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DIETARY EFFECTS OF *SCLEROCARYA BIRREA CAFFRA* NUT MEAL IN GROWING-FATTENING MALE DORPER SHEEP

Ingrid Marumo Mokgadi Malebana

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, School of Physiology, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2018.

DECLARATION

I, Ingrid Marumo Mokgadi Malebana declare that this thesis, hereby submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, is my own work and has not been submitted to any other institution for an award. Where other sources have been used; they have been appropriately cited and acknowledged.

_____

(Signature of candidate)

_____day of _____20 _____in _____

DEDICATION

In memory of my dearly loved father

Moetji Jan “Rocky” Malebana

1952 - 2007

PRESENTATIONS ARISING FROM THIS RESEARCH PROJECT

Poster presentations

i. **Malebana, I.M.M.,** Nkosi, B.D., Erlwanger, K.H. and Chivandi, E. (2015). *Sclerocarya birrea caffra* (Amarula) seed: a potential dietary protein source high in energy and oleic acid for ruminants. 43rd Conference of the Physiology Society of Southern Africa, 6-9th of September 2015, Parys, South Africa.

ii. **Malebana, I.M.M.,** Nkosi, B.D., Erlwanger, K.H. and Chivandi, E. (2015). A comparison of the proximate, mineral, amino acid and fatty acid of industrially processed *Sclerocarya birrea caffra* seed meals and soyabean meal. 48th Congress of the South African Society for Animal Science, 21-24th of September 2015, Empangeni, South Africa.

Oral presentations

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iii. **Malebana, I.M.M.,** Nkosi, B.D., Erlwanger, K.H. and Chivandi, E. (2016). Dietary effects of *Sclerocarya birrea caffra* (Marula) nut meal on growth performance, metabolic substrates, visceral organs and general health of growing male Dorper lambs. 3rd Congress of the Professional Development Programme, 24-26th of October 2016, ARC-VOPI, Roodeplaat, Pretoria, South Africa.

iv. **Malebana, I.M.M.,** Nkosi, B.D., Erlwanger, K.H. and Chivandi, E. (2017). Effect of dietary Marula nut meal on carcass characteristics and meat quality of male Dorper lambs. 50th Congress of the South African Society for Animal Science, 18-21st of September 2017, Boardwalk Hotel, Port Elizabeth, South Africa.

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ii. **Malebana, I.M.M.,** Nkosi, B.D., Erlwanger, K.H. and Chivandi, E. (2017). Dietary effects of the substitution of Soyabean (*Glycine max*) meal with Marula (*Sclerocarya birrea caffra*) nut meal on the growth performance of Dorper lambs. *Journal of Animal Physiology and Animal Nutrition.* (Manuscript under review).

ABSTRACT

In Sub-Saharan Africa, the cost of producing livestock products is increasing due to the use of imported soyabean meal (SBM) as a dietary protein source in feeds. Reliance on the costly SBM can be reduced by developing alternative dietary protein sources for feeds. Seeds from indigenous trees are potential sources of nutrients including protein. In a series of experiments, the potential of Marula nut meal (MNM) to substitute SBM in lamb fattening diets was evaluated. Chapter 3 characterised and compared the nutrient and anti-nutrient composition of two MNMs (MNM1: produced using hydraulic filter press, MNM2: produced using cold press) and SBM.

The MNMs'crude protein (CP) content, which ranged from 33% (MNM1) to 39% (MNM2), was lower than that of SBM (51%). The MNMs' essential amino acid content ranged from 40 to 70% compared to that of SBM. The gross energy (GE), ether extract (EE) and total monounsaturated fatty acid (TMUFA) content of the MNMs were significantly higher ($P<0.05$) compared to that of SBM. The MNMs had a higher ($P < 0.05$) phosphorus and magnesium content than SBM. Compared to SBM, MNM2 had a higher oxalate and phytate-phosphate content, but a lower saponin and tannin content ($P<0.05$). MNM2 was used in the *in vivo* study (chapter 4) as a dietary protein source, due to it having a higher CP content and *in vitro* digestibility compared to MNM1.

Chapter 4 interrogated the effects of MNM2: denoted as MNM, as a dietary protein source on growth performance, feed efficiency and health of Dorper lambs. Dietary MNM neither affected the growth, feed efficiency nor the liver and kidney function of the lambs. In chapter 5 the effect of dietary MNM2: denoted as MNM, due to its higher CP content than MNM1, was evaluated on meat quality. Substituting SBM with MNM at 0, 50 and 75% resulted in meat with high CP ($P<0.05$) but low EE content while substituting at 25 and 100% resulted in meat with high EE ($P<0.05$) but low CP content. Dietary MNM significantly increased the total saturated and TMUFA concentration in the meat. Complete substitution of SBM with MNM yielded fatty meat rich in oleic acid, manganese and selenium ($P <0.05$).

The MNM can replace SBM in lamb fattening diets without compromising growth, feed efficiency, liver and kidney function and meat quality. However, substitution of SBM with MNM at high inclusion levels results in increased lipid content in the meat.

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NOMENCLATURE

ADF: Acid detergent fibre

ADG: Average daily gain

ADL: Acid detergent lignin

ALP: Alkaline phosphatase

ALT: Alanine transaminase

ANFs: Anti-nutritional factors

ANOVA: Analysis of variance

AOAC: Association of Official Analytical Chemists

API: Animal Production Institute

ARC: Agricultural Research Council

BWG: Body weight gain

BUN: Blood urea nitrogen

Ca: Calcium

Cl: Chloride

CLA: Conjugated linoleic acid

Co: Cobalt

CP: Crude protein

Cu: Copper

DAFF: Department of Agriculture, Forestry and Fisheries

DM: Dry matter

DP: Dressing percentage

EAAs: Essential amino acids

EE: Ether extract

EFA: Essential fatty acid

EPA: Eicosapentaenoic acid

FA: Fatty acid

FAO: Food and Agricultural Organisation

FAOSTAT: Food and Agriculture Organization Corporate Statistical Database

FCR: Feed conversion ratio

Fe: Iron

FI: Feed intake

GDP: Gross domestic product

GE: Gross energy

GIT: Gastrointestinal tract

IBW: Induction body weight

IVOMD: *In vitro* organic matter digestibility

K: Potassium

LDL: Low density lipoprotein

MDA: Mine workers Development Agency

MFL: Myofibrillar fragmentation length

Mg: Magnesium

M.LL: *Musculus longissimus lumborum*

MNM: Marula nut meal

MNMs: Marula nut meals

Mn: Manganese

Mo: Molybdenum

MUFA: Monounsaturated fatty acid

Na: Sodium

NAMC: National Agricultural Marketing Council

n-3 fatty acid: Omega-3 fatty acid

n-6 fatty acid: Omega-6 fatty acid

N: Nitrogen

NDF: Neutral detergent fibre

NRC: National Research Council

OA: Oleic acid

OM: Organic matter

P: Phosphorus

pH_i: Initial pH

pH_u: Ultimate pH

Pi: Inorganic phosphate

PUFA: Polyunsaturated fatty acid

S: Sulphur

SA: South Africa

SFA: Saturated fatty acid

SBM: Soyabean meal

Se: Selenium

SSA: Sub-Saharan Africa

TBW: Terminal body weight

TMUFA: Total monounsaturated fatty acid

TPUFA: Total polyunsaturated fatty acid

TSFA: Total saturated fatty acid

USD: United States of America Dollar

USFA: Unsaturated fatty acid

VFA: Volatile fatty acid

WBSF: Warner Bratzler shear force

CHAPTER ONE - INTRODUCTION AND JUSTIFICATION

1 THESIS OVERVIEW

The major challenge to the intensification of sheep production in South Africa (SA) has been noted to be the high cost of imported soyabean meal (SBM). Due to inadequate local production, SA imports SBM to meet the livestock feed industry's requirements. The resultant imported SBM-induced increased feed costs have created a window of opportunity to explore the potential of locally available non-conventional dietary protein sources for livestock feeds. One such locally available potential non-conventional dietary protein source is Marula nut meal; a by-product of oil extraction from Marula nuts. This study, therefore, sought to interrogate the potential of the Marula nut meal to substitute SBM as a dietary protein source in lamb fattening diets. Chapter 1 of this study gives the background information regarding the global, Sub-Saharan Africa (SSA) and South African demand for livestock products. The demand for livestock products is observed to be increasing. In SSA, the demand is increasing at an increasing rate. With regards to SA, it was noted that with the provision of adequate feed resources, sheep production could be intensified to reduce the country's dependency on costly sheep product imports. The second chapter is a review of the pertinent literature relevant for the study.

While oil can be extracted from Marula nuts by physical and or chemical processes, physical processes (hydraulic filter press and cold press) are mainly used in South Africa.
Chapter 3 gives a description of the chemical characterisation of the Marula nut meals (MNMs) derived from the two physical processes. In addition, nutrient and anti-nutrient composition of the MNMs and SBM was undertaken with a view to compare and determine the MNMs chemical nutrient potential as dietary protein sources in lamb fattening diets in place of SBM. The chemical characterisation focuses on the proximate, mineral, amino acid, fibre and fatty acid content and the determination of the anti-nutrient factors content of the MNMs and their comparison to the SBM. In the fourth chapter, the potential of the MNM to substitute SBM as a dietary protein source is evaluated *in vivo* using intact male Dorper lambs. The effects of the substitution of SBM with the MNM on the feed (nutrient) intake, growth performance, viscera macro-morphometry, rumen fluid characteristics (pH and volatile fatty acids content), rumen papillae score, circulating and stored metabolic substrate content, surrogate markers of liver and kidney function and other markers of general health are evaluated. Dietary composition is known to influence meat quality, thus Chapter 5 gives an account of the effect of substituting SBM with

MNM on carcass characteristics (warm and cold carcass weight, dressing percentage, muscle pH and temperature), meat physical attributes (colour, drip loss, moisture characteristics and tenderness) and meat chemical composition (proximate content, mineral and fatty acid profiles) . Chapter 6 summarises the major study findings, identifies some limitations of the study and makes recommendations for future studies. A list of references is cited in the thesis.

1.1 INTRODUCTION

Globally, the livestock sector is the most important agricultural sub-sector in terms of value-added products and land uses (Ali, 2007). The sector plays a critical role in the provision of employment, income, risk insurance/security and food (Herrero et al., 2009). The livestock sector employs about 1.3 billion people and maintains, directly and indirectly, the livelihood of 830 million communal farmers in developing countries [Gerber et al., 2010; Food and Agriculture Organisation (FAO), 2011; Herrero et al., 2016]. Approximately 300 million of the rural communities in the Sub-Saharan Africa (SSA) depend on livestock to sustain livelihoods (Thornton, 2010). Livestock contribute to household livelihoods by providing draught power for cultivation and transport, manure, security against contingencies and wealth (Nampanya et al., 2014). They (livestock) are also used in many cultural ceremonies (Randolph et al., 2007; Peden et al., 2009). Livestock products (eggs, meat and milk) which account for one-fifth of the global trade of agricultural products provide humans with nutrients for balanced diets (Perry and Sones, 2007). Meat, which is an energy dense food, is an excellent source of protein, minerals and vitamins; and contains essential amino acids and essential fatty acids (Marangoni et al., 2015) that the human body requires for normal physiological perinatal development of the human brain (Marangoni et al., 2016). Meat and meat products contribute 17% to the global food balance in terms of calorific intake per person per day and 33% of protein consumption (Rosegrant et al., 2009), making them important sources of both dietary energy and protein. Livestock also contribute to products (hides, wool, fur, mohair, feather meal, blood meal, meat and bone meal, tallow and lard) that are of industrial importance (Moll, 2005). Due to their multiple important roles (contribution to employment and the economy, industrial raw products, human nutrition and use for cultural ceremonies), livestock production needs to be sustained.

1.2 Global, regional and local demand for livestock products

There is a worldwide increase in the demand for livestock products (Cawthorn et al., 2013; Regmi and Meade, 2013) and this demand is estimated to continue growing until 2030/2050 (Alexandratos and Bruinsma, 2012). In the developed world where the demand for livestock products is increasing at a decreasing rate (Thornton, 2010), the consumption of livestock

products is reported to contribute 1000kcal per person per day (Van Vuuren et al., 2009). In these countries, the sector accounts for 53% of the total agricultural gross domestic product (World Bank, 2009). Unlike in the developed world, in developing countries where the livestock sector contributes 33% of the total agricultural GDP, the demand for livestock products is increasing at an increasing rate (Assan, 2014; Msangi et al., 2014) and is projected to increase from 200 to 400kcal per person per day from the year 2000 to the year 2050 (Van Vuuren et al., 2009). Perry et al. (2013) reported that the increase in population, growth in disposable income and the expansion in urban settlements as the major drivers of the observed increase in demand.

In SSA, livestock production contributes 35% to the agricultural GDP (Ehui et al., 2002). In the region (SSA), the demand for livestock products is rapidly expanding (Alexandratos and Bruinsma, 2012) such that production is failing to meet the demand (Notenbaert et al., 2017). Thornton (2010) asserts that the demand for livestock products in SSA will double by 2050. The increase in livestock product demand in SSA is fuelled by the increase in human population, at about 1.2% per year (Thornton, 2010), urbanisation and an improvement in incomes (Assan, 2014). Compared to SSA, per capita consumption of meat in South Africa is four times higher [Organisation for Economic Co-operation and Development/Food and Agriculture Organisation (OECD/FAO, 2016)].

The consumption of meat in South Africa (SA) surpasses 45kg per capita per year and current livestock production levels are failing to meet consumer demand for poultry, pork and beef (Scholtz and Palmer, 2013; Webb, 2013; Jankielsohn, 2015). The country therefore relies on imports from Australia and New Zealand to make up for the shortfall (Taha and Hahn, 2012). Similarly, the demand for sheep products (lamb, mutton and wool) is high (Olivier and Roux, 2007; Cloete et al., 2014) such that local production is insufficient. In 2013, South Africa had approximately 24.3 million sheep [Department of Agriculture Forestry and Fisheries (DAFF, 2014)] split as 21.7 million and 2.6 million in the commercial and communal farming sectors, respectively (Scholtz and Palmer, 2013). According to the DAFF (2011), SA reached its mutton production peak in 2008 with 1.88 million tonnes of mutton produced largely from the commercial farming sector. However, production has since been on the decline (DAFF, 2011). Sheep production in SA contributes 2.5% to the total gross value of the agricultural contribution to GDP (DAFF, 2013). Despite the seemingly large sheep flock, the country is a net importer of

sheep products (lamb, mutton and wool) due to an increasing demand (DAFF, 2012). Although according to FAO (2012), in 2011 South Africa consumed 1.49 million tonnes of mutton of which 1.40 million tonnes (about 94%) was produced locally (FAO, 2012). However, Hough et al. (2013) contend that South Africa imports 28% of its lamb and mutton requirement and 751 tonnes of wool annually. These imports are primarily from New Zealand, Australia and Namibia (DAFF, 2013). By virtue of it being a net importer of poultry meat and red meat (lamb and mutton included), South Africa loses a significant amount of foreign currency as it funds the imports (Taha and Hahn, 2015). In order to meet the consumer demand for sheep products, there is need to intensify sheep production particularly from the smallholder sector where currently, production and off take are low due to inadequate feeding (Molotsi et al., 2017). The intensification of sheep production will enable SA to reduce the import bill and channel the saved resources to other pertinent societal needs resulting in an improvement of the livelihoods.

1.3 Problem statement

The intensification of sheep production and any other livestock species is dependent on the availability of feed ingredients necessary for the formulation of nutritionally adequate diets (Wanapat et al., 2013). The shortage of feed ingredients is a major limiting factor towards the intensification of sheep (and other livestock) production in SA (Mapiliyao, 2010). Feed costs account for 80% of total sheep production costs (Omar and Naser, 2011). Of the major ingredients for formulating sheep fattening diets, protein accounts for 15% of the total production costs (Singh, 2001). Conventional plant-derived dietary protein sources for feeds are primarily derived from oilseed crops such as cotton, soyabean, sunflower and canola (DAFF, 2013) and secondarily from leguminous trees browse (Mokoboki et al., 2005). Animal-derived sources of dietary protein in feeds include meat and bone meal, blood meal, fishmeal and poultry litter (Meeker and Hamilton, 2006). In SSA and SA, oilseed-derived dietary protein sources are in short supply (Adhikari et al., 2015; Dlamini et al., 2014) due to inadequate production of the oilseed crops. The shortage of oilseed-derived dietary protein sources (meals and cakes) results in increased feed costs. The increase in feed cost negatively impact on the envisaged intensification of livestock (sheep) production (Mlambo et al., 2011), thus resulting in failure to meet the increased demand for sheep products leading to dependency on costly imports (Erb et al., 2012).

Coupled with the shortage of plant-derived dietary protein sources, the South African legislation (ACT 36, 1947) restricts the use of animal-derived products as dietary protein sources in feeds (DAFF, 2012). The use of meat and bone meal is associated with bovine spongiform encephalopathy (Ducrot et al., 2013), while the use of fish meal in finisher diets results in odour tainting of the products (Meeker, 2009). The seasonality of legume-tree derived browse (Yusuf and Muritala, 2013) and the presence of anti-nutritional factors in browse limit its (browse) use as dietary protein sources in feeds (Akande et al., 2010; Njidda and Ikhimioya, 2012).

Soyabean meal (SBM) is the major dietary protein source utilised in the livestock feed industries of SSA (Wang et al., 2011) and SA (Dlamini et al., 2014). The widespread use of SBM as a dietary protein source in livestock feeds stems from its nutritionally important characteristics: palatability, high protein content, digestibility, and a well-balanced amino acid composition (Wang et al., 2011). However, the production of soyabean in SSA (SA included) is inadequate to generate SBM, in quantities large enough to meet the requirements of the livestock feed and the food (human) industries (Adhikari et al., 2015; Dlamini et al., 2014). South Africa produces less than a third of its SBM requirements annually and depends on imports for the shortfall [National Agricultural Marketing Council (NAMC), 2011]. For instance, in 2009/2010 season, the annual SBM requirement for the South African livestock feed industry was 1.63 million tonnes against a local annual production of 566 000 tonnes (NAMC, 2011). The country thus relies on imports to meet its SBM requirements.

1.4 Justification of the study

In order to reduce the country's dependence on imported SBM, there is a need to find alternative dietary protein sources for sheep and other livestock feeds. Current research on animal nutrition in SSA and SA largely focuses on the potential of browse plants (Franzel et al., 2014) and that of agriculture by-products from plantations (Khan et al., 2015). Research has interrogated the potential of browse plants such as *Acacia nilotica, Carpobrotus edulis, Combretum hereroense, Dichrostachys cinerea, Diospyros mespiliformis, Ficus sycomorus, Mangifera indica, Terminalia sericea, Psidium guajava and Persea americana* as dietary protein sources (Chepape et al., 2011). The chemical composition of agriculture by-products from plantations (citrus fruit peel,

fruit pulp and pomace) has been characterised to some extent, and these by-products are being utilised as nutrient supplements in livestock feeds (Bhila et al., 2010). However, their use has not managed to meet the increased demand of dietary protein for livestock feeds. There is a dearth of information on the potential of seeds/nuts from indigenous fruit bearing trees (IFBTs), which are adapted to the agro-ecological conditions of SSA and SA and are sources of biomass (Chivandi et al., 2011; Chivandi et al., 2012). The shortage of SBM for the livestock feed industry of SA calls for the need to explore alternative dietary protein sources in order to ameliorate the shortage and to be able to intensify sheep (and other livestock) production. One of such alternatives is *Sclerocarya birrea caffra* nuts.

