dry season when the cost of most conventional protein sources is expensive due to high demand.

6.5. Conclusion

In conclusion, incorporation of MKC at 12% inclusion level of total diet dry matter may be fed without depressing sheep growth performance in similar fashion as the commercial diet or sunflower seed cake. In addition, MKC has the potential to improve meat sensory attributes as evidenced by numerically higher-ranking scores on overall eating quality. Positive gross margins are dependent on live weights of sold animals and cost of added weight to marketed animal. MKD and SCD had similar gross margins. Therefore, use of alternative non-conventional protein sources like MKC can help reduce feed costs without any detrimental effect on the growth, carcass yield and meat quality especially for smallholder farmers.

6.6. Recommendation

- Evaluate inclusion level of MKC in complete diets of dairy cows, testing effects on milk production and milk quality.
- Evaluate 12% inclusion level of MKC in complete diets of feedlot cattle to determine feedlot performance and meat sensory attributes.
- Measure enteric methane output of ruminants (cattle, sheep and goats) feeding on diets having 12% inclusion level of MKC in complete diets.
- Evaluate rumen fermentation characteristics in animals fed similar diets used in the current study.

The thesis final chapter (chapter 7) is a summary of major findings from each study and also outlines major conclusions, recommendations and future research.

6.7. **References**

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CHAPTER 7

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

7.1. Summary

Feed costs contribute to most costs in a livestock enterprise. This calls for search and evaluation of alternative cheaper and novel feed ingredients or underutilised feed ingredients for the sustenance of livestock production. In study 1, MKC as one of the potential feed ingredients suited for ruminant feeding was chemically characterised. The major nutrients found in MKC were crude protein (45.3%) and ether extract (33.9%). Given the high crude protein (CP) and ether extract (EE) content in MKC, it can be classified as a protein and energy source thus with potential to be used in total mixed rations and/or a supplement. However, the amount of EE in MKC was about 8.4 times greater than the recommended conservative maximum fat level (4%) in diets for ruminants. When compared to sunflower seedcake (SSC), MKC had superior nutrient content with respect to CP, EE, NDF, ADF, ADL, IVDMD, GE and ash. On the other hand, SSC was found to be rich in NFE, cellulose and hemicellulose (Chapter 3: Table 3.1). The measured mineral content of MKC was found satisfactory for ruminants except for calcium, potassium and sodium. MKC amino acid profile was comparable to SSC amino acid profile. However, MKC was deficient in lysine that is very important for the growth of young non-ruminant animals. Both MKC and SSC were found to be rich in unsaturated fatty acids. MKC had a lot of oleic acid which is known to prevent heart-related diseases in humans. Therefore, animal products rich in oleic acid can be regarded as nutraceutical. The chemical composition of oilseed cakes such as MKC is mainly influenced by oil extraction methods, tree genotype and the environment.

Chemical composition of a feed ingredient determines how it is fermented in the rumen. At 24h of *in situ* rumen incubation 77.5% of MKC was digested while 54% of SSC was digested. The slow disappearance of SSC in the rumen was attributed to high NDF and ADL content. In ruminants, diets with high NDF content usually have long retention time in the rumen and this culminates on low daily dry matter intake by the animal. On the other hand, the high EE content (33.9%) in MKC seemed not to have impeded the rumen microbes that digested the dry matter. This was reflected by the higher dry matter effective degradability of 77.7% for MKC compared to SSC effective degradability of 56.6%. With regard to CP, the rumen disappearance of CP from MKC and SSC were similar at 24h time point. However, degradation

rate of MKC (13%/h) was faster than that of SSC (9.8%/h). Rumen microbes use readily fermentable crude protein to digest fibre. MKC was found to be degraded rapidly in the rumen as a result of its rich RDP when compared to SSC (Chapter 3; Table 3.7).