Sclerocarya birrea caffra (Marula) is an IFBT, which is generally drought resistant (Hall et al. 2002; O'Connor, 2010) and adapted to the agro-ecological conditions of SSA and SA (Shackleton and Shackleton, 2002; DAFF, 2010). The tree grows best in frost-free areas under warm conditions and is well adapted to dry and hot weather conditions (Shackleton and Shackleton, 2002). It naturally grows in woodlands with sandy or sandy loam soil (Shackleton and Shackleton, 2002). In SA, it is mostly found in arid and semi-arid areas, and or low altitude savannah areas receiving between 250 and 800 mm of rainfall per annum (DAFF, 2011). Its distribution extends to parts of the northern Gauteng, KwaZulu-Natal, Limpopo and the Mpumalanga provinces (Helm, 2011). A mature Marula tree yields between 21 000 - 91 000 fruits per year (Mkwezalamba et al., 2015). The tree's fruit has been and is being commercially exploited in the production of beer, Amarula liqueur, juice, jam and jelly that are marketed locally, regionally and internationally (Shackleton et al., 2002). In SA, households where the Marula tree is found collect Marula fruit to make beverages and jam (Wynberg et al., 2003). However, about half of the Marula fruit and seed collected is sold to two major Marula fruit processing cooperatives: Mirma/Distell products and Mine Workers Development Agency Marula project (MDA) for Amarula liqueur production and Marula seed oil extraction, respectively (Wynberg et al., 2002; Shackleton et al., 2006). The Amarula liqueur is produced from the fruit pulp, while the seed nuts are pressed for extraction of Marula oil. The extraction of oil from Marula nuts generates Marula nut meal (MNM) as a by-product. Several cooperatives (Marula Natural Products/Limpopo Marula Products, Makhushwane Secondary cooperative, Mine workers Development Agency, Marula Nut Collectors Close Cooperation, Phosani co-operative and Home of Phadima Marula Oil) in Limpopo Province, SA extract oil from the

Marula nuts. The production figures from each cooperative are not readily available; however, approximately 80 tonnes of the nuts are processed by the Mine workers Development Agency cooperative annually (Mr Andrea Brink; Personal Communication). Thus, a significant quantity of the MNM biomass is produced in South Africa annually. The MNM is a potential source of dietary nutrients for feeds including protein. There is thus a need to characterise, both chemically and *in vivo*, the potential of MNM as a dietary protein source with the target being to reduce feed cost and be able to intensify sheep production.

1.5 Aim of the study

This study sought to determine the chemical nutrient potential of Marula nut meals (MNMs) and compare it to that of the SBM and to test, *in vivo*, the effects of graded dietary substitution of SBM with MNM as a dietary protein source on the growth performance, feed utilisation efficiency, viscera macro-morphometry, rumen health status and rumen fluid characteristics, and on the general health profile of intact male Dorper, as well as the carcass and meat quality derived from the MNM-fed lambs.

1.6 Objectives of the study

Specifically, the study sought to:

i. determine the proximate (dry matter, ash, crude protein and ether extract), gross energy, fibre [acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (aNDF)] content, amino acid composition, mineral and fatty acid profile; anti-nutritional factors [(ANFs): oxalate, phytate-phosphate, saponin and tannin] content and *in vitro* organic matter digestibility of the Marula nut meals (MNMs); and compare them to that of the SBM.
ii. formulate diets where MNM would substitute SBM on crude protein basis in lamb fattening diets and to determine the effects of the graded dietary substitution of SBM on:

a. growth performance [terminal body weight, body weight gain, average daily gain, feed intake, feed conversion ratio and linear growth (femur and tibia weight, length, Seedor ratio) and bone mineral composition].
b. rumen digesta pH and rumen liquor volatile fatty acids content
c. visceral organ macro-morphometry.
d. blood/serum metabolic substrate (glucose and triglyceride) concentration.
e. liver metabolic substrate (glycogen and lipid) storage.
f. serum surrogate markers of liver function [total protein, albumin, globulin and total bilirubin concentration and alanine transaminase (ALT) and alkaline phosphatase (ALP) activity] and kidney function [blood urea nitrogen (BUN) and creatinine concentration] as well as other markers (inorganic phosphate, calcium, cholesterol, and glucose concentration and amylase activity) of general health.
g. carcass characteristics (warm and cold carcass weight, dressing percentage and muscle pH and temperature).
h. meat physical attributes [drip loss, colour, moisture characteristics (thaw, total cooking loss, cooking drip loss and cooking evaporation loss) and tenderness (Warner Bratzler shear force and myofibrillar fragmentation length)]
i. meat (lamb) chemical composition [proximate content (dry matter, moisture, ash, organic matter, crude protein and ether extract), mineral composition and fatty acids profile.

1.7 Hypotheses of the study

The hypotheses of this study were:

a. H_0: There are no differences in the proximate (dry matter, ash, crude protein and ether extract), gross energy, fibre [acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (aNDF)] content, amino acid composition, mineral and fatty acid profile, anti-nutritional factors [(ANFs): oxalate, phytate-phosphate, saponin and tannin] content and *in vitro* organic matter digestibility between the MNMs and SBM.

H_1: There are differences in the proximate (dry matter, ash, crude protein and ether extract), gross energy, fibre [acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (aNDF)] content, amino acid composition, mineral and fatty acid profile, anti-nutritional factors (oxalate, phytate-phosphate, saponin and tannin) content and *in vitro* organic matter digestibility between the MNMs and SBM.

b. H_0: Graded dietary substitution of SBM with MNM, on crude protein basis, has no effect on the growth performance [terminal body weight, body weight gain, average daily gain, feed intake, feed conversion ratio and linear growth (femur and tibia weight, length, Seedor ratio and bone mineral composition)], rumen digesta pH and rumen liquor volatile fatty acids content, viscera macro-morphometry, blood and liver metabolic substrate content, plasma surrogate markers of liver and kidney function and general health; as well as carcass characteristics and quality of meat (lamb) of and from intact male Dorper lambs.

H_1: Graded dietary substitution of SBM with MNM, on crude protein basis, affect the growth performance [terminal body weight, body weight gain, average daily gain, feed intake, feed conversion ratio and linear growth (femur and tibia weight, length, Seedor ratio and bone mineral composition)], rumen digesta pH and rumen liquor volatile fatty acids content, visceral macro-morphometry, blood and liver metabolic substrate content, plasma surrogate markers of liver and kidney function and general health; and carcass characteristics and meat (lamb) quality of and from intact male Dorper lambs.

CHAPTER TWO – LITERATURE REVIEW

2 INTRODUCTION

Successful intensive livestock production occurs when nutritionally adequate diets are provided to meet the maintenance and productive requirements of the target animal (Villalba et al., 2010; NRC, 2007). Dietary protein and energy are the main ingredients (macronutrients) required in the formulation of livestock feeds (Abbasi et al., 2014). Their absolute quantities in diets make them (dietary protein and energy) critical ingredients to formulate and produce nutritionally adequate diets (Ingale and Shrivastava, 2011). The total cost of animal production is largely dependent on the cost of feed ingredients used to formulate feeds (Donohue and Cunningham, 2009). Dietary protein is the most expensive feed ingredient (Meissner et al., 2013; Wickramasuriya et al., 2015). While feed costs account for 60-70% of the total production cost (Njarui et al., 2016) in intensive livestock production systems, dietary protein accounts for 15% of the total production cost in intensive animal production (Singh, 1990). In order to minimise on high costs in animal production, it is imperative to evaluate the nutrient composition of feed ingredients so as to be able to formulate least cost rations/diets while meeting the nutrient requirements for the target animal (Weiss and St-Pierre, 2015). Additionally, the evaluation of feed ingredients and feeds allows for quality control in the livestock feed production chain (Glencross et al., 2007).

Feed ingredients that are utilised in livestock feed formulation are derived from animal and plant sources (Sapkota et al., 2007). Animal-derived feed ingredients used in livestock feed production include among others by-products from poultry production (litter and feather meal), meat and bone meal, blood meal, and fishmeal (Meeker et al., 2006). These animal-derived feed ingredients have a high crude protein content and are rich sources of essential amino acids (Bureau et al., 2000). Importantly, they are generally high quality feed ingredients characterised by high dry matter and energy digestibility (Bureau et al., 2000). By-products of oilseeds (soyabean, sunflower, cottonseed and canola) processing in the form of cakes and meals constitute plant-derived dietary protein sources for feeds (Francis et al., 2001). Leguminous browse leaf meals and root tuber meals are used as dietary protein and energy sources, respectively, in the feeds (Francis et al., 2001; Chandrasekara and Kumar, 2016). The leguminous browse leaf meals consist of high mineral content and low crude fibre (Amata, 2010) but are known to contain anti-nutritional factors (ANFs) such as tannins, lectins, gossypol, protease inhibitors, saponins, and phytic acid in varying concentrations (Francis et al., 2001).

The ANFs are plant-derived secondary metabolites whose main function is to protect the plant against herbivory (Mithöfer and Boland, 2012). Generally, these ANFs affect palatability (hence feed intake), digestion as well as nutrient absorption (Aruwayo and Maigandi, 2013; Katole et al., 2013). Specifically, lectins bind to the intestinal mucosa and cause intestinal epithelial lesions lowering the efficiency of nutrient absorption (Santiago et al., 1993). Tannins, which are polyphenolic compounds, form complexes with proteases in the gastrointestinal tract and dietary protein resulting in reduced protein digestion (Enujiugha, 2003). Saponins bind to and damage erythrocyte membranes resulting haemolysis (Cheeke, 1996). One of the many plant-derived polyphenolics, gossypol, is found largely in cottonseed by-products and has toxic effects on livestock. Gossypol toxicity results in respiratory distress, anorexia, and emaciation (Gadelha et al., 2014). It also weakens and interferes with the immune system, which can result in reduced efficiency of livestock vaccines (Gadelha et al., 2014). Phytic acid or phytate, chemically known as inositol hexaphosphate forms insoluble complexes with dietary protein and the products of protein digestion (peptides and amino acids) and minerals in the gastrointestinal tract (GIT) making them unavailable (Prieto et al., 2010). Digestive enzyme inhibitors, for example, protease inhibitors/phytohaemaglutinins (Mezhlum'yan et al., 1997) and amylase inhibitors (Giri and Kachole, 1998) inactivate digestive enzymes in the GIT. Inactivation of the GIT enzymes results in sub-normal protein and carbohydrate digestion and ultimately causes reduced animal productive performance as a consequence of reduced nutrient supply. Due to the detrimental effects of ANFs on livestock growth and reproductive performance, it is essential to establish their concentration in feed ingredients in order to process the feed ingredient(s) in a way that results in the inactivation and or the reduction of the effects of the ANFs. It is thus essential to have detailed information on the chemical nutrient and anti-nutrient composition of feed ingredients prior to using them in ration/diet formulation both for purposes of preventing potential feed-based toxicity (Adesogan, 2002) and to generate data on the nutritional potential of the feed ingredients (Căpriţă et al., 2013; Van der Klis et al., 2014). There are several methods that can be used to determine chemical nutrient and ANFs (toxin) content in plant-derived feed ingredients (FAO, 2010).

2.1 Approaches to feed evaluation

There are two major approaches to the evaluation of the potential nutritive value of feed ingredients and feeds: the *in vitro* and *in vivo* approach. Encompassed within these two major approaches are several individual evaluation methods such as chemical, enzymatic and biological methods, and digestibility and nitrogen balance as well as animal growth performance trials (Cheli et al., 2012; Kim et al., 2012; Kumar et al., 2015; Jha and Tiwari, 2016).

2.2 Feed ingredient and feed evaluation methods

2.2.1 Chemical methods of evaluation

The proximate analysis of feed ingredients and feeds is one of the most basic chemical analytical methods (Cheli et al., 2012) that were developed in the mid-nineteenth century at the Weende Experimental Station in Germany (Choct, 2015). It is a quantitative analysis that breaks down and quantifies feed ingredients into 6 components: moisture, ash, crude protein and ether extract, and crude fibre and nitrogen free extracts (Cherney, 2000). While this method is credited with ease of execution and saving on time and is generally deemed economical (Bureau and Hua, 2007), the method makes use of environmentally unfriendly chemicals. Additionally, a major important technical flaw with significant biological and physiological impact in animal production is that this analytical procedure deems crude fibre as a single entity. The latter (crude fibre) is made up of digestible and indigestible components, thus this does not give a true reflection of the nutrient potential of the feed ingredient and or feed (Kerr and Shurson, 2013). This is particularly true with regards to non-ruminants which have a limited capacity to digest fibre (Knudsen et al., 2012). Modifications of the proximate analysis by Van Soest (1967) helped to break down the crude fibre into neutral detergent fibre (NDF), which represent readily digested non-structural components, acid detergent fibre (ADF) and acid detergent lignin (ADL) that represent poorly digested cell-wall components (Căpriţă et al., 2010). By itself, the proximate analysis cannot provide information regarding potential intake and digestibility of the feed ingredient/feed (Cheli et al., 2012). Due to the shortcomings associated with the use of the

"proximate analysis" other physico-chemical and biological evaluation methods have been developed.

2.2.2 Physical methods of evaluation

Spectroscopic analytical methods, for example, nuclear magnetic resonance (NMR) and the near infrared reflectance (NIR) spectroscopy are analytical methods which make use of the absorbance of electromagnetic radiation to relate to chemical components within the feed ingredient(s) and or feeds (de Jesus et al., 2015). While NMR spectroscopy does not require a calibration standard of an identical material for the analysis (Saito et al., 2004), NIR spectroscopy has to be calibrated using large frequently updated data sets that are similar in nature and variability to the samples that are tested (Beever and Mould, 2000). Compared to the proximate analysis, the NMR spectroscopic method is environmentally friendly (Majid and Pihillagawa, 2014). The NIR does not depend on the use of chemicals for feed ingredients analysis (Manley, 2014). Importantly, NMR and NIR are rapid and predict a wide range of parameters which allows for a more detailed characterisation of the feed ingredient and or sample (Chatham and Blackband, 2001; Swart et al., 2012).

Analytical chromatography is a technique used in physical methods to evaluate the nutritional potential of feed ingredients and or feed (Cifuentes, 2012). As is the case with NIR spectroscopy, chromatography depends on calibration using standards of the same conditions with the tested samples (Smith et al., 1986). Chromatography distributes and separates feed components between the stationary and mobile phase of a chromatogram (Shabir et al., 2007). Although chromatography is used in feed evaluation, the method is beset by limitations such as cumbersome sample pre-treatments and improper experimental conditions that may lead to incomplete separation of feed components which then results in contamination (Petterson and Langseth, 2002). Additionally, the chromatography cannot be relied upon to predict feed value as the experimental conditions under which the assay is done are far removed from what happens *in vivo* (Jung, 1997).

2.2.3 Enzymatic methods of evaluation

There are several enzymatic methods that are used to predict the nutritive value of different feed ingredients and or feeds (roughages, by-products of agro-processing industry concentrate), digestibility of feeds and feed mixes (Aufrère and Guérin, 1996; Ribeiro and Moreira, 1998). Aufrère and Michalet-Doreau (1990) state that, these methods differ according to the nature of the enzyme preparation and reaction conditions (temperature, pH and duration) used in the analyses. In general, these methods give a nutritive value index that provides a biologically significant parameter in the estimation of feed quality (Wilfart et al., 2008). The *in vitro* enzymatic methods include the use of pepsin-cellulase (Aufrère et al., 2007), neutral detergent cellulase (Stakelum et al., 1988), proteases, lipases and amylases individually or as mixtures (Mohamed and Chaudhry, 2008). The use of *in vitro* enzymatic methods does not require fistulated animals (to be described later). Importantly, the methods do mimic the conditions of how feed nutrients are digested in and absorbed from the animal gastrointestinal tract, and are more ethically acceptable compared to the chemical methods (Cheli et al., 2012). Additionally, when compared to chemical methods of feed evaluation, the enzymatic methods are better predictors of the feed value and give estimates that are closer to values generated by the more expensive, time-consuming and cumbersome *in vivo* digestibility trials (Aufrère and Guérin, 1996).

2.2.4 Biological methods of evaluation

The biological methods are designed to simulate a part or a series of parts of the GIT with regards to the digestion and absorption of nutrients in the GIT (Ribeiro and Moreira, 1998). The *in vitro* two stage technique (Tilley and Terry, 1963), the *in situ* degradability technique (Mehrez and Ørskov, 1977; Ørskov and McDonald, 1979), the *in vitro* gas production technique (Menke and Steingass, 1988) and the *in vivo* digestibility trials (Khan et al., 2003) are key examples of biological methods of feed evaluation. Although these methods provide useful information about feed potential, a criticism is their failure to generate similar information and the use of different units of measurement making it difficult to correlate one method to another (Ribeiro and Moreira, 1998).

2.2.4.1 In vitro two stage technique

The *in vitro* two stage digestibility technique consists of a 48-hour fermentation of feed samples in buffered rumen liquor (Kitessa et al., 1999). The fermentation process is followed by pepsin digestion in an acidic solution, which mimics digestion in the glandular stomach (Kitessa et al., 1999). Goering and Van Soest (1970) modified this technique by subjecting the residues from the fermentation and pepsin digestion to digestion with neutral detergent fibre solution in order to allow for the estimation of the true dry matter digestibility (Aufrère and Gèurin et al., 1996). The technique allows for instituting controlled experimental conditions, saves on time and allows for repeatable and accurate prediction of the nutritive value of the feed ingredient and or feed.

2.2.4.2 In situ degradability technique

The *in situ* degradability technique (*in sacco* and or nylon bag technique) was developed by Quin et al. (1938). In this technique, ruminant feeds packaged in specialised nylon bags (Rodriguez, 1968) are placed in the rumen via a rumen fistula (Gosselink et al., 2004; Damiran et al., 2008) and then the feed is fermented over specified time periods to measure the disappearance of feed (dry matter) from the bag (Kitessa et al., 1999). This time-based measurement of the disappearance of feed from the bags provides information about the kinetics of feed degradation in the rumen (Kitessa et al., 1999), thus predicting feed value (Ørskov, 2000). Compared to *in vitro* methods, the *in situ* degradability technique has minimal dependence on electricity. This is an important factor to consider in countries with an unreliable supply of electricity. Importantly, it provides accurate predictions of forage intake and digestibility (Ørskov, 2000) and also provides information regarding nitrogen flow to the ruminant and to the rumen resident microbiota. In addition to the difficulty in trying to standardise the procedure (Vanzant et al., 1998), the large quantity of nylon bags made use of, makes the technique labour intensive (Olaisen et al., 2003) and expensive.

2.2.4.3 In vitro gas production technique

The *in vitro* gas production technique is used to evaluate the potential nutritive value of feed ingredients and or feeds by measuring the volume of gas produced during fermentation (Makkar

et al., 1995). Feed samples are incubated in buffered rumen fluid and gas production is measured at fixed time points. The technique is easy and highly reproducible (Herrero et al., 1996) and generates useful data on the digestion kinetics of soluble and insoluble components of feed (Schofield, 2000).

2.2.4.4 In vivo digestibility

An *in vivo* digestibility trial in which feed ingredients and or feeds are exposed to physiological processes responsible for digestion and absorption, gives some indication with regards to the relationship between feed ingredient/feed nutrient content and the availability of nutrients (from digestion) to the animal (Safwat et al., 2015). The *in vivo* digestibility trial, unlike other biological methods, allows for the interaction between the animal and the feed, but it is a time consuming and labour intensive trial (Khan et al., 2003). The key role of energy and protein as dietary components in livestock feeds makes it crucial to determine, by physico-chemical and biological techniques, their concentration in potential feed ingredients and feeds to ensure adequacy.

2.3 Dietary energy and protein sources for feeds in Sub-Saharan Africa

2.3.1 Dietary energy sources

Cereals (maize, sorghum, wheat, and barley and their milling by-products) as well as tubers (cassava mostly) are the major dietary sources of energy for the Sub-Saharan livestock feed industry (Jayasuriya, 2002; Chakeredza et al., 2008). Other agro-industrial by-products, for example, brewer's grain, and dried brewery yeast are also used as dietary energy sources (Kassahun et al., 2012). Additionally, by-products from plantation agriculture and fresh fruit processing are also used as dietary energy sources (Jayasuriya, 2002). Oilseed cakes are used as dietary protein sources (Teferedegne, 2000).

2.3.2 Dietary protein sources

2.3.2.1 Conventional dietary protein sources

Oilseed cakes and meals from canola, copra, cotton, groundnut, palm, sesame and sunflower and animal by-products (bone and blood meal, fishmeal, shrimp waste and hydrolysed feather meal) are utilised as dietary protein sources for livestock feeds in SSA (Shipton and Hecht, 2005). Of the plant-derived dietary protein sources for livestock feeds, SBM is the major conventional dietary protein source in SSA and SA (Chianu et al., 2009). Thus, for the rest of this study, I will focus my discussion on soyabean.

Soyabean production in SSA does not meet the SBM requirements for the human food and livestock feed industries of the region, which thus makes the region to rely on costly imports (Sihlobo and Kapuya, 2016). The dependence on imported SBM results in increased feed costs. An increase in feed costs negatively impacts on the intensification of animal production, hence making it a challenge to meet the increasing demand for animal products. This shortage of dietary protein sources for livestock feeds demands that research be directed at finding alternatives. Most of the research on potential dietary protein sources for animal feeds has focused on browse from leguminous trees and plantation agriculture by-products (Alemayehu, 2004; Valbuena et al., 2015). The potential of seeds/nuts from indigenous fruit bearing trees (IFBTs) as non-conventional sources of dietary protein for livestock feeds has not received much attention.

2.3.2.2 Non-conventional dietary protein sources

The IFBTs are adapted to the region's climate and soil conditions and thus require fewer inputs compared to farmed crops in order to produce fruit (Fukushima et al., 2010). Most of these IFBTs produce edible fruit whose pulp is generally consumed by humans and wildlife (Rathore, 2009) but their seeds/nuts, despite being potential sources of nutrients, are discarded and or left to decompose in the veld (grasslands). There is, therefore, a dire need to interrogate the nutritive potential of seeds and nuts from IFBTs with a view to exploiting them as sources of dietary protein in feeds. Recently, some work on the chemical characterisation of the nutritive potential of seeds from IFBTs has shown promising results (Chivandi et al., 2013; Chivandi et al., 2016;

Ezeagu, 2017 and Van Wyk, 2011). Seeds from the IFBTs such as *Schinziophyton rautanenii* and *Adansonia digitata*) are commercially exploited in South Africa, Namibia and Zimbabwe (Vermaak et al., 2011; Sundarambal et al., 2015; Chidumayo, 2016), while fruit pulp and nuts from *Sclerocarya birrea caffra* (Marula) are commercially exploited in South Africa (Street and Prinsloo, 2013).

The Marula tree is an IFBT with multiple purposes (Shackleton, 2002). For instance its stem bark, leaves and root bark are used in ethnomedicine to treat, in humans, dysentery, fevers, diarrhoea, stomach ailments, sore eyes, headaches, toothaches and body pains (Gouwakinnou et al., 2011; Ojewole et al., 2010) as well as tonsillitis, skin allergies, stomach ache and cough (Cheikhyoussef et al., 2013). The use of Marula tree in ethnomedicine is attributed to the presence of a host of phytochemicals (tannins, polyphenols, triterpenoids, phytosterols and coumarins) which impart to extracts from the tree's parts health beneficial properties (Ojewole et al., 2010) and biological activities including antibacterial, antioxidant (Masoko et al., 2008) and anti-inflammatory (Ndifossap et al., 2010). The root and stem bark and leaf preparations are also used as ethnoveterinary medicines (Cheikhyoussef et al., 2013). The tree offers shade and provides a habitat for birds and edible caterpillars (Shackleton et al., 2003).

The Marula tree's fruit and seeds are extensively used in rural communities for nutrition (Shackleton, 2002; Hiwilepo-van Hal et al., 2014). While the fruit can be eaten raw, it can be pressed for juice and processed into jam, jelly, chewing gum (Leakey, 1999) or into alcoholic beverages (Gouwakinnou et al., 2009). In addition to the Marula fruit and seeds being utilised for food, its leaves provide nutritious browse to game, domestic livestock (Gouwakinnou et al., 2011) and insects (Shackleton et al., 2003). Its (Marula tree) nuts have been identified as potential source of nutrients (carbohydrates, protein, fat, vitamins, and minerals) and antioxidant in livestock feeds (Gouwakinnou et al., 2011). The nuts can be pressed to produce oil which (oil) is used for culinary and cosmeceutical purposes (Vermaak et al., 2011) and in the manufacture of margarine, soap and candles (Belsito et al., 2011). The by-product of Marula oil production is Marula nut meal (MNM). The MNM, due to its biomass could, be used as a potential source of nutrients in livestock feed, which can be used to mitigate the cost of feed and be able to intensify sheep production. The intensification of sheep production subsequently leads to improved growth performance, which (growth) is a critical trait in intensified animal production (Thornton, 2010).

Hence measurements and factors that impact on animal growth taking into consideration the health cost of the animal, are worth interrogating.

2.4 Animal growth

A critical trait of living organisms, animals included, is growth, a description of the changes in shape, volume and size of an organism over time (Lupi et al., 2015). Animal growth is reflected by an increase in body size (body height, length and girth) and body weight over time (Ojedapo et al., 2012, Sampurna et al., 2016). Growth is brought about by cell hypertrophy and or hyperplasia until a mature size is reached (Sampurna et al., 2016). When using body weight as an indicator of growth, animal growth is described in two major ways: biological and commercial growth (Lupi et al, 2015). The former describes an increase in animal body weight until an animal reaches adulthood while the latter is a description of the weight gain by the animal from birth to slaughter (Lupi et al., 2015).

A plot of the animal's growth over time gives a growth curve, which mathematically, represents a function of the animal's age and live weight over its (animal's) lifespan (Loaiza-Echeverri et al., 2013). Modifications to growth rate as the animal develops occur (Ojedapo et al., 2012; Lupi et al., 2015). Early life growth rate is typified by accelerated weight gain per unit time in comparison to near adulthood when a deceleration in weight gain per unit time is experienced (Lupi et al., 2015). In the growth curve, from the point which identifies the highest growth rate, the inflection point, growth gradually decreases to a point where it coincides with the horizontal asymptote of the growth curve (Gómez et al., 2008); thus resulting in a sigmoid growth curve. Figure 2.1 below is an illustration of a sigmoidal growth curve of an animal.

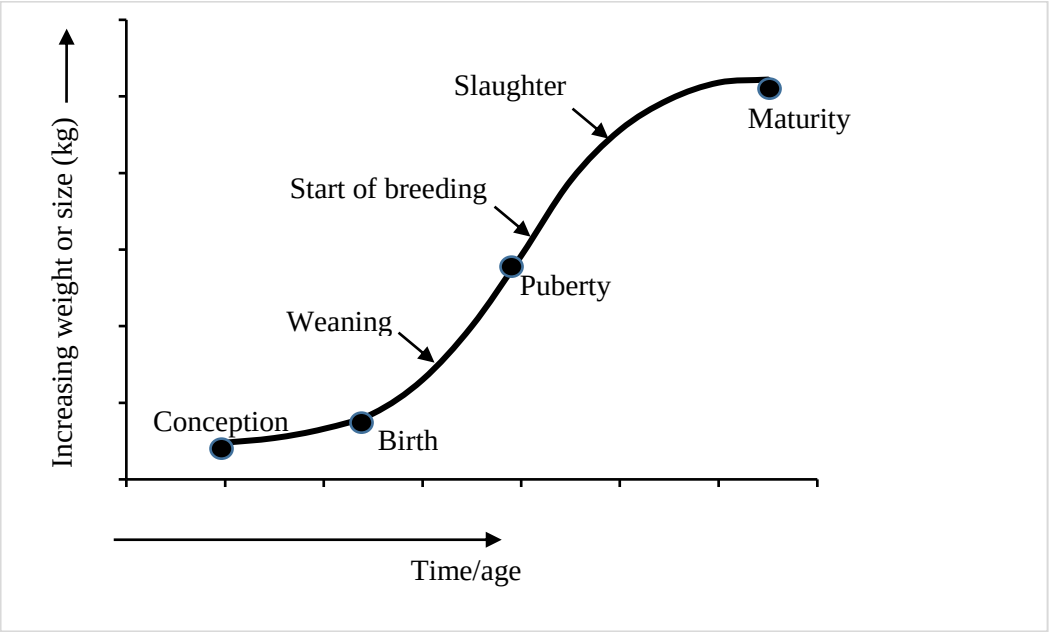

Figure 2.1: Animal growth curve (Adapted from Warriss, 2000)

The age at slaughter, which is dependent on several factors including carcass size (as demanded by the market) and the expected level of marbling in the carcass, make commercial growth a key growth phase from an economic point of view (Lupi et al., 2015). Genetic and environmental factors, which impact commercial growth and hence slaughter age, contribute to differences in the commercial growth period of different breeds and these (differences in commercial growth) have a bearing on the slaughter age, which (slaughter) is also influenced by cultural and technical aspects (Lupi et al., 2015). Consumers from different regions and customs prefer different carcass size with regard to the time (age) and manner of slaughter (Lupi et al., 2015).

The commercial animal growth curve is critical and can be applied for animal breeding and economic purposes. In view of the variations among breeds and populations regarding growth behavior in the context of the animal's biological and commercial lifespan, it is imperative to determine the best fitting curve for each breed taking cognisance of the animal's whole lifespan and the restricted period between birth and slaughter (Lupi et al., 2015).

Over and above being used to describe animal body weight development in different animal species, different mathematical growth models are also used to combine information from various measurements to a few critical parameters (Lambe et al., 2006). The essence of

modelling of growth curves lies in the explanations of the growth patterns of animals over time and ability to predict, from the generated equations, the envisaged animal weight at a particular age (Norris et al., 2007). Waheed et al. (2011) contend that several mathematical models, including Brody (Brody, 1945), Richards (Richards, 1959), Von Bertalanffy (Bertalanffy, 1957), Logistic (Nelder, 1961) and Gomperts (Laird, 1965), have been proposed to describe animal growth behaviour. These models have reported differences of fit accuracy among breeds (Tariq et al., 2013; Hamouda and Atti, 2011). The Brody, Bertalanffy, Verhulst, Logistic and Gompertz models have been shown to best demonstrate sheep growth (Topal et al., 2004; Hamouda and Atti, 2011; Tariq et al., 2013).

The three main types of tissues that develop with animal growth are muscle, bone and fat (de Oliveira et al., 2011). The rates of growth of the different tissues depend on the tissue being deposited and the animal age (de Oliveira et al., 2011). Muscle tissue forms and develops from the prenatal stage and through to maturity stage where its development slows down (Bruns et al., 2004; Oksbjerg et al., 2004). Similarly, bone develops from the prenatal to postnatal stages with bone elongation ceasing after the animal reaches physiological maturity although bone remodelling (deposition and resorption) continues throughout the animal's life (Zoetis et al., 2003). The deposition of fats occurs after muscle tissue and continues to develop (de Oliveira et al., 2011). The variation in the size of fat tissue is dependent on the nutritional status of the animal postnatally (Du et al., 2013). Fat deposition starts with subcutaneous fat, the largest amount of fat deposited under the skin, and is followed by the deposition of visceral fat in the abdominal cavity and around the viscera. Intermuscular fat is deposited between the animal muscle fibres while intramuscular fat is deposited last within the muscles (Hausman et al., 2014). Figures 2.2 and 2.3 below are a pictorial representation of the trends/patterns for weight and percentage of muscle, bone and fat in an animal, respectively, over time.

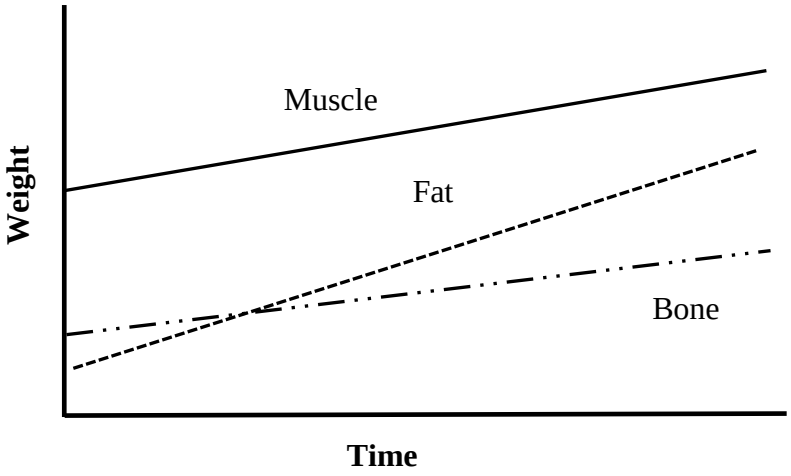

Figure 2. 2: Growth pattern of muscle, fat and bone over time on a weight basis (Adapted from Aberle et al., 2012)

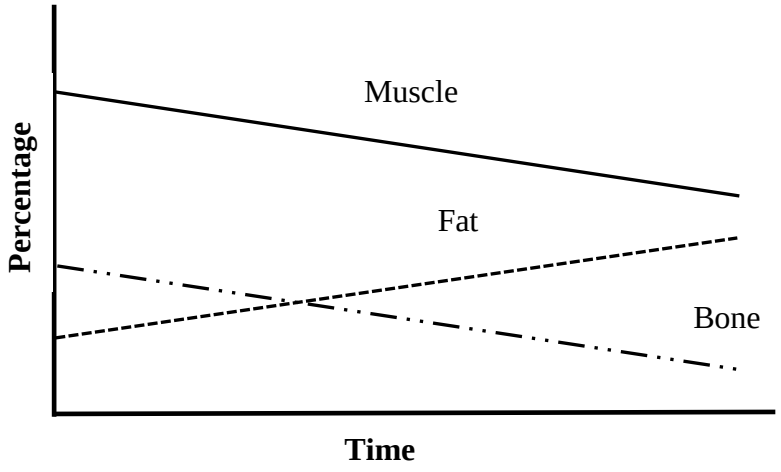

Figure 2.3: Growth pattern of muscle, fat and bone over time on a percentage basis (Adapted from Aberle et al., 2012)

2.5 Factors affecting animal growth and body composition

Animal growth and body composition are influenced by several factors: animal genetic makeup, hormone effect, the environment (as influencing the plane of nutrition - causing optimal, under- and or over-nutrition) and physiological status are critical factors that impact animal growth performance and body composition (Conlon and Raff, 1999).

2.5.1 Effect of animal genetic makeup

The genotype of an animal, which is heritable, imparts into each animal its inherent growth potential (Kononoff et al., 2005; Rehfeldt et al., 2011). However, the expression of the genotype's phenotype is subject to environmental modifiers such as the plane of nutrition, ambient temperature and disease (Bova et al., 2014). There is thus, an understanding and an acceptance that an optimal genetic makeup requires a conducive environment (optimal nutrition, environmental temperature, humidity, radiation, space, disease management) for optimal expression of the desired phenotype. Importantly, complex traits such as animal growth and body composition are dependent on age of the animal, management and an interaction of the genetic and environmental factors (Yi et al., 2006). Epigenetic changes, defined as variations in the genetic code that brings about an alteration in gene expression without changing the DNA sequence, also play a significant role in the development, growth, behaviour and well-being of livestock (Scholtz et al., 2014). In addition, animal growth and body composition are controlled by several genes including myostatin and callipyne (Joo et al., 2013). The former (myostatin) influences the growth and movement of muscle cells (Clop et al., 2006) and the latter enhances the percentage of lean meat (Fahrenkrug et al., 2000).

2.5.2 Effect of hormones

The major hormones that influence animal growth and body composition include growth hormone (GH), insulin, thyroid hormones [triiodothyronine (T3) and thyroxine (T4)], leptin and adiponectin (Jiang and Ge, 2014; Werner and LeRoith, 2014). Growth hormone, whose secretion by pituitary somatotropes following stimulation by the hypothalamic growth hormone releasing hormone (GHRH) from the hypothalamus, influences growth indirectly by stimulating the liver

to synthesise and secrete the somatomedins: insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-2 (IGF-2) (McMahon et al., 2001). These growth factors then stimulate muscle growth in animals (Dayton and White, 2013; Kasuya, 2016). Skeletal growth is mediated mostly by IGF-1 (Pääkkönen and Leppäluoto, 2002). Importantly, growth hormone impacts body composition and fat distribution through its influence on energy metabolism, lipolytic and nitrogen sparing effects (Wauters et al., 2000). Insulin sustains the normal blood glucose concentration by mediating uptake of glucose from the blood into cells (Qaid and Abdelrahman, 2016). It also regulates lipid, carbohydrate and protein metabolism (Wilcox, 2005). Through interaction with its specific cell surface receptor, insulin activates the Sos-Ras-Raf-Map kinase branch of cellular signalling that promotes cell division and animal tissue growth through its mitogenic effects (Draznin, 2011), thus contributing to animal growth.

Thyroid hormones, triiodothyronine (T3) and thyroxine (T4) are synthesised and secreted by the thyrotropes in the thyroid gland (Zimmermann, 2009) following stimulation of the thyroid gland by the thyroid stimulating hormone (TSH) released by the pituitary gland (Dietrich et al., 2012). These thyroid hormones stimulate metabolism in mammalian cells (Gereben et al., 2008; Dietrich et al., 2012), which (metabolism) triggers cascades of reactions some that lead to cell division (hyperplasia), cell growth (hypertrophy) and differentiation (Wiliams, 2008; Cheng et al., 2009); processes that contribute to animal growth.