A growth study (Chapter 4) which was the first to report sole use of MKC as a protein source in ruminants was conducted to establish the inclusion level of MKC in complete diets for sheep. Graded inclusion level of MKC at 0, 4, 8 and 12% in complete diets for sheep resulted in linear increase of DMI. This finding corroborated data from chapter 3, that MKC ether extract content (6.3%) does not poison or impede rumen microbes that digest ingested dry matter. Fat or EE in the diet may prevent rumen bacteria to digest feed by forming a physical barrier on feed particles or by destroying bacterial cell membrane. It was observed that measured nutrient digestibility coefficients were similar across the treatments with treatment D (12% MKC inclusion) having numerically superior digestibility values. Nitrogen retention (NR) was similar across the treatments although MKC treatments tended to have improved NR by 88% compared to the control. This resulted in increased mean ADG of 43.2% by MKC treatments over the control (0% MKC). MKC protein contain RUP which might have contributed to difference in growth compared to the control as the latter (0% MKC) diet's source of protein was urea. Blood parameters like total protein or blood urea are important indicators of nutritional and physiological status of animals. In the current study (Chapter 5), graded inclusion of MKC in complete diets for sheep resulted in a similar content of blood metabolites except for blood urea which showed a linear increase. It affirms that MKC is also a rich source of RDP that promote active microbial fermentation (Chapter 3). On the other hand, the blood profile (red blood cells, white blood cells, neutrophils, lymphocytes, eosinophils, monocytes, basophils, haemoglobin and packed cell volume) of the lambs was similar across the treatments and were within the recommended range indicating that the diets were safe and supported proper animal growth of which was reflected by similar morphometric measures across the treatments.

The last growth experiment (Chapter 6) compared MKC and SSC as alternative protein sources in complete diets for sheep, using the best inclusion rate from chapter 4. Dry matter intake is an important parameter that determines weight gain in animals. Also, weight gain is an important profit indicator in a livestock enterprise. The lambs fed on MKC diet, sunflower seed cake diet (SD) or the control diet (CD; commercial diet) had similar dry matter intake.

The intake of digestible organic matter was also similar across the treatments. Organic matter contains all the nutrients that are useful for animal growth. Despite the high ether extract (9.4%) on MKC diet, DMI was not depressed and all the treatments in the current study had similar intake of digestible NDF. The NR was higher in CD, intermediate in MKC diet and lower in SCD. Commercial diet (control) had better NR probably because it had a significantly higher CP digestibility coefficient than the other treatments. Sunflower seed cake diet and MKD treatments had similar NR and is probably attributable the two oil seedcakes having comparable total amino acid profile in terms of essential amino acids (Chapter 3; Table 3.3). However, the final body weight was similar across the treatments and this was attributable to the diets that were supplying similar nutrient profile. Live weight at slaughter is an important variable as it determines both hot carcass weight and cold carcass weight. Meat quality is also determined by composition of fatty acids and cholesterol level. Saturated fatty acids concentration in red meat is associated with coronary heart disease in humans. *Longissimus dorsi* (LD) muscles of lambs from MKC treatment had abundant oleic acid which is known to prevent heart-related diseases. On the other hand, LD muscle of lambs from SD had no oleic acid. This is attributable to SSC being rich in linoleic fatty acid (Chapter 3: Table 3.4). Fatty acids from meat are also known to have a bearing on eating quality of meat. In the current study, consumers rated sensory attributes of meat from the current treatments in a similar way. However, meat sensory ratings of MKC treatment were numerically superior to other treatments. The gross margin analysis of the current study showed that the economic returns of fattening animals are mainly dependent on feed costs and finishing weight. Cost minimisation of feed in livestock production is the goal of most enterprises. Morula kernel cake diet and SD had higher gross margins than CD. Therefore, MKC low price makes it an alternative protein source that can be used in livestock diets especially during the dry season to take advantage of premier prices in the market.

7.2. Conclusions

MKC is a feed ingredient rich in energy, crude protein and phosphorus than SSC. MKC also provides RUP in the diet that contributes to supply of metabolisable protein. The major fatty acid in MKC is oleic acid. Inclusion of MKC in ruminant diets can range from 4-12% of total diet dry matter. Inclusion of MKC at 12% of the total diet dry matter does not negatively affect

proximate composition and sensory attributes of meat. Sheep fed diets containing SSC or MKC had highest gross margin than the commercial diet (control diet).

7.3. Recommendations

- MKC can be used to replace SSC in diets for ruminants.
- MKC at 12% inclusion in sheep diets (treatment D) can be used in feeding strategies aimed at maximising animal growth

7.4. Further research

- The effects of MKC inclusion level in diets for dairy cattle; milk production and composition
- Effect of MKC on reduction of enteric methane in ruminants.
- Effect of Morula oil on rumen fermentation parameters and rumen microbe's ecology.