Leptin is secreted by adipocytes (Amitani et al., 2013). It (leptin) controls body weight by acting in the hypothalamus to suppress appetite (Perry and Wang, 2012) and also by stimulating beta-oxidation of fatty acids in muscle tissues via the adenosine monophosphate (AMP)-dependent kinase pathway (Minokoshi et al., 2002). Several factors stimulate the secretion of leptin, among them, hormones such as insulin (Tsai et al., 2012), glucocorticoids (Leal-Cerro et al., 2001) and oestrogens (Rosenbaum et al., 2001). The expression of leptin receptors in the hypothalamic nuclei points to its (leptin) involvement in the stimulation of GH secretion, thus leptin is an essential chemical messenger to the GH neuroendocrine axis (Park and Ahima, 2015). By virtue of leptin participating in the stimulation of GH release and the latter's (GH) response to GHRH, it thus causes a pulsatile release of GH which is critical in regulating animal growth performance (Tannenbaum et al., 1998). Over and above its regulatory effect on GH secretion, leptin, whose

concentration increases in proportion to body fat mass, regulates feed intake as well as energy expenditure to maintain stored body fats (Máčajová et al., 2004).

Ghrelin is produced by the ghrelinergic cells in the gastrointestinal tract and is a ligand for the growth hormone secretagogue receptor (GHSR) (Wu and Kral, 2004; Sakata and Sakai, 2010). In ruminants, the ghrelinergic cells are localized primarily in the abomasal mucosa (Hayashida et al. 2002), suggesting that the abomasum may be a major pool for circulating ghrelin in ruminants (Sugino et al., 2010). The secretion of ghrelin increases during fasting, feeding of low-protein diet and decreases with a feeding of high-fat diet (Lee et al., 2002). Physiologically, ghrelin affects animal growth and body composition by regulating energy balance (Hayashida et al., 2002), stimulating feed intake (Kamegai et al., 2001), promoting accretion of adipose tissue (Tschöp et al., 2000) and stimulating gastric acid secretion (Dieguez and Casanueva, 2000; Masuda et al., 2000).

Adiponectin, a hormone that regulates energy homeostasis, glucose and lipid metabolism is secreted by adipocytes (Yamauchi et al., 2002) following their (adipocytes) stimulation by insulin (Motoshima et al., 2004). By activating adenosine monophosphate-activated protein kinase, the expression of adiponectin is distinctly increased during adipocyte differentiation in ruminants (Roh et al., 2016). Through regulation of energy homeostasis, glucose and lipid metabolism, adiponectin acts in the brain to decrease body weight (Liu et al., 2016).

2.5.2.1 Effect of synthetic growth promoters

Synthetic growth promoters (anabolic steroids, β-adrenergic agonists, GH, antimicrobials and ionophores) have been used in meat (particularly beef) production to improve production efficiency as characterised by increased growth performance (Du, 2014). Synthetic hormones, for example, zeranol (an oestrogen analogue), trenbolone acetate (an androgen) and melengestrol acetate (a progestin) have also been used to increase animal growth performance (Al-Dobaib and Mousa, 2009). However, due to their residual effect in animal products, their use is facing consumer resistance such that their use has been largely banned **(**Bridges and Bridges, 2001).

2.5.3 Effect of the environment on animal growth performance

2.5.3.1 Plane of nutrition

Nutrition is one of the major determinants of animal growth performance and body composition (Alejandro et al., 2014). Animal feed should be formulated so as to deliver adequate nutrients to meet the nutrient requirements for both maintenance and production for each class/age of the animal and for the target physiological status. Optimal nutrition results in optimal animal productivity and body composition (Alejandro et al., 2014). Under-nutrition leads to suboptimal growth performance (Richardson, 2009), malformed organs (Fuller, 2004), susceptibility to parasites and diseases (Paul and Dey, 2015) and poor reproductive performance (Boland et al., 2001). Over-nutrition may lead to problems such as dystocia (Everett-Hincks, 2007) and excessive deposition of fat in meat (Yang et al., 2010). In growing animals, when the problem of under-nutrition is addressed by a nutritionally adequate diet, such animals exhibit compensatory growth (Keogh et al., 2016); a phenomenon whereby animals previously deprived of adequate nutrition show recovery upon supply of a higher plane of nutrition that meets their nutrient requirements (Mitchell, 2007). During compensatory growth, animals show improved appetite and feed intake, increased efficiency of energy utilisation, enhanced body tissue composition and large body weight gain when compared to their counterparts that had continual access to nutritionally adequate diets (Ryan et al., 1993).

Various nutrition-dependent approaches have been used in order to achieve desired animal growth performance and body composition. In order to increase lean muscle growth, manipulation of dietary protein content and or the dietary protein: energy ratio has been employed (Baracos, 2005). Feeding ruminants diets with a protein content high for their physiological requirements has been observed to lead to a tax on growth performance due to the energy required to metabolise and excrete nitrogenous wastes from excess amino acids (Miyada and Silva, 1999). Importantly, there is contradictory data on the maximal beneficial amount of crude protein (CP) to include in ruminant diets. Feeding ruminants diets with a CP content greater than 14% reportedly has no extra benefit on growth performance (Van Emon et al., 2012; Ríos-Rincón et al., 2014), but is actually deemed to result in a loss of the expensive dietary protein. In contrast, some studies have reported improved growth performance and body

composition in fattening lambs fed diets consisting 16, 18 and 20% CP content, respectively (Rocha et al., 2004; Abbasi et al., 2014). In lambs, diets with a high plane of energy have been observed to promote and increase lean muscle growth (Barreto et al., 2012; Ríos-Rincón et al., 2014). Although increased levels of dietary protein and energy result in improved animal growth performance, the interaction of the high energy and high protein levels has been observed to promote fat accretion at the expense of lean growth in lambs (Ríos-Rincón et al., 2014).

2.5.3.2 Heat stress

The thermo-neutral zone is defined as ambient temperatures within which the normal body temperature is maintained with minimal energy expenditure (Belhadj et al., 2016). Ambient temperatures above the thermo-neutral zone and exerting a total heat load exceeding the animal's capacity for heat dissipation (Bernabucci et al., 2010) lead to heat stress, which in turn reduces feed intake and production efficiency (Sunil-Kumar et al., 2011). Reduced feed intake in heat-stressed animals is a survival strategy to reduce the heat load, since the nutrient digesting process (particularly in ruminants) generates heat (Chauhan et al., 2014). This reduction in feed intake triggered by heat stress leads to reduced animal growth performance. Heat stress also reduces the metabolic rate and changes post-absorptive metabolism independent of feed intake (Belhadj et al., 2016), thus negatively impacting on animal growth performance and reproduction (Marai et al., 2007). Furthermore, heat stress compromises the health status of the animals, which deteriorates due to stress-mediated impairment of metabolism (Belhadj et al., 2016). Temperatures above thermo-neutral zone lead to increased metabolic reactions taking place at a higher rate that result in a reduction in the body's capacity to fight disease (Padodara and Jacob, 2013), which (disease) then compromises growth. For instance, a 10ºC temperature increase in ruminants will increase the animals' metabolic rate; a process known as the Q10 effect, which functionally depends on the animal's total shifts in fundamental physico-chemical rates (Chaui-Berlinck et al., 2002).

2.5.3.3 Diseases

Livestock diseases are identified by behavioural changes in animals (Weary et al., 2009). These changes that are indicative of sickness (depression, inactivity, sleepiness, abnormal drinking and

feeding) are commonly referred to as sickness behaviour (Hart, 2010). Diseases are known to cause reduced feed intake (Konsman and Dantzer, 2001). The reduced feed intake leads to the induction of nutrient deficiencies resulting in compromised growth performance (Padodara and Jacob, 2013). Over and above causing a direct reduction in animal growth, nutrient deficiencies (from reduced feed intake) compromise the ability of the animal to mount efficient immune response against diseases, further negatively impacting growth performance (Padodara and Jacob, 2013). The observed reduction in animal growth performance due to diseases is mediated by the partitioning of nutrients away from the muscles (whose accretion is responsible for animal growth) to the liver and lymph nodes, tissues directly associated with the immune response (Le Floc'h et al., 2004).

2.5.3.4 Physiological status

Energy and protein are the major nutrients required for animal production (Wanapat et al., 2013) and growth performance (Abbasi et al., 2014). The physiological status of the animal affects its nutrient requirements (Picciano, 2003; Alejandro, 2016). Unlike mature animals, young growing animals require more dietary energy for maintenance and dietary protein for muscle accretion (Radley-Crabb et al., 2014). During early pregnancy (embryonic growth) nutrient requirement is lower compared to later stages of pregnancy where increased intake of dietary protein and energy is required for maintenance and foetal development (Waterland and Jirtle, 2004). Post-parturition, lactating animals require large dietary energy in order to support milk production (Dunlap et al., 2015). In contrast to high dietary energy requirement by animals during the lactation period, moderate energy intake is recommended for animals in dry period (Silva-del-Rio et al., 2010). In growing and productive animals, increased dietary intake of energy and protein is required to support production (Tedeschi et al., 2010). Nutrient deficiencies in such classes of animals lead to reduced productivity (Tedeschi et al., 2010). While there are several factors that affect the growth performance and body composition of animals, measurements of the former and the latter are essential for efficient animal production.

2.6 Measurement of animal growth performance

Several methods and indices are used in the estimation of animal growth performance. Total body weight (Ibom and Okon, 2012), lean body weight (Yardimci et al., 2008), body weight gain (Alves and Franzolin, 2015), percentage body weight gain (Yusuf et al., 2014), average daily gain (Kamruzzaman et al., 2013) and linear growth (Agamy et al., 2015; Lukuyu et al., 2016) are some of the indices used in the measurement of animal growth performance. Animal body weight can be full, shrunk or empty body weight (Waldo et al., 1990). Although live body weight is a commonly used index of animal growth performance due to its ease of determination (Elhashmi et al., 2011), it is not a very accurate measure of growth as it is influenced by gut fill and hydration status (Pearson and Ouassat, 2000). Body weight dependent indices such as average daily gain (ADG) and body weight gain (BWG) although used to determine growth performance, their computation hinges on body weight, thus carry the error of the influence of the hydration status and or the gut fill of the animal. Anti-gravity bones, tibiae and femora, respond to growth hormone in a dose-dependent manner (Cheek and Hill, 1974 as cited by De Lama et al., 2000). Thus, the determination of linear growth (before the growth plates of the bones fuse), based on the indices (length, weight and density) of such long bones gives a more accurate measure of animal growth performance (Applegate and Lilburn, 2002; Eshet et al., 2004). Over and above long bones being the accurate measure of growth performance, the bones support and give shape to the body, resist all of forms of mechanical stresses and provide a surface for muscles, ligaments and tendons attachments (Rauf, 2014). Additionally, bones protect the viscera, house the marrow and serve as a reservoir for calcium homeostasis (Boskey and Coleman, 2010). Hence, bones are critical for the normal growth and development of vertebrates (Almeida Paz and Bruno, 2006).

2.7 Development of the gastrointestinal tract and animal growth performance

Through its digestive and absorptive role, the gastrointestinal tract (GIT) plays an essential role in the supply of nutrients to ruminants (Yildirim et al., 2014). Thus, a functional GIT is important for the survival and optimal postnatal growth performance and development of animals (Applegate, 2010). The development of the GIT mainly occurs prenatally (Sangild et al., 2002).

In new-born ruminants, the rumen is basically non-functional due to its smooth, stratified, squamous epithelia without prominent papillae and the undeveloped microbiota (Wang et al., 2016). During the first 7 days of postnatal life, the development of the gut mucosa in suckling lambs follows a proximal-distal pattern in which the crypts and villi appear in the duodenum (Guilloteau et al., 2009). The forestomach, small and large intestines, and the crypt depth in the duodenum become greater at 42 days postnatal; the period during which the sizes of the abomasum, villus height in the jejunum and ileum are reduced (Guilloteau et al., 2009). At this stage, the development of the rumen structure is minimum and might affect the animal growth (Wang et al., 2016).

During weaning period, the transition of lambs from milk to solid feeds causes the GIT to undergo marked structural changes, which adapt the animal to efficiently digest and absorb nutrients (Guilloteau et al., 2009). The evolution of the GIT postnatally is dependent on nutritional factors and may be a limiting factor in animal growth (Guilloteau et al., 2009). Feeding grain-based and or forage-based diets to weaned ruminants increases the density and length of the rumen papillae compared to ruminants fed milk alone (Connor et al., 2013). The introduction of solid feeds to weaners further changes the architecture of the rumen, omasum (Boyd et al., 2013) and gut microbiota by stimulating the development of rumen papillae, epithelial and rumen motility (Khan et al., 2016). These developmental changes are critical for the creation of a conducive environment for the proliferation of rumen-resident microbiota, which has a symbiotic relationship with the host (Nathani et al., 2015; Khan et al., 2016). Ruminants as host animals, benefit from microbial crude protein (MCP), vitamin-like metabolites and volatile fatty acids generated from the fermentation of complex carbohydrates by exogenous enzymes from the microbiota (Nathani et al., 2015). Therefore, it is critical that in ruminants, the architecture of the rumen and omasum develop optimally as this is important in enhancing them to create a good environment for the optimal development of the requisite microbiome necessary for the digestion and supply of critical nutrients to the host animal that impacts on growth performance (Baldwin, 1999).

2.8 Effect of feed ingredients and diet on digestion and growth of ruminants

Ruminants possess a special fermentation vat, the rumen, which anatomically is placed before the glandular stomach, where fermentative digestion of feedstuffs takes place (Henderson et al., 2013). Rumen resident microbiota (fungi, protozoa and bacteria) contribute to the digestion of feedstuffs and generate MCP and volatile fatty acids (VFAs), both utilised by the host. As a result of their special digestive system, ruminants are able to digest almost all types of roughages largely through the fermentative activity of rumen resident microbiota (Singh et al., 2014; Li et al. 2016). The VFAs and MCP, which provide almost 80% of the host's energy requirements and 60-85% of the protein requirements, constitute the major products of rumen fermentation (Patil et al., 2012; Li et al., 2016). The total concentration and proportion of individual VFAs in the rumen are highly dependent on the type of diet fed to the animal (Attaelmannan et al., 1999). The feeding of forage-based diets to weaned ruminants has been observed to increase the rate of rumen passage resulting in reduced fermentation and availability of VFAs (Suarez-Mena et al., 2016); the major source of energy to ruminants. A reduction in VFA production causes reduced energy availability to the animal, which negatively impacts on animal growth performance. With regards to VFA (energy) production, feeding of high-forage diets is discouraged especially during the pre-weaning period due to inadequate utilisation of cellulose and a concomitant increase in rumen residence time due to the accumulation of undigested fibre. Feeding weaned or mature ruminants high concentrate diets, however, leads to increased ruminal VFA production. The increased ruminal VFA concentration reduces the ruminal pH (Penner et al., 2011) resulting in the production of endotoxins (Gozho et al., 2005) and anions (butyrate, lactate and succinate) (Bide, 1983) by the rumen microflora. Such toxins stimulate the rumen epithelium to initiate an adaptive response (Penner et al., 2011; Hernández et al., 2014): the epithelial layer, through tissue proliferation, becomes denser. The increase in rumen epithelial density increases the surface area for nutrient absorption (Mentschel et al., 2001). Thus, the feeding of high-concentrate diets triggers an increase in the absorptive function of the rumen important for increased VFA absorption thus, positively impacting on growth performance (Baldwin, 1999).

2.8.1 Effect of dietary fibre on rumen fermentation and growth performance

Dietary fibre is essential for maintaining rumen health and fermentation activity (Haddad and Grant, 2000). The provision of forages whose fibre is more susceptible to degradation by fibrolytic bacteria encourages increased colonization of the digesta with such bacteria (fibrolytic) leading to increased degradation resulting in increased forage utilization (Sirohi et al., 2013). Owens and Goetsch (1988) noted that feeding ruminants a high forage (high fibre) diet caused a proliferation of cellulolytic and saccharolytic bacteria in the rumen. The feeding of high fibre diets, compared to high concentrate diets, results in a relative decrease in VFA production which translates to a relative increase in rumen pH (Dohme et al., 2008). The relative increase in rumen pH results from increased saliva secretion (with bicarbonate ions) due to increased rumination associated with fibrous/high forage diet (Yang et al., 2001), and the relative decrease in overall VFA production (Dohme et al., 2008). Rumen fermentation of high fibre diets results in a relatively lower concentration of propionic acid but relatively higher acetic acid concentration compared to high concentrate diet (Beauchemin and McGinn, 2005) resulting in a high acetic: propionic acid ratio (Van Baale et al., 2004). The acetic acid is channelled to the liver where it is utilized as a substrate for the synthesis of glucose which is used to provide the energy necessary for support animal growth.

Forage quality is one of the major determinants of rumen ammonia nitrogen concentration and MCP (Uddin et al., 2015). High-quality forage diets characterised by a high content of readily degradable carbohydrates are readily fermented in the rumen resulting in increased production of ammonia nitrogen which in turn is used for MCP synthesis (Hoover and Stokes, 1991). High fibre diets containing undegradable protein lead to a decrease in ruminal ammonium nitrogen concentration and an increase in MCP flow from the rumen to the small intestines resulting in increased protein (amino acid) supply leading to increased growth performance (Erasmus et al., 1992).

2.8.2 Effect of high concentrate diet on rumen fermentation and growth performance

Feeding high concentrate diets to ruminants is credited with a number of positive effects inclusive of increased digestibility which leads to reduced rumen residence time resulting in

increased feed intake (Cantalapiedra-Hijar, 2014). The increase in digestive efficiency and the increased generation of fermentation products (VFAs and MCP) supplies requisite nutrients that promote production including growth performance. In terms of the rumen microbial biochemical environment, the feeding of high concentrate diets results in the proliferation of amylolytic bacteria at the expense of fibrolytic and cellulolytic bacteria (Tajima et al., 2001). Similarly, the proportion of protozoa in the rumen microflora is decreased by the feeding of high concentrate diets (Hristov et al., 2011). An increase in the amylolytic bacterial population leads to increased propionic acid production which is utilised in gluconeogenesis (Puddu et al., 2014). Zhang et al. (2015) contend that high concentrate diets lead to a channelling of ammonia nitrogen into MCP synthesis effectively causing a reduction in rumen ammonia nitrogen concentration. The increased MCP synthesis coupled with the increased VFA generation, especially propionic acid, provides the nutrient base necessary for supporting increased animal growth performance. Several studies (Serment et al., 2011; Kargar et al., 2012) have observed that the feeding of high concentrate: low forage ratio diets improved milk yield in dairy cows and growth performance in goats and lambs compared to the feeding of more fibrous diets. It is important to also note that feeding of high concentrate diets to animals has been associated with the development of acute acidosis as a result of excessive increase in VFA generation leading to an abnormal decrease in rumen pH (Chen et al., 2015). Thus, the benefits of feeding of high concentrate diets may be outweighed by the undesirable effects on rumen health if uncontrolled.

2.8.3 Effect of high lipid diet on rumen fermentation and growth performance

Via its negative effect on gram positive cellulolytic bacteria, high dietary lipid has been reported to cause reduced degradation of cellulose which translates to reduced energy production (Jenkins and Palmquist, 1984). High lipid diets have been shown to stimulate a proliferation of gram negative bacteria (Jouany, 1996). Variation in the gut microbiota composition in favour of the gram negative bacteria increases the production and permeability of lipopolysaccharides resulting in metabolic endotoxemia, which then manifests in obesity and obesity-induced inflammation (DiBaise et al., 2012). Szumacher-Strabel and Cieslak, (2012) reported that dietary lipids, in general, reduce ruminal protozoa population. A reduction in protozoa concentration is associated with low ammonia nitrogen concentration (Morais et al., 2006) as a result of a decrease in proteolysis of bacterial protein (Broderick et al., 1991). The reduced proteolytic activity

contributes towards an increase in the efficiency of microbial protein synthesis (Doreau and Ferlay, 1995), which is beneficial to the host.

2.8.4 Effect of type of fatty acid on rumen fermentation and growth performance

The fatty acid composition, length and the degree of saturation determine the physical and chemical properties of the dietary lipids (Baião and Lara, 2005). According to Ferlay et al. (1993), dietary lipids high in polyunsaturated fatty acids have negative effects on the metabolism of cellulolytic bacteria compared to those high in saturated fatty acids. The polyunsaturated fatty acids reduce the feed intake, cellulolytic bacteria and protozoa concentration as well as total ruminal volatile fatty acids (Yang et al., 2009). In addition to polyunsaturated fatty acids having negative effects on growth performance and rumen fermentation, dietary lipids high in unsaturated fatty acids with the presence of two (e.g. linoleic acid) and three (e.g. linolenic acid) double bonds reduce the ruminal microorganism' profiles (Yang et al., 2009). Linolenic acid is more toxic to ruminal microorganisms than linoleic acid, whereas docosahexaenoic (six double bonds) and eicosapentaenoic (five double bonds) acids are more toxic than linolenic acid (Yang et al., 2009). An increase in the degree of the fatty acid bonds decreases the concentration of protozoa (Oldick and Firkind, 2000). A decrease in the concentration of protozoa due to an increase of the degree of unsaturation, results in an increase in the concentration of proteolytic bacteria, which is likely to accelerate protein degradation (Yang et al., 2009). Acceleration of protein degradation increases the concentration of ruminal ammonia nitrogen, which is associated with a decrease in microbial protein synthesis by ruminal microbes (Yang et al., 2009). Reduced microbial protein synthesis translates to insufficient energy and protein supply for the host animal, thus compromising the growth performance of the animal.

2.9 Effect of non-conventional dietary protein sources on growth and health

The utilisation of non-conventional oilseed cakes/meals as dietary protein sources in feed can be limited by the presence of anti-nutritional factors (ANFs) such as saponins, gossypol, oxalic acid and phytate (Woyengo et al., 2016), tannins, cyanogen, nitrates, protease inhibitors, alkaloids, mycotoxin and aflatoxins (Tadele, 2015). Anti-nutritional factors adversely affect viscera (Das

and Singhal, 2005) and can cause toxicity, negatively impacting animal productivity (Kumar, 1992).

2.9.1 Effect of anti-nutritional factors

Tannins, which are polyphenolic compounds, form complexes with dietary protein and digestive enzymes, thus reducing the digestion and absorption of protein (Enujiugha, 2003) negatively impacting animal growth performance (Panhwar, 2005). Tannins also chelate key divalent metal ions (calcium, zinc, copper and magnesium), thus reduce their bioavailability (Gemede and Ratta, 2014). The deficiency of such key minerals compromises animal growth performance. Saponins which are characterised by bitterness and foaming when ingested by animals cause reduced feed intake and nutrient absorption (Ogunbode et al., 2014). Additionally saponins haemolyse red blood cells (Soetan and Oyewole, 2009) and inhibit the fermentative activity of rumen microflora, thus causing a reduction in VFA production and MCP synthesis (Lu and Jorgensen, 1987); negatively impacting animal growth performance.

Phytates have a high density of negatively charged phosphate groups which form protein- and mineral-phytate complexes (Lopez et al., 2002). The complexes produced result in reduced bioavailability of protein and minerals, compromising animal growth performance (Lopez et al., 2002). Importantly, phytates also form complexes with key digestive enzymes (tyrosinase, trypsin, pepsin, lipase and amylase) and result in reduced nutrient supply to the animal due to a reduction in digestive and absorptive efficiency (Lopez et al., 2002). Oxalates bind the calcium ions to form insoluble calcium-oxalate complexes that cause gastrointestinal tract irritation, renal tubule blockage, urinary calculi and hypocalcaemia (Akande et al., 2010). They also cause nephrotic lesions (Jones et al., 1997). Thus, the absorption and utilisation of calcium for animal skeletal muscle growth are adversely affected.

The use of non-conventional plant-derived feed ingredients in animal diets can result in toxicity (Ogbuewu et al., 2015). An assessment of the blood metabolites can be used to determine feed-mediated and other causes of toxicity in animals (Olafadehan, 2011). Haematological and biochemical indices of the blood are influenced by the quantity or the concentration of anti-nutrients factors in the diet ingested by the animal (Togun and Oseni, 2003; Akinmutimi, 2004).

The liver is the metabolic citadel of the body and is involved in key functions among them the synthesis of key proteins including albumin, fibrinogen and clotting factors (Kaneko et al., 2008) as well as hormones such as hepcidin (Bartnikas, 2014) and betatrophin (Maurer et al., 2017). The liver also metabolises carbohydrates, proteins and lipids in the process of maintaining homeostasis (Lee et al., 2015). Importantly the liver also metabolises hormones and drugs thereby regulating cell signalling (Rui, 2014; Jeschke, 2009) and eliminating drug residues, respectively. The synthetic and metabolic function of the liver is largely undertaken by hepatocytes which contain enzyme systems that catalyse the various metabolic reactions (Wiśniewski et al., 2016). Damage to liver cells result in decreased synthetic, metabolic and excretion/conjugation capacity. The dysfunctionality of the hepatocytes and cells of the biliary system manifest with the accumulation in blood, waste products such as bilirubin, creatinine and increased serum alanine transaminase, aspartate aminotransferase and alkaline phosphatase activity (Jeschke, 2009; Subramaniam et al., 2015).The kidneys, consist of complex nephrons that filter the blood to remove waste generated by metabolism and regulate body fluid and electrolytes balance thus maintain homeostasis (Kardasz, 2012). Residues of chemical messengers and drugs, urea and creatinine are some of the wastes filtered from the blood by the kidneys. The gold standard for kidney function is the glomerular filtration rate (Hsu and Bansal, 2011). However, surrogate markers such as plasma creatinine and blood urea nitrogen can be used in the determination of kidney function (Lopez-Giacoman and Madero, 2015).

While biochemical indices of liver and kidney functions are sensitive to elements of toxicity in feeds, they can also be used to assess the quality of feeds (Akinmutimi, 2004). From the literature cited above, it is evident that the use of non-conventional feed ingredients as dietary protein sources might elicit varied effects on the functionality of vital organs; some might trigger negative health and growth effects, while others might have no negative effect on the animal growth performance and health. Other than the effects that non-conventional dietary protein sources might have on the animal growth and health, the composition of the dietary sources might affect the product of the animal

2.10 Meat quality

Meat is an animal product and a major source of essential amino acid rich protein, metabolically important minerals (iron, selenium and potassium), fat-soluble vitamins (Gabryszuk et al., 2014) and essential fatty acids (Williams, 2007). While meat is a source of nutrients essential for human growth (Pighin et al., 2016), meat quality is considered as a complex and diverse component of the meat (Joo et al., 2013). Claasen (2008) contends that the quality of meat, including that of lamb, is a function of its physical (pH, temperature, colour, drip loss, cooking loss and tenderness), sensory (aroma, flavour and juiciness) and chemical nutrient composition. Hence, the quality of meat (carcass included) signifies complex characteristics that can be a challenge to evaluate (Rodrigues et al., 2006). When determining meat quality, several factors such as breed, age, body condition at slaughter, sex and plane of nutrition play a crucial role (Erasmus et al., 2016). *Ante-mortem* procedures such as handling, temperature (Adzitey et al., 2011), transportation, duration and conditions in lairage (Guerrero et al., 2013); and dehydration and feed restriction impact on the quality of meat (Ferguson and Warner, 2008). Storage conditions, electrical stimulation, ageing, processing, packaging, (Adzitey and Huda, 2012) and cooking conditions also affect meat quality (Listrat et al., 2016).

2.11 Effects of diet on meat quality

Of the multiplicity of factors that affect meat quality, diet (plane of nutrition) is one of the critical factors that has a strong bearing on the physical, sensory and chemical nutrient properties of meats including lamb (Guerrero et al., 2013). Dietary manipulation, especially targeting the type and proportion of certain feed ingredients, can and is used to influence meat (product) quality (Scollan et al., 2006; Joo et al., 2013). According to Kim et al. (2014), high concentrate diets improve the meat and carcass quality traits through modification of the fatty acid composition of the muscle lipid. Li et al. (2015) point that high fibre diets result in a decrease in muscle glycolytic potential, which improves meat quality by limiting the rate and degree of *post-mortem* pH decline (Li et al., 2015). Dietary lipids consist of different fatty acids (saturated and unsaturated) with different melting points that influence the firmness of the meat. The presence of unsaturated fatty acids and the location of their double bonds impact on the oxidation of the

fatty acids, which in turn affects the shelf life and colour of meat (Wood et al., 2004). Research shows diets containing high levels of anti-nutritional factors such as saponins, terpenes (Vasta and Luciano, 2011; Jerónimo et al., 2012) and tannins (Priolo and Vasta, 2007), can affect the quality of meat through their (ANFs) effects on metabolism of rumen fatty acids (Morales et al., 2015)

2.11.1 Effect of concentrate and forage diets on meat quality

Concentrate-diet fed animals yield higher carcass weights compared to forage-diet fed animals (Priolo et al., 2002). Their carcasses (concentrate-diet fed animals) have a consistent composition (Webb and Erasmus, 2013). Their meat has a high concentration of intramuscular fat and cholesterol (Descalzo et al., 2005). The meat from the high concentrate fed animals is tenderer and has favourable odour and flavour (Priolo et al., 2001) compared to those fed high forage diet. However, feeding concentrate diets results in meat with a high proportion of saturated, monounsaturated and omega-6 fatty acids (Ryan et al., 2007; Montossi et al., 2013) but low in linolenic acid (Priolo et al., 2001), conjugated linoleic acid (Aurousseau et al., 2007) and high polyunsaturated: saturated fatty acid ratio (Ponnampalam et al., 2014).
The meat from forage-diet fed animals has a lower lipid content (Sañudo et al., 2004), is darker and has off-flavour (Webb and Erasmus, 2013), is less tender (Nuernberg et al., 2005) and has a high proportion of polyunsaturated fatty (linolenic acid and conjugated linoleic acid) acids (Descalzo et al., 2005) compared to that from animals fed a high concentrate diet.

2.11.2 Effect of dietary lipid profile on meat quality

Dietary lipid profile affects the lipid fraction of the meat and hence the physical and chemical properties of the meat (Oliveira et al., 2011; Moloney et al., 2012). Unlike MUFAs, PUFAs increase the susceptibility of low-density lipoprotein (LDL)-cholesterol to oxidation modification (Scheffer et al., 2001; Kratz et al., 2002), which results in meat rancidity (Wood et al., 2008) and reduced shelf life (Ladeira et al., 2014). Meat lipid oxidation results in the production of potentially toxic compounds such as malondialdehyde, hydroxynonenal and hydroxyhexenal during processing (Tang et al., 2000).

2.11.3 Effect of secondary plant metabolites on meat quality

Oil seed cakes and or meals contain secondary plant metabolites (Ogori et al., 2017). Hydrolysable and condensed tannins, saponins, flavonoids and terpenes are some of the major secondary plant metabolites (Saxena et al., 2013; Geetha and Geetha, 2014). Although these secondary plant metabolites, when found in high concentrations in diets, can trigger anti-nutritional effects, research has demonstrated that they have the potential to improve meat quality (Vasta and Luciano, 2011; Jerónimo et al., 2012). In ruminants, tannins, for instance, inhibit ruminal biohydrogenation of linoleic acid (Morales and Ungerfeld, 2015), resulting in increased concentration of conjugated linoleic acid, vaccenic acid and omega-3 fatty acids in the meat (Vasta et al., 2012). The inhibition of the biohydrogenation of linoleic acid decreases the concentration of saturated and trans-fatty acids in the meat (Morales and Ungerfeld, 2015) and concomitantly leading to a reduction in the LDL-cholesterol concentration in the meat. The reduced concentration of LDL reduces the prevalence of cardiovascular diseases (CVDs) in meat consumers (De Souza et al., 2015).

Having considered how feed and its constituents can impact animal performance, health and product quality, it is essential that the nutrient and anti-nutrient composition of the MNMs as potential non-conventional protein sources be determined. Additionally, an estimation of their digestibility, which shows potential to release nutrients, is also important to be evaluated. Thus, the next chapter focuses on the nutrient and anti-nutrient factors characterisation and *in vitro* digestibility of the MNMs.

CHAPTER THREE – CHEMICAL CHARACTERISATION AND *IN VITRO* ORGANIC MATTER DIGESTIBILITY OF MARULA NUT AND SOYABEAN MEALS

3 INTRODUCTION

Prior to being tested *in vivo* any potential feed ingredient needs to be chemically characterised (Kumar et al., 2015). Chemical characterisation of potential feed ingredients generates information that assist to classify (establishing the chemical nutrient and anti-nutritional factors composition) feed ingredients (Glencross et al., 2007). The classification of the feed ingredients is important to grade the ingredients as energy and or protein sources (Glencross et al., 2007). Importantly, chemical characterisation gives pointers to whether prior processing of the ingredient is required in order to minimise the impact of the anti-nutritional factors (Abdulrazak and Oniwapele, 2014; Rahman et al., 2015).

Digestibility of feed ingredients is similarly important to establish information that can help in qualifying the feed ingredients as having adequate nutrients that can be utilised by the animal efficiently (Harmon, 2007). Importantly, i*n vitro* digestibility allows observations of the dynamics associated with the breaking down of a nutrient by using small quantity of raw materials (Dimes and Haard, 1994). Although the *in vitro* digestibility does not replace apparent digestibility trials, it can be used to evaluate the potential digestibility of feed ingredients (Lee and Lawrence, 1997).

Although some preliminary chemical characterisation of the Marula nut meal (MNM) has been done (Mlambo et al., 2011; Mdziniso et al., 2016; Mthiyane and Mhlanga, 2017), the evaluation did not cover important parameters such as amino acid composition, mineral and fatty acid profiles. These parameters have a significant impact on animal performance and productivity (Devendra and Leng, 2011). Additionally, where a near full chemical characterisation was done, it was with Marula provenances from Burkina Faso, Botswana, Nigeria, Kenya and Mozambique (Glew et al., 2004; Gandure and Ketlogetswe, 2011; Muhammad et al., 2011; Wairagu et al., 2013; Magaia et al., 2013). According to Hiwilepo-van Hal et al. (2014), the chemical composition of the Marula nuts varies according to a place of origin. Furthermore, edaphic conditions affect the nutritional composition of plants and their products (Mariod and Abdelwahab, 2012). While edaphic conditions affect the nutritional composition of plants and the products, processing of feed ingredients results in physical and chemical variations of the feed ingredients, which can increase or decrease the nutritional value of the feed ingredients (Kim et

al., 2016). Therefore, there is a need to characterise MNM generated from Marula tree provenances from South Africa before testing *in vivo*, the potential of the nut meals to supply nutrients. Thus, this study sought to fully chemically characterise the nutritive potential of Marula nut meals (MNM1: produced using hydraulic filter press and MNM2: produced using cold press) for purposes of generating the chemical nutrient data necessary to formulate test diets for evaluating *in vivo* trials where the MNM would be used as a dietary protein source. Additionally, the study sought to determine the potential of the MNM to release nutrients by evaluating the meals' in *vitro organic* matter digestibility.

3.1 Objectives of the study

The specific objectives of the study were to:

i. determine the proximate (dry matter, ash, crude protein and ether extract), gross energy, fibre [acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (aNDF)] content, mineral content, amino acid composition, fatty acid profile and anti-nutritional factors [(ANFs): oxalate, phytate-phosphate, saponin and tannin] content of Marula nut meals (MNMs) and compare them to that of the SBM.
ii. determine the *in vitro* organic matter digestibility of the MNMs and compare them to that of the SBM.

3.2 Hypotheses of the study

a. H_0: There are no differences in the proximate (dry matter, ash, crude protein and ether extract), gross energy, fibre [acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (aNDF)], mineral content, amino acid, fatty acid profile and anti-nutritional factors [(ANFs): oxalate, phytate-phosphate, saponin and tannin] content between the MNMs and SBM.

H_1: There are differences in the proximate (dry matter, ash, crude protein and ether extract), gross energy, fibre [acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (aNDF)] content, mineral content, amino acid composition, fatty acid profile and anti-

nutritional factors [(ANFs): oxalate, phytate-phosphate, saponin and total tannin] content between the MNMs and SBM.

b. H_0: There is no difference between the *in vitro* organic matter digestibility of the MNMs and that of SBM.

   H_1: There is a difference between the *in vitro* organic matter digestibility of the MNMs and that of SBM.

3.3 Materials and methods

3.3.1 Sources of the meals

The MNMs were sourced from two small-scale Marula nut processing industries: Mineworkers Development Agency Marula Project (MDA) in Bushbuckridge and Home of Phadima Marula Oil (HPMO) in Phalaborwa, Limpopo, South Africa. Solvent extracted soyabean meal (SBM) was procured from Greenbridge Group (Pty) Ltd, Pretoria, South Africa.

3.3.3.1. Production of the Marula nut meals

The MNM from MDA, a by-product of hydraulic filter pressing of Marula nuts, was designated Marula nut meal 1 (MNM1) while that from HPMO, a by-product of cold pressing of Marula nuts, was designated Marula nut meal 2 (MNM2). Prior to oil extraction, the Marula nuts were air-dried to 3% moisture. For the MNM1, the dried nuts were preheated in an industrial oven at 60ºC for 30 minutes after which they were pressed at a pressure of 100MPa in a hydraulic press with a contact time of 60 minutes per 1000kg of nuts. To produce MNM2, the dried nuts were dry pressed at 6.1-9.5 kgh^{-1} and 35-40 min^{-1} rotations of the screw at 55-60ºC.

3.3.2 Chemical analyses

Representative samples of the MNMs (MNM1 and MNM2) and SBM were collected on three different days from three different batches and three different sides of the batches. The samples were pooled and evaluated for proximate, gross energy (GE), fibre, mineral and anti-nutritional factors content as well as for amino acid and fatty acid profile. The GE content, amino acid and fatty acid profile of the meals were determined at the Agricultural Research Council's Irene Diagnostic Analytical Laboratory, Pretoria, South Africa. The fibre and mineral content of the meals were assayed at University of Pretoria's Nutrilab, Pretoria, South Africa. The anti-nutritional factors content of the MNM2 and SBM were determined at the Agricultural Research Council's Roodeplaat Vegetation and Ornamental Plant Institute, Pretoria, South Africa. Each of the assays on the pooled samples was done in triplicate.

3.3.2.1 Proximate analysis

The dry matter (DM), ash, crude protein (CP) and ether extract (EE) content of the meals were determined as described by Association of Analytical Chemists [(AOAC, 2006: method numbers 930.15, 942.05, 990.03, and 920.39, respectively)].

3.3.2.2 Determination of gross energy

Each meal's GE was determined using an MC-1000 Modular Calorimeter (Energy Instrumentation, Centurion, South Africa) equipped with a PC and MC1000 software.

3.3.2.3 Determination of the fibre fraction

The neutral detergent fibre (aNDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) content of each meal were determined as outlined by Van Soest et al. (1991). In brief, the aNDF was determined with a heat stable alpha-amylase using a Tecator Fiber System (Fibertec System, Tecator, Hoganas, Sweden). Approximately, 1g of each sample was refluxed for 60 minutes at 100ºC in 100ml of neutral detergent solution (NDS) followed by filtration and rinsing. The residue was then dried in an oven at 100ºC overnight, cooled and then weighed. The acid

detergent fibre (ADF) content of each meal was determined using similar procedure to that of the determination of aNDF except that the acid detergent solution (ADS) was used in place of NDS for the extraction. After rinsing, the residue was oven dried at 100ºC for overnight, cooled and weighed. Similar procedure to that of the determination of aNDF was also used to determine ADL except that after rinsing each sample residue, 72% of concentrated sulphuric acid was added to the residue and boiled at 700ºC for 3 hours. After boiling, the sulphuric acid was washed off with hot water and the residue was dried in an oven at 100ºC for 12 hours, cooled and then weighed.

3.3.2.4 Determination of mineral content

Prior to determination of calcium, cobalt, copper, chloride, iron, magnesium, manganese, phosphorus, potassium, sodium, sulphur and zinc content in each meal, each meal sample was prepared for the assay as described by AOAC (2000; method number 953.13). Briefly, 1g of each sample was digested in a 25ml of 65% concentrated nitric acid and 5ml of perchloric acid at 200ºC. The digest solution was allowed to cool before adding 50ml of distilled water. Absorbance was then measured as described by Giron (1973) using atomic absorption spectrophotometer (Perkin Elmer 2380 model, USA). The molybdenum concentration of each meal was determined by digesting 0.5g of the meal sample in 20ml of 20% hydrochloric acid at 200ºC. The digested sample was made to 100ml with distilled water and filtered using Whatman's number 4 filter paper (Whatman's No. 4, Fisher Scientific, Georgia, USA) before rinsing the filtrate with 8N ammonium hydroxide solution. The pH of the filtrate was adjusted to 1.6 using hydrochloric acid. Chloroform was then added to the filtrate followed by reading of the absorbance of the molybdenum concentration at 540nm on an atomic absorption spectrophotometer (Perkin Elmer 2380 model, USA) as described by Giron (1973).

3.3.2.5 Determination of the amino acid composition

The composition of amino acids in each meal was determined as described by Einarsson et al. (1983). Briefly, each meal sample was subjected to acid hydrolysis with 6M hydrochloric acid at 110ºC for 24 hours. The acid hydrolysis was followed by pre-column fluorescence derivatisation of amino acids using 9-flourenylmethyl chloroformate after which the amino acids were extracted

with pentane and then separated by gradient elution on a chromatograph. The chromatograph consisted of a SpectraSystem P4000 Quaternary high performance liquid chromatography (Rigas Labs S.A., Thessaloniki, Greece) equipped with a SpectraSystem FL3000 fluorescence detector and Rheodyne 7125 valve with 20µl injection loop. For separation of the amino acids, an OmniSper 5 C18 150 × 4.6 analytical column and guard-column were used. The amino acids were then identified at an excitation wavelength of 264 nm and an emission wavelength of 340 nm. A PC equipped with TSP software was used for quantification, which was performed by using an external calibration procedure.

3.3.2.6 Determination of the fatty acid profile

Prior to determination of fatty acid profile of each meal, fat from each sample was extracted by the Soxhlet method as described by AOAC (2006; method number 920.39). The extracted oil was then used to determine the fatty acid profile as described by Christopherson and Glass (1969). In summary, the oil extracts were methylated with 2M methanol sodium hydroxide. The resulting fatty acid methyl esters were extracted in heptane, filtered and dried under nitrogen after which they were separated by a temperature gradient over 45 minutes on a gas chromatography with nitrogen as carrier gas on a DB-23 capillary column (90cm × 250µm × 0.25µm) (Supelco, Sigma-Aldrich). The chromatograph consisted of an HP6890 gas chromatography (Hewlett Packard, Bristol, United Kingdom) with flame ionisation detector (FID). Both the detector and injector temperatures were set at 300ºC. A PC equipped with Chemstation software was used for quantification. Nonadecanoic acid was used as an internal standard.

3.3.3 Determination of the anti-nutritional factors

3.3.3.1 Determination of tannin content

The tannin content of each meal was determined from the total phenolic content using the Folin Ciocalteu phenol (Follin C) assay as described by Makkar (1993). Briefly, 2g of each meal sample was put in a test tube followed by addition of 10ml of 50% methanol. The mixture was then sonicated (in a cold water bath) for 20 minutes. Following sonication, 50µL of extract was harvested and transferred into 950µL of distilled water and mixed with 0.5ml of 1N Folin

Ciocalteu phenol reagent. The mixture was shaken well and kept at room temperature for 30 minutes after which absorbance for each sample was measured at wavelength of 725nm with UV/Visible spectrophotometer (Perkin Elmer, California, USA). Gallic acid was used as reference solution. Tannin content was expressed as gallic acid equivalents (GAE) in mg/g of the extract.

3.3.3.2 Determination of the saponin content

The saponin content of each meal was determined using a spectrophotometric method as described by Hiai et al. (1976) with modifications. In summary, 10g of each meal sample was defatted with 50ml hexane in a Soxhlet apparatus for 3 hours. After air-drying, the remaining meal sample was extracted twice with 100ml of 50% aqueous methanol by incubating at room temperature for 12 hours with continuous stirring. The extract was then centrifuged at 3000 × g for 10 minutes and the supernatant was collected. The first and second supernatants (from the two respective extractions) were then combined and filtered under vacuum through Whatman No.1 filter paper. Methanol from the filtrate was evaporated under vacuum at 40ºC following which the aqueous extract was centrifuged at 3000 x g for 10 minutes. The aqueous extract was then transferred to a separating funnel and extracted three times with 20ml of chloroform. The concentrated saponin in the aqueous solution was then extracted twice with 60ml of n-butanol. The n-butanol was evaporated under vacuum at 45ºC followed by dissolving the dried fraction in 10ml of distilled water and freeze-drying. The freeze-dried extract was then dissolved in 100ml of 50% aqueous methanol to a concentration of 10mg/ml. From the solution, 250µl aliquots of each sample were added into test tubes into which 0.25ml of vanillin reagent (8g/100ml ethanol) was added followed by 2.5ml of 72% (v/v) sulphuric acid and vortexed. Each mixture was incubated in a water bath at 60ºC for 10 minutes. After incubation, the test tubes were cooled on ice-cold water bath for 3-4 minutes and absorbance was immediately measured at 544nm using a UV-visible spectrophotometer (Perkin Elmer, California, USA) against a blank. The blank contained 50% aqueous methanol (without an extract). The saponin concentration, calculated from the standard curve, was expressed as diosgenin equivalents.

3.3.3.3 Determination of the phytate-phosphate content

The phytate-phosphate content of each meal was determined colorimetrically as described by Wheeler and Ferrel (1971) using a Perkin Elmer Lambda25 UV/Vis Spectrometer (Perkin Elmer, California, USA) equipped with a desk top computer and Lambda25 software. Briefly, 10g of each meal sample was weighed into 125ml of an Erlenmeyer flask and extracted with 50ml of 3% trichloroacetic acid at a constant shake for 30 minutes. The extract was centrifuged at 1600 × g for 30 minutes before the supernatant was transferred into 40ml of conical centrifuge tube to which 4ml of ferric chloride solution was added. The mixture was boiled in a water bath for 45 minutes at 100ºC. The aliquot was then centrifuged for 15 minutes at 1600 × g and the supernatant was decanted. The precipitate was immediately rinsed with distilled water before adding 3ml of 1.5N sodium hydroxide and 30ml of distilled water. The mixture was boiled in a water bath at 100ºC for 30 minutes before being filtered through a Whatman's No. 2 filter paper (Whatman's No. 2, Fisher Scientific, Georgia, USA). The filtrate was then dissolved in 40ml of 3.2N nitric acid. Five millilitres (5ml) of the solution was transferred into a 100ml volumetric flask to which 65ml of water and 20ml of 1.5M potassium thiocyanate were added. The absorbance of the mixture was immediately read at 480nm against a blank. Iron nitrate was used as a standard. The phytate phosphate concentration was expressed as mg/g dry weight.

3.3.3.4 Determination of the oxalate content

The oxalate content of each meal was determined as described by Munro and Bassiro (1969). Briefly, 2g of each meal sample was placed into 250ml volumetric flask to which 190ml of distilled water was added. A 10ml 6M hydrochloric acid solution was then added to each sample in the flask and the mixture was digested for 1 hour in a water bath at 100ºC. After cooling, each sample was made up to 250ml with distilled water and immediately filtered through a Whatman's No. 30 filter paper (Whatman's No. 30, Fisher Scientific, Georgia, USA). The pH of the filtrate was adjusted with 30% of ammonium hydroxide solution until the colour of the mixture changed from pink to yellow. Each mixture was then heated on a hot plate at 90ºC for 10 minutes, cooled and filtered to remove the precipitate containing ferrous iron. The filtrate was then treated with 10ml of 5% calcium chloride solution to precipitate the insoluble oxalate. The suspension was centrifuged at 1048 × g for 5 minutes, after which the supernatant was decanted and the precipitate dissolved in 10ml of 20% sulphuric acid. The suspension was then filtered and made up to 300ml using distilled water from which 125ml was removed and heated until near boiling.

The aliquot was immediately titrated against 0.05M standardised potassium permanganate titrant to a pink colour which persisted for 30 seconds. The oxalate content, calculated by taking 1ml of 0.05M potassium permanganate as equivalent to 2.2 mg oxalate, was expressed as mg/g dry weight. (Chinma and Igyor, 2007).

3.3.4 Determination of the meals' organic matter digestibility

The *in vitro* organic matter digestibility (IVOMD) of each meal was determined according to the two-stage technique of Tilly and Terry (1963). In summary, 0.2g of the dried meal sample was incubated in a mixture of rumen fluid, urea solution and artificial saliva in a test tube for 48 hours at 39ºC in a water bath. After incubation, each sample mixture was then centrifuged at 4695 × g for 15 minutes and then subjected to a 48-hour acid pepsin digestion at 39ºC in a water bath. The residue (following filtration) was weighed and then ashed using a muffle furnace at 550ºC for 2 hours and then the resultant ash was weighed. The IVOMD of each sample was then calculated as described by Larsen and Jones (1973) using the equation: percentage (%) D = 100 – [undigested residue (OM) / sample weight (g)] x 100; where: D = % IVOMD digestibility; OM = organic matter.

3.4 Statistical analysis

Data are presented as proximate, gross energy, fibre and mineral content, amino acid and fatty acid profile and anti-nutritional factors content; as well as the *in vitro* organic matter digestibility of the meals. Genstat statistical software (Genstat, 2000) was used to analyse data using a one-way ANOVA. Means were separated using the Fisher's Least Significant Difference (LSD) test. The level of significance was set at $P < 0.05$.

3.5 Results

3.5.1 Chemical composition of the meals

The proximate, gross energy, fibre content and the *in vitro* organic matter digestibility of Marula nut meals and soyabean meal are shown in Table 3.1.

Table 3.1: Proximate, gross energy, fibre content and the *in vitro* organic matter digestibility of the Marula nut meals and soyabean meal

	Meal					
Parameter	**MNM1**	**MNM2**	**SBM**	**LSD**	**SE**	***P***
Proximate (% DM)						
Dry matter	95.84[a]	95.69[a]	92. 32[b]	0.34	0.17	0.001
Ash	5.19[b]	4.65[c]	6.14[a]	0.13	0.07	0.001
Crude protein	32.47[c]	39.10[b]	51.18[a]	2.03	1.02	0.001
Ether extract	49.85[a]	41.13[b]	1.98[c]	1.71	0.86	0.001
Energy (MJ/kg DM)						
Gross energy	30.69[a]	28.49[b]	19.02[c]	2.14	1.07	0.001
Fibre fraction (% DM)						
Neutral detergent fibre	7.93[a]	14.54[a]	7.03[a]	13.89	6.95	0.409
Acid detergent fibre	4.93[a]	7.77[a]	4.86[a]	5.72	2.87	0.421
Acid detergent lignin	10.85[a]	1.31[c]	5.45[b]	1.50	0.66	0.001
Digestibility (%)						
In vitro organic matter digestibility	81.97[c]	88.40[b]	94.83[a]	5.41	2.39	0.003

[a, b, c] Means in the same row with different superscripts differ significantly at $P < 0.05$. The DM content of MNM1 was similar ($P > 0.05$) to that of MNM2. The DM content of the MNMs was significantly higher ($P = 0.001$) compared to that of SBM. The ash content of MNM1 was significantly higher ($P < 0.05$) than that of MNM2. The MNMs had a lower ash content compared to the SBM. The CP content of MNM2 was significantly higher ($P < 0.05$) than to that of MNM1. The MNMs had a lower CP content than the SBM. The EE content of MNM1 was significantly higher ($P < 0.05$) than that of MNM2. The MNMs' EE content was significantly higher ($P < 0.05$) than SBM's. The MNM1 had a higher ($P = 0.001$) GE content compared to MNM2. The GE content of the MNMs was

significantly higher (P = 0.001) than that of the SBM. The ADF and aNDF content of the MNM1 and MNM2 was similar (P > 0.05) to that of the SBM. The MNM2 had a lower ADL content compared to MNM1. The ADL content of the SBM was significantly higher (P < 0.05) than that of MNM2. The IVOMD of MNM2 was significantly higher (P = 0.003) than that of MNM1. The SBM's IVOMD was significantly higher (P < 0.05) compared to the MNMs'. MNM1 = Marula nut meal produced by hydraulic filter press; MNM2 = Marula nut meal produced by cold press; SBM = solvent extracted soyabean meal; n = 3.

Although the Marula nut meals had higher DM and EE content compared to the SBM, their CP and ash content was lower compared to that of SBM (Table 3.1). While the aNDF and ADF content of the meals were similar, the hydraulic filter produced MNM (MNM1) had the highest ADL content. Soyabean meal had significantly higher *in vitro* organic matter digestibility compared to the MNMs.

3.5.2 Mineral content of the meals

The mineral content of the Marula nut meals and soyabean meal is shown in Table 3.2.

Table 3.2: Macro- and micro-mineral content of the Marula nut meals and soyabean meal

	Meal					
Mineral	**MNM1**	**MNM2**	**SBM**	**LSD**	**SE**	***P***
Macro-mineral (g/kg DM)						
Calcium	1.43[b]	1.10[c]	2.73[a]	0.34	1.17	0.001
Phosphorus	10.0[b]	10.9[a]	7.43[c]	0.07	0.03	0.001
Magnesium	4.87[b]	5.50[a]	3.10[c]	0.13	0.07	0.001
Potassium	6.33[c]	9.30[b]	25.80[a]	0.93	0.46	0.001
Sodium	0.03[a]	0.10[a]	0.10[a]	0.07	0.03	0.079
Chlorine	0.00[c]	0.30[a]	0.17[b]	0.07	3.03	0.001
Micro-mineral (mg/kg DM)						
Copper	24.50[a]	24.97[a]	16.30[b]	13.57	6.79	0.001
Iron	694.33[a]	44.03[c]	142.00[b]	292.11	146.20	0.001
Manganese	18.43[b]	12.37[c]	41.50[a]	12.13	6.07	0.001
Zinc	54.57[c]	61.37[a]	57.93[b]	8.24	4.12	0.001
Cobalt	0.07[b]	3.33[a]	0.14[b]	37.11	18.57	0.012
Molybdenum	<0.01[b]	<0.01[b]	0.92[a]	7.90	3.96	0.045
Sulphur	4230.66[a]	5920.67[a]	3372.87[a]	2974.68	1488.91	0.184

[a, b, c] Means in the same row with different superscripts differ significantly at $P < 0.05$. Calcium was significantly higher ($P < 0.05$) in MNM1 than in MNM2. Calcium in the SBM was the highest ($P < 0.05$). Phosphorus and magnesium were significantly higher ($P < 0.05$) in MNM2 than in MNM1. Phosphorus and magnesium in SBM were lower compared to those in the MNMs. Potassium was significantly higher ($P < 0.05$) in MNM2 than in MNM1. Potassium in SBM was highest ($P < 0.05$). Sodium and sulphur content in MNM1 and MNM2 were similar ($P > 0.05$) to those in SBM. Chlorine in MNM2 was significantly higher ($P < 0.05$) than that in MNM1. Chlorine in SBM was significantly higher ($P < 0.05$) than that in MNM1 and lower than that in MNM2. Copper in MNM1 was similar ($P > 0.05$) to that in MNM2. The SBM's copper was lower compared to the MNMs'. Iron was significantly higher ($P < 0.05$) in MNM1 than that in MNM2. Iron in SBM was significantly higher ($P < 0.05$) than that in MNM2 and lower than that in MNM1. Manganese in MNM1 was significantly higher ($P < 0.05$) than that in MNM2. Manganese was highest ($P < 0.05$) in SBM. Zinc was significantly higher ($P <0.05$) in MNM2 than in MNM1. Zinc in SBM was significantly higher ($P < 0.05$) than that in MNM1 and lower than that in MNM2. Cobalt was significantly higher ($P < 0.05$) in MNM2 compared to MNM1. Cobalt in SBM was lower compared to that in MNM2, but similar ($P > 0.05$) to that in MNM1. Molybdenum was similar ($P> 0.05$) MNM1 and MNM2. Molybdenum was highest ($P < 0.05$) in SBM. MNM1 = Marula nut meal produced by hydraulic filter press; MNM2 = Marula nut meal produced by cold press; SBM = solvent extracted soyabean meal; n = 3.

The macro-minerals' (phosphorus, magnesium and chlorine) and micro-minerals' (zinc and cobalt) concentration were highest ($P < 0.05$) in MNM2 than in SBM. Of the micro-minerals, manganese and molybdenum were significantly higher ($P < 0.05$) in SBM compared to in the Marula nut meals. While copper was significantly more concentrated in the Marula nut meals compared to its concentration in SBM, the concentration of iron was highest ($P < 0.05$) in MNM1. The sodium and sulphur concentration of the meals were similar ($P > 0.05$).

3.5.3 Amino acid profile of the meals

The amino acid profile of the Marula nut meals and soyabean meal is shown in Table 3.3.

Table 3.3: Amino acid profile of the Marula nut meal and soyabean meal

	Meal					
Amino acid	**MNM1**	**MNM2**	**SBM**	**LSD**	**SE**	***P***
Essential amino acid (g/100g DM)						
Arginine	5.09^{b}	7.63^{a}	4.49^{b}	0.64	0.31	0.001
Histidine	0.57^{a}	0.68^{a}	1.14^{a}	0.50	0.24	0.068
Isoleucine	1.23^{b}	1.29^{b}	1.85^{a}	0.29	0.14	0.005
Leucine	1.69^{b}	1.67^{b}	3.27^{a}	0.65	0.31	0.002
Lysine	0.77^{b}	0.77^{b}	3.11^{a}	0.16	0.08	0.001
Methionine	0.63^{b}	0.74^{b}	1.02^{a}	0.19	0.09	0.009
Phenylalanine	1.24^{b}	1.19^{b}	2.05^{a}	0.27	0.13	0.001
Threonine	0.69^{b}	0.61^{b}	1.67^{a}	0.16	0.08	0.001
Tryptophan	0.34^{b}	0.63^{ab}	0.92^{a}	0.35	0.17	0.020
Valine	1.30^{b}	1.40^{b}	2.17^{a}	0.50	0.24	0.012
Non-essential amino acid (g/100g DM)						
Alanine	0.89^{b}	0.81^{b}	1.84^{a}	0.31	0.15	0.001
Aspartic acid	1.98^{b}	1.94^{b}	3.75^{a}	1.38	0.66	0.032
Cysteine	0.27^{c}	0.97^{b}	1.64^{a}	0.16	0.07	0.001
Glutamic acid	6.61^{ab}	6.15^{b}	7.46^{a}	0.95	0.45	0.041

Glycine	1.36[a]	1.15[a]	1.35[a]	0.62	0.29	0.636
Hydroxy-proline	0.06[b]	0.03[c]	0.09[a]	0.02	0.02	0.001
Proline	1.30[b]	0.91[b]	2.79[a]	0.64	0.30	0.001
Serine	1.22[b]	1.04[b]	2.01[a]	0.23	0.11	0.001
Tyrosine	0.97[b]	1.26[b]	2.04[a]	0.40	0.19	0.002

[a, b, c] Means in the same row with different superscripts differ significantly at $P < 0.05$. The essential amino acids concentration in MNM1 were similar ($P > 0.05$) to that in MNM2. The essential amino acid concentration was highest ($P < 0.05$) in SBM, except for arginine, which was highest ($P < 0.05$) in MNM2 than in MNM1. Arginine in SBM was lower than that in MNM2 and similar ($P > 0.05$) to that in MNM1. Histadine in MNM1 was similar ($P > 0.05$) to that in MNM2 and SBM. Alanine, aspartic and glutamic acids in MNM1 were similar ($P > 0.05$) to that in MNM2. Alanine, aspartic and glutamic acids were highest ($P < 0.05$) in SBM. Cysteine was significantly higher ($P < 0.05$) in MNM2 than in MNM1. Cysteine in the SBM was the highest ($P < 0.05$). Glycine in MNM1 was similar ($P > 0.05$) to that in MNM2 and SBM. Hydroxy-proline was significantly higher ($P < 0.05$) in MNM1 compared to MNM2. Hydroxy-proline was highest ($P < 0.05$) in SBM. Proline, serine and tyrosine acids in MNM1 were similar ($P > 0.05$) to that in MNM2. The SBM's proline, serine and tyrosine acids concentration were the highest ($P < 0.05$). MNM1 = Marula nut meal produced by hydraulic filter press; MNM2 = Marula nut meal produced by cold press; SBM = solvent extracted soyabean meal; n = 3.

The amino acid composition (essential and non-essential amino acids) of the MNMs (MNM1 and MNM2) was lower compared to that of SBM, except for arginine which was highest ($P = 0.01$) in MNM2. The histidine and glycine content of the meals were not significantly different ($P > 0.05$).

3.5.4 Fatty acid profile of the meals

The fatty acid profile of the Marula nut meals and soyabean meal is shown in Table 3.4.

Table 3.4: Fatty acid profile of Marula nut meals and soyabean meal

Fatty acid (% DM)	Meal			LSD	SE	*P*
	MNM1	MNM2	SBM			
Saturated						
C12:0 (Lauric acid)	0.01[b]	0.02[b]	0.06[a]	0.01	0.01	0.001
C14:0 (Myristic acid)	0.08[b]	0.13[b]	0.27[a]	0.09	0.04	0.005
C15:0 (Pentadecyclic acid)	0.01[b]	0.03[ab]	0.06[a]	0.03	0.01	0.016
C16:0 (Palmitic acid)	11.83[c]	13.57[b]	17.19[a]	1.21	0.58	0.001
C17:0 (Margaric acid)	0.13[a]	0.2[a]	0.16[a]	0.16	0.08	0.365
C18:0 (Stearic acid)	6.52[b]	7.66[a]	5.14[c]	0.34	0.16	0.001
C20:0 (Eicosanoic acid)	0.57[a]	0.25[a]	0.38[a]	0.50	0.24	0.333
C21:0 (Heneicosylic) acid)	0.02[b]	0.04[b]	0.14[a]	0.07	0.03	0.012
C22:0 (Behenic acid)	0.15[b]	0.17[b]	2.13[a]	0.25	0.12	0.001
C23:0 (Tricosylic acid)	0.01[b]	0.01[b]	0.11[a]	0.02	0.01	0.001
C24:0 (Lignoceric acid)	0.16[a]	0.12[a]	0.16[a]	0.12	0.06	0.648
TSFAs	**19.40[a]**	**15.11[a]**	**25.60[a]**	**16.81**	**8.01**	**0.352**
Monounsaturated						
C14:1 (Myristoleic acid)	0.00[a]	0.00[a]	0.03[a]	0.04	0.02	0.177
C16:1 (Palmitoleic acid)	0.17[b]	0.02[c]	0.74[a]	0.04	0.02	0.001
C17:1 (Heptadecanoic acid)	0.06[a]	0.05[ab]	0.05[b]	0.01	0.00	0.042
C18:1n9c (Oleic acid)	72.91[a]	74.45[a]	26.54[b]	2.83	1.35	0.001
C18:1n9t (Elaidic acid)	0.11[b]	0.07[b]	1.12[a]	0.17	0.08	0.001
C20:1 (Gadoleic acid)	0.33[a]	0.04[a]	0.16[a]	0.23	0.11	0.054
C22:1n9 (Erucic acid)	0.00[a]	0.03[a]	0.00[a]	0.04	0.02	0.201
C24:1 (Nervonic acid)	0.00[b]	0.00[b]	0.08[a]	0.04	0.0	0.003

TMUFAs	**73.00[a]**	**81.60[a]**	**27.90[b]**	**15.61**	**7.44**	**0.001**
Polyunsaturated						
C18:2n6c (Linoleic acid)	6.91[b]	6.84[b]	39.25[a]	0.27	0.13	0.001
C18:2n6t (trans-linolenic acid)	0.00[a]	0.28[a]	0.00[a]	0.62	0.30	0.460
C20:2 (Eicosadienoic acid)	0.00[b]	0.01[b]	0.14[a]	0.04	0.02	0.001
C22:2 (Docosadienoic acid)	0.00[a]	0.03[a]	0.11[a]	0.09	0.04	0.061
C18:3n3 (α-Linolenic acid)	0.06[b]	0.04[b]	5.33[a]	0.04	0.02	0.001
C18:3n6 (γ-linolenic acid)	0.00[b]	0.00[b]	0.19[a]	0.04	0.02	0.001
C20:3n3 (Eicosatrienoic acid)	0.00[a]	0.12[a]	0.16[a]	0.25	0.12	0.297
C20:3n6 (Dihomo-gamma-linolenic acid)	0.00[a]	0.03[a]	0.08[a]	0.07	0.03	0.060
C20:4n6 (Arachidonic acid)	0.00[a]	0.00[a]	0.14[a]	0.18	0.09	0.177
C20:5n3 (Eicosapentaenoic acid)	0.00[b]	0.36[a]	0.30[a]	0.17	0.08	0.005
TPUFAs	**6.97[b]**	**5.21[b]**	**45.75[a]**	**5.18**	**2.46**	**0.001**
Trans fatty acid	0.11[a]	2.59[a]	1.20[a]	6.00	2.38	0.500
Cis fatty acid	79.83[a]	53.64[a]	65.78[a]	61.76	29.40	0.586
Omega 3	0.07[b]	0.19[b]	5.85[a]	0.39	0.18	0.001
Omega 6	6.90[b]	2.85[b]	39.77[a]	5.76	2.75	0.001
Omega 9	73.01[a]	27.90[a]	27.93[a]	56.40	26.87	0.151
Eicosapentaenoic acid	0.00[a]	0.21[a]	0.00[a]	0.23	0.11	0.094
Docosahexaenoic acid	0.00[a]	0.09[a]	0.00[a]	0.16	0.08	0.320

[a, b, c] Means in the same row with different superscripts differ significantly at $P < 0.05$. Total saturated fatty acids content in MNM1 was similar ($P > 0.05$) to that in MNM2 and SBM. Total monounsaturated fatty acids content in MNM1 was similar ($P > 0.05$) to that in MNM2. Total monounsaturated fatty acids content was lowest in the SBM. Total polyunsaturated fatty acids content in MNM1 was similar ($P > 0.05$) to that in MNM2. The SBM's total polyunsaturated fatty acids content was the highest ($P = 0.001$). TSFAs = total saturated fatty acids; TMUFAs = total monounsaturated fatty acid; TPUFAs = total polyunsaturated fatty acid; MNM1 = Marula nut

meal produced by hydraulic filter press; MNM2 = Marula nut meal produced by cold press; SBM = solvent extracted soyabean meal; n = 2.

The total saturated fatty acid content was similar ($P > 0.05$) across the meals. However, the TMUFAs content was significantly higher ($P < 0.05$) in MNMs than in SBM with oleic acid being the dominant fatty acid. The TPUFAs content was significantly higher ($P < 0.05$) in SBM compared to its content in the MNMs. Proportionately; linoleic acid was the most abundant polyunsaturated fatty acid across the meals and was highest in SBM.

3.5.5 Anti-nutritional factors content of the meals

The anti-nutritional factors content of the meals (marula nut and soyabean meals) are shown in Table 3.5.

Table 3.5: Anti-nutritional factors content of Marula nut meal and soyabean meal

Anti-nutrient	Meal					
(mg/100g DM)	MNM1	MNM2	SBM	LSD	SE	*P*
Oxalate	nd	15.03[a]	2.53[b]	2.11	0.93	0.001
Phytate-phosphate	nd	218.30[a]	72.00[b]	13.91	6.14	0.001
Saponin	nd	21.21[b]	43.63[a]	6.13	2.70	0.001
Tannins	nd	0.07[b]	0.11[a]	0.01	0.01	0.001

[a, b] Means in the same row with different superscripts differ significantly at $P \leq 0.05$. Oxalate and phytate-phosphate content were significantly (P = 0.001) concentrated in MNMs than in SBM. Saponins and tannins were significantly (P = 0.001) more concentrated in SBM than in MNM2. nd = not done. MNM2 = Marula nut meal produced by cold press; SBM = solvent extracted soyabean meal; n = 3.

While the oxalate and phytate-phosphate concentration in the Marula nut meal produced following cold pressing of the Marula nuts (MNM2) was significantly higher (P = 0.001) compared to that in SBM, its saponin and tannin concentration was significantly lower (P = 0.001) compared to that in SBM.

3.6 Discussion

3.6.1 Proximate and amino acid content

Determining the chemical nutrient composition of potential feed ingredients and or feeds on a dry matter basis allows for comparison between and among different ingredients and or feeds (Thiex and Richardson, 2003). The higher DM content of the MNMs compared to SBM points to them having a higher amount of biomass (nutrients). The CP and the EE content of MNM1 (82.32%) and MNM2 (80.23%; Table 3.1) constituted the bulk of the dry matter content (nutrients) in the MNMs compared to the SBM's 53.16% (CP and EE) contribution to the dry matter. With regards to the CP and EE of the meals, it could be inferred that the MNMs potentially have a higher nutrient density per unit mass compared to SBM. While the MNMs had a lower CP content (Table 3.1) compared to that in SBM, their CP (content (MNM1: 33% CP and MNM2: 39% CP) was higher compared to the 23.5% CP and 32.2% CP content of sunflower meal and rapeseed cake that are by-products of mechanical oil extraction (Lardy, 2008; Kaldmäe et al., 2010). The latter (sunflower meal and rapeseed cake) are conventional dietary energy and protein sources in livestock feeds (Amores et al., 2014). The CP content (33%) of Marula nut meal produced from hydraulic filter pressing (MNM1) is comparable to the 32% CP content reported for sesame seed meal (Ranganayaki et al., 2012); while the 39% CP content of the cold press produced Marula nut meal (MNM2) is comparable to the 38.6% CP content reported for solvent extracted groundnut meal (Atasie et al., 2009).

The oil extraction method (mechanical vs solvent) affects the residual oil content of the meal and its CP content (Ramachandran et al., 2006). The MNMs evaluated in this experiment are by-products of mechanical oil extraction (inefficient method compared to solvent extraction) from the nuts. Thus, if the high residual oil of the MNMs (at 20 times more than in SBM) was to be reduced through solvent extraction, their respective CP content could go up and be comparable to that of the solvent extracted SBM. Substantial reduction in the residual oil content of the MNMs would also help guard against oil-mediated rancidity of feed positively affecting the shelf life of feeds produced with MNMs as part of the ingredients.

Results of the current study suggest that the MNMs, due to their higher and at times similar CP content to other commonly used plant-derived dietary protein sources could potentially be utilised as dietary protein sources in livestock feeds.

While the CP content is a reasonable estimate of a feed ingredient to supply and or meet dietary protein requirements for a given class of animal, the amino acid composition and proportion (which impact protein quality) is a critical determinant in the evaluation of nutritional potential of feed ingredients (Püssa et al., 2009). Findings from this study show that on average the Marula nut meals had lower essential amino acid content (except for arginine) compared to those in SBM. Thus, despite the relatively high and comparable CP content of the MNMs, their utilisation, especially as dietary protein source in monogastric animal feed, would require supplementation with synthetic lysine for monogastric and methionine for growing ruminants, in order to avoid deficiencies.

3.6.2 Energy content

Energy-dense feed is vital in the early growth phase of animals as at this stage animals require higher energy intake in order to meet maintenance and production (lean accretion) requirements (Ahmed et al., 2013). They are also vital (energy dense feeds) for fattening animals in order to facilitate the deposition of intramuscular fat (de Oliveira et al., 2007). Intra-muscular fat is known to improve the eating quality of meat (Listrat et al., 2016). Results from the current study show that the GE value of the MNMs was on average 56% higher compared to that of SBM. The high GE value in MNMs is attributed to their higher residual oil content when compared to SBM. Due to their high GE value and relatively high CP content, the MNMs could potentially be exploited both as dietary energy and protein sources in the feeds of growing and fattening animals. Prior to the use of the MNMs in livestock feeds, the fatty acid profile of the residual oil in the MNMs needs to be determined since fatty acid composition and quantity impact digestion and absorption of nutrients (Baião and Lara, 2005), hence animal growth in addition to the fatty acid composition's potential effects on animal health and product (meat) quality.

3.6.3 Fatty acid profile

My findings show that on average TSFAs made up 17.3%, TMUFAs constituted 77.3% and TPUFAs made up 6.1% of the residual oil in the MNMs. Oleic acid (OA) at 74.2% made up a large proportion of the MNMs'oil content. The TSFAs (17.3%) and TPUFAs (6.1%) content of the MNMs closely resembled the 20%, and 6.4%, TSFAs and TPUFAs in the Marula nut oil from a variety of Marula tree in Nigeria (Robinson et al., 2012). Also interesting is the almost similar content of OA in the MNMs' residual oil, which averaged 74.2% and the reported 73.6% OA in the Marula nut oil from the Nigerian Marula tree variety (Robinson et al., 2012). With regards to the Marula nut oil fatty acid profile, my results pointed to similarities with studies done in Nigeria which is interesting when one considers that environmental factors usually result in differences in the chemical nutrient profile of plants (Cardinale et al., 2006). Unlike in the MNMs, the bulk (45.8%) of the residual oil from SBM was made up of PUFAs and almost equal proportions of the SFAs (25.6%) and MUFAs (27.9%).

Oleic acid protects muscles against being damaged by reactive oxygen species (Carvalho et al., 2012), improves carcass characteristics (Leheska et al., 2008), and the fatty acid composition of adipose tissue (Pereira et al., 2012). The *in vivo* effects of OA in total positively impact meat quality (Suksombat et al., 2016). It could be inferred that the high OA in the MNMs could be exploited in the formulation of livestock feeds with benefits on animal health and product (meat) quality.

3.6.4 Mineral content

Appropriate dietary mineral content is required for normal animal function (Özcan, 2004). Both macro- and micro-minerals contribute to structural regulatory, catalytic, and physiological roles in animals (Özcan, 2004). It is thus imperative that the mineral content of potential feed resources be evaluated (López-Alonso, 2012). My findings show that the phosphorus content of the MNMs (range: 1.00 to 1.09g/100g) was higher than that of SBM (0.74g/100g). Importantly, the phosphorus content in my findings was higher than that of SBM (range: 0.70 to 0.77g/100g) reported by García-Rebollar et al. (2016). The copper content of the MNMs in this study was higher (range: 24.50 to 24.97mg/kg DM) than that of SBM (16.30mg/kg DM). The MNMs'

copper content was higher than the SBM's copper content (range: 15.30 to 16.60mg/kg DM) reported by García-Rebollar et al. (2016). Results in this study show that zinc content of MNM2 (61.37mg/kg DM) was higher than that of MNM1 (54.57mg/kg DM) and SBM (57.93mg/kg DM). Importantly, zinc content of MNM2 was higher than that of SBM (50.90 to 56.90mg/kg DM) reported by García-Rebollar et al. (2016). The higher concentration of phosphorus, magnesium, copper and zinc in MNMs compared to that in SBM, could be exploited to reduce the amount of mineral supplementation in feeds when the MNMs are utilised as feed ingredients. Phosphorus is a key element in the fertility of ruminants (Yasothai, 2014); while copper, magnesium and zinc constitute co-factors that derive metabolic processes in animals (Soetan et al., 2010). Their abundance in the MNMs could be exploited to formulate nutritionally balanced feeds. The sulphur content of MNM2 (5920.67mg/kg DM) was generally comparable to the 6200.00mg/kg DM in canola meal (Spragg and Mailer, 2007), a conventional dietary protein source in feeds. The similarities in the sulphur content of the MNMs and the SBM in this study suggests that utilisation of the MNMs as feed ingredients is not likely to result in compromising the synthesis of essential sulphur-containing amino acids (methionine, cystine and cysteine), hormones and chondroitin (Nimni et al., 2007).

3.6.5 Fibre content

Neutral detergent fibre (cellulose, hemicelluloses and lignin) and acid detergent fibre (cellulose fraction, lignin and silica) are structural carbohydrates (McDonald et al., 2002) with different functions in animals (Tekçe and Gül, 2014). Among their functions are: protection of the rumen function via regulation of pH through rumination, generation of energy and prevention of acidosis, laminitis, and rumen parakeratosis (Tekçe and Gül, 2014). Results of this study show similarities in the fibre (aNDF and ADF) content of the MNMs and SBM. The observed similarities in the aNDF and ADF suggest that MNMs could be exploited in formulating animal feeds without compromising the fibre content of the feeds. One of the major dietary energy source in animal feeds, maize, has an aNDF and ADF content of 10.8% and 2.8%, respectively (FAO, 1992). While the aNDF of the MNM1 and MNM2 (average 11.23%) was relatively similar to the aNDF content of maize, their (MNMs) ADF averaged 6.4%, which was about 1.3 times higher than that reported for maize. The similarities in aNDF and near similarities in the ADF content of the MNMs and that of maize suggest that the MNMs could have a dual function

(as dietary protein and energy sources) in feeds with limited effects on nutrient digestibility. However, due to their low aNDF and ADF content, use of the MNMs as the main dietary ingredients could trigger physiologic and metabolic disorders in ruminants. Thus, it would be prudent to include a source of dietary fibre to such diets in order to protect against potential low dietary fibre mediated physiological and metabolic derangements such as acidosis, laminitis and lameness (Westwood et al., 2003).

Lignin is a primary component that limits forage digestion (Agbagla-Dohnani et al., 2001) and reduces the degradation of cellulose and hemicelluloses (Van Soest, 1994). It (lignin) is known to increase retention time of digesta in the rumen (Van Soest, 1994) and hence reduces feed intake (Van Soest, 1994). The concentration of lignin in feed ingredients and feeds is an important predictor of digestible energy (Jung et al., 1999). In the current study MNM1, MNM2 and SBM had 10.85%, 1.31% and 5.45% of acid detergent lignin (ADL), respectively. The 1.31% of ADL content in MNM2 was, although higher, near the 1.16% of ADL content in SBM reported by Das et al. (2014). The results of this study indicate that compared to MNM1, MNM2 could potentially be utilised as a source of dietary energy and protein in ruminant feeds without compromising digestion and degradation in the rumen. Importantly, like the SBM, the MNM2 has the potential to be used as a dietary protein source in feed without reducing the digestibility of the nutrients in feed and or reducing feed intake.

3.6.6 Anti-nutritional factors content

Utilisation of plant-derived by-products as sources of nutrients in livestock feeds is limited by the presence of the anti-nutrient factors (Sindhu et al., 2002). The commonly used plant-derived dietary protein sources in feeds (groundnut cake, sesame seed cake, sunflower meal and soyabean meal) contain anti-nutrient factors, which depending on concentration in diets, can be detrimental to animal health (Akande et al., 2010) nutrient digestion and absorption (Enujiugha, 2003), thus compromise growth performance (Tadele, 2015). In the current study, focus was on the meals' saponin, phytate-phosphate, and oxalate and tannin concentration. Although the saponin and tannin concentration of the MNM2 was higher than that in SBM, considering that the MNMs would be used as dietary ingredients, the ANFs concentration in the diet would be expected to be

lower than the toxic concentration that would otherwise negatively impact digestion and absorption of nutrients.

3.6.7 *In vitro* organic matter digestibility

The digestibility of non-conventional feed resources is major determinant of their potential as dietary sources of nutrients (Safwat et al., 2015). Nutrient digestibility is a crude, but useful measure of nutrient availability to the animal and it (nutrient digestibility) is a key factor in formulating nutritionally adequate feeds (Căpriţă et al., 2012). One of the many biological methods used in determining the potential of a test material to supply nutrients is to determine its (material) *in vitro* organic matter digestibility (IVOMD). In this study, SBM had an IVOMD of 94.83%, which was higher compared to the 81.97% and 89.77% IVOMD of MNM1 and MNM2, respectively (Table 3.1). Despite the lower IVOMD of the MNMs compared to that of the SBM, the MNMs' IVOMD was higher than the 54% and 79.2% IVOMD of macadamia oilcake and avocado meal (Das et al., 2014) suggesting that the MNMs could be better suppliers of nutrients when compared to macadamia and avocado meals. According to Meissner and Paulsmeier (1995), feed intake increases with an increase in IVOMD. Hence, the higher IVOMD of MNM2 compared to that of MNM1, which was attributed to its (MNM2) lower ADL content, could lead to an increase in feed intake. These findings suggest that MNM2 can potentially be utilised as a dietary protein source in livestock feeds without compromising the feed intake and hence growth performance of the animals.

3.7 Conclusion

The chemical nutrient evaluation showed that the MNMs, had a CP content that was lower than that of SBM, however their mean CP content was found to be equal to and at times higher than the CP content reported in literature, of other commonly used plant-derived dietary protein sources (sunflower meal, rapeseed cake, sesame seed meal and groundnut meal) used in feed formulation. The GE content of the MNMs was shown to be higher than that of maize meal and the residual oil from the MNMs had a preponderance of oleic acid. The MNMs could therefore be potentially exploited as energy-dense oleic acid-rich dietary protein sources that can be

utilised in the formulation of livestock feeds. The meals' high oleic content could be exploited to positively manipulate the fatty acid content of meat, hence influence the quality of the meat. Due to variations in the composition of feed ingredients as a result of processing, the chemical composition of the MNMs generated using two different mechanical methods varied. Cold press processed MNM (MNM2) could be exploited as a feed ingredient without compromising feed intake and digestibility of the nutrients due to its low ADL content and high IVOMD. While utilisation of the MNM2 as a feed ingredient might require supplementation of key minerals, which could be chelated by the oxalates and phytate-phosphate, utilisation of the meal is not expected to compromise digestion of protein (meal has low tannin concentration) and is unlikely to cause the destruction (hemolysis) of erythrocytes since the meal has a low saponin concentration.

While the *in vitro* evaluation of the nutritive potential of feed ingredients and or feeds generates useful estimates on the potential of the test materials to supply nutrients, they do not completely mimic the *in vivo* interaction between the test material (feed ingredient and or feed) and the physiological digestive and absorptive machinery and microbiological ecology of the gastrointestinal tract. Although results from the chemical analyses of the test feeds could show a tremendous potential, *in vivo*, negative associative effects could result in poor animal responses, thus it is imperative to test the potential feed ingredient and or feed *in vivo* in order to evaluate its true potential. Thus, in Chapter 4 (next chapter of this thesis), the potential of the Marula nut meal (MNM2) as a substitute to SBM (conventional dietary protein source) was evaluated *in vivo*, using intact male Dorper lambs. The effects of the graded dietary substitution of SBM with the MNM on a crude protein basis, on feed (nutrient) intake, growth performance, rumen epithelial health, rumen fluid traits (digesta pH and rumen liquor volatile fatty acid content), visceral macro-morphometry, metabolic (blood and liver) substrate concentration, surrogate markers of liver and kidney function and the general health profile are interrogated.

CHAPTER FOUR - EFFECT OF DIETARY MARULA NUT MEAL ON THE GROWTH PERFORMANCE, RUMEN DIGESTA TRAITS, VISCERAL MACRO-MORPHOMETRY AND HEALTH PROFILE OF GROWING LAMBS

4 INTRODUCTION

Intensified animal production is premised on the supply of the requisite nutrients to meet the nutrient requirements of a given class of animal (Wanapat et al., 2013). Of the basket of nutrients required to meet animal nutrient requirements for maintenance and production, protein and energy are the major macro-nutrients, making them (protein and energy) essential feed ingredients that play a pivotal role in the formulation of nutritionally adequate diets (Ingale and Shrivastava, 2011). The shortage of dietary protein and energy sources for the production of livestock feed in SSA and SA is a major limiting factor to the intensification of animal production. Dietary protein, one of the most costly dietary constituents in livestock feeds in SSA (Chakeredza et al., 2008) and SA (Brand, 2007), is normally supplied by imported SBM making animal production costly. In order to meet the ever increasing demand of animal products in SSA and SA, the search and development of alternative dietary protein sources for livestock feeds is of paramount importance. In the previous Chapter (Chapter 3), chemical characterisation of the Marula nut meals revealed that the meals could potentially be utilised as energy-dense and oleic acid-rich dietary protein sources. Of the two MNMs, the cold press produced MNM2 was selected to evaluate its potential *in vivo* based on its higher CP content and higher digestibility as measured by its IVOMD (Table 3.1) when compared to MNM1.

While it is known that feed ingredients with high residual oil in animal diets can cause excessive metabolic burden to the liver (Jin et al., 2013) that stimulate excessive fat deposition in the animal (Ghanawi et al., 2011); the high residual oil can be exploited as a dietary lipid particularly when used in fattening diets. In ruminants, high dietary lipid reduces fermentation (rumen) efficiency, negatively impacting on fibre digestion and feed intake (Manso et al., 2006). A decrease in fibre digestion causes a concomitant decrease in VFA production that translates into a decrease in rumen digesta pH (Montgomery et al., 2008). This cascade of events negatively impacts energy (VFA production) supply to the animal, thus resulting in reduced animal growth performance (Gonzalez-Felix et al., 2015). Importantly, the MNM2 was observed to contain ANFs (Table 3.5) that could impact on nutrient digestion and absorption efficiency and the normal health (physiology) of animals. Thus, in light of its (MNM2) significant nutrient content (protein, energy and oleic acid), the *in vivo* evaluation of the potential of MNM2 (designated MNM for the rest of this chapter) as a dietary protein source in feeds became critical to evaluate

its potential to replace the costly SBM in lamb fattening diets. Importantly, due to the complex biochemical and physiological interactions that occur *in vivo*, the favourable chemical nutritive potential exhibited by the MNM2 as a possible dietary protein source needed to be evaluated *in vivo*. Research shows that most studies exploring alternative feeds tend to focus on the growth performance and product quality but under-report on the health status and well-being of the animals. Hence, this study sought to determine, in intact growing-fattening male Dorper lambs, the effects of graded dietary substitution of SBM with MNM, on a CP basis; on the growth performance, rumen health status, rumen digesta pH and VFA content, GIT and other viscera macro-morphometry and on kidney and liver function and general health.

4.1 Objectives of the study

The objectives of this study were to determine the effect of graded dietary substitution of SBM, on a CP basis, with partially defatted MNM in fattening diets fed to male Dorper lambs on:

i. the growth performance [weekly body weight, terminal body weight, body weight gain, average daily gain, feed intake, feed conversion ratio and linear growth (femur and tibia weight, length, Seedor ratio) and bone mineral composition].
ii. rumen papillae health status, rumen digesta pH and rumen liquor volatile fatty acid (VFA) content.
iii. visceral organ macro-morphometry.
iv. circulating (blood/serum) metabolic substrates (glucose and triglyceride) concentration.
v. stored metabolic substrate (liver glycogen and lipid) content.
vi. serum surrogate markers of liver function [total protein, albumin, globulin and total bilirubin concentration, and alanine transaminase (ALT) and alkaline phosphatase (ALP) activities], and kidney function [blood urea nitrogen (BUN) and creatinine)].
vii. markers of general health (inorganic phosphate, calcium, cholesterol and amylase activity) and packed cell volume.

4.2 Hypotheses of the study

The hypotheses of this study were:

a. H_0: dietary substitution of SBM with MNM, on a CP basis, in lamb fattening diets does not affect the growth performance (body weight based indices of growth, linear growth), viscera macro-morphometry, rumen digesta pH and liquor volatile fatty acid content of growing-fattening male Dorper lambs.

   H_1: dietary substitution of SBM with MNM, on a CP basis, in lamb fattening diets affects the growth performance (body weight based indices of growth, linear growth), viscera macro-morphometry, rumen digesta pH and liquor volatile fatty acid content of growing-fattening male Dorper lambs.

b. H_0: dietary substitution of SBM with MNM, on a CP basis, in lamb fattening diets does not affect the concentration of circulating (blood glucose and triglyceride content) and stored (hepatic glycogen and lipid content) metabolic substrates of growing-fattening male Dorper lambs.

   H_1: dietary substitution of SBM with MNM, on a CP basis, in lamb fattening diets affects the concentration of circulating (blood glucose and triglyceride content) and stored (hepatic glycogen and lipid content) metabolic substrates of growing-fattening male Dorper lambs.

c. H_0: dietary substitution of SBM with MNM, on a CP basis, in lamb fattening diets does not affect the blood/serum markers of liver and kidney function, general health and packed cell volume of intact male Dorper lambs.

   H_1: dietary substitution of SBM with MNM, on a CP basis, in lamb fattening diets affects the blood/serum markers of liver and kidney function, general health and packed cell volume of intact male Dorper lambs.

4.3 Materials and methods

4.3.1 Feed ingredients and diet formulation

Feed ingredients such as *Eragrostis curvula* (hay) and *Medicago sativa* (lucerne) were sourced from the Agricultural Research Council's Vegetable and Ornamental Plant Institute (ARC-VOPI), Roodeplaat, Gauteng, South Africa. Canola oil was purchased from Energy Oil, Johannesburg, Gauteng, South Africa. The Marula nut meal was sourced from Home of Phadima Marula Oil, Phalaborwa, Limpopo, South Africa. The dietary treatments were formulated in a manner that they met the National Research Council (NRC) requirements for growing lambs (NRC, 2007) in a complete diet. The MNM substituted SBM on crude protein (CP) basis to generate iso-nitrogenous and iso-caloric diets. The dietary ingredients and chemical composition of the dietary treatments are shown in Table 4.1.

Table 4.1: Ingredient and chemical composition of the dietary treatments

	Diet				
Ingredient (g/kg)	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
Hay	80.00	80.00	80.00	80.00	80.00
Lucerne	100.00	100.00	100.00	100.00	100.00
Maize	470.00	470.00	470.00	470.00	470.00
Wheat bran	110.00	110.00	110.00	110.00	110.00
Molasses	50.00	50.00	50.00	50.00	50.00
Marula nut meal	0.00	47.30	94.900	142.20	189.50
Soyabean meal	150.00	112.50	75.00	37.50	0.00
Canola oil*	70.00	52.50	35.00	17.50	0.00
Salt	5.00	5.00	5.00	5.00	5.00
Limestone	15.00	15.00	15.00	15.000	15.00
Total	**1050**	**1042**	**1035**	**1027**	**1020**
Chemical composition					
Proximate (% DM)					
Dry matter	92.66	92.06	92.18	92.43	92.30
Organic matter	87.15	85.97	86.36	87.17	86.51
Crude protein	14.70	14.43	14.40	14.98	15.07
Ether extract	11.54	11.50	11.98	13.62	13.29
Energy (MJ/kg DM)					
Gross energy	19.67	20.12	19.78	20.49	19.98
Fibre fraction (% DM)					
Neutral detergent fibre	33.11	31.38	32.14	32.66	32.79
Acid detergent fibre	14.10	14.24	14.01	15.73	16.00

Proximate, energy and fibre assays where done in triplicate and chemical composition values in the table are means. *Canola oil, whose fatty acid profile closely resembles that of the Marula nut oil, was used in order to balance the dietary lipid and energy content.

4.3.2 Ethical approval and study site

This study was approved by the University of the Witwatersrand Animal Ethics Screening Committee (Ethics clearance number: AESC 2014/50/B) and the Agricultural Research Council-Animal Production Institute Ethics Committee (Ethics clearance number: APIEC 14/019). The study was carried out at the Agricultural Research Council-Animal Production Institute (ARC-API), Irene, Gauteng, South Africa.

4.3.3 Animals and animal management

The forty male Dorper lambs (intact) used in the study were sourced from Farm Number 156, Mamogalies Kraal, Brits, North-West, South Africa. Following veterinary inspection and acquisition of the requisite permit (permit number: 57/1959 ART/SEC 6/8), the lambs were transported to the ARC-API. Upon arrival, the lambs were treated for external parasites with 1% Flumethrin [(Drastic Deadline); Bayer PTY Limited, Isando, Gauteng, South Africa], an acaricide, at 1ml/10kg body weight as a pour on. For control of internal parasites, the lambs were orally administered a broad spectrum anthelmintic [Albendazole, 11.36% (Valbazen®), Pfizer South Africa, Sandton, Johannesburg, South Africa] at 2ml/10kg of body weight. The lambs were ear-tagged for identification purposes. Induction body weight was measured using a digital weighing scale (TAL-TEC®, Brits, Gauteng, South Africa) after which the lambs were allowed to adapt to the environment and dietary treatments for 21 days. The lambs had *ad libitum* access to feed and water. Each lamb was housed individually in clean disinfected metabolic crates (1.2 m length x 0.74 m width x 0.92 m height) with wooden slatted floors in a well-insulated and ventilated animal house. Each crate was equipped with a water drinking nipple and feed trough.

4.3.4 Experimental design

Forty 112-day old intact male Dorper lambs with a mean induction body weight of 20.3±3.3 kg were used. Following a 21-day adaption period, the lambs were randomly allocated to five dietary treatments (Diet 1 to 5). Diet 1 through to 5 had the SBM substituted with MNM, on a CP basis, at 0, 25, 50, 75 and 100%, respectively in a completely randomised design (CRD). Each dietary treatment was replicated 8 times with each lamb acting as an experimental unit. The

lambs on the respective dietary treatments were fed for 63 days, by which age (about 6 months) fattening Dorper lambs are expected to have attained the expected market weight.

4.3.5 Body weight and feed intake measurements

Induction body weight (IBW) of each lamb was measured prior to execution of the trial. Thereafter, body weight was measured weekly. Feed intake (FI) of each lamb was recorded daily based on the amount of feed offered every morning and the refusals collected the following morning before the next feeding. The amount of feed offered to each lamb was adjusted every three days to cater for changes in body weight of the lamb and to ensure that each lamb continued to have *ad libitum* access to feed. The total body weight gain (BWG) and average daily gain (ADG) of each lamb were computed from their induction body weight and terminal body weight (TBW). Feed conversion ratio (FCR) of each lamb was computed as described by Arthur and Herd (2008) using the equation: feed conversion ratio = feed intake (g) / body weight gain (g).

4.3.6 Terminal procedures and measurements

4.3.6.1 Measurement of terminal body weight and collection of blood

On study termination, the TBW of each lamb was measured using a digital weighing scale (TAL-TEC®, Brits, Gauteng, South Africa) following a 15-hour overnight fasting period. Blood from each lamb was collected via jugular vein puncture using 18G needles and 10ml syringes. The aspirated blood was transferred into 10ml heparinised and 10ml plain (with no coagulant) blood collection tubes. The blood in the heparinised blood collection tubes was used for determination of packed cell volume and fasting blood glucose and triglyceride concentration immediately after collection. The blood collected into plain blood collection tubes was allowed to clot, then centrifuged and the harvested serum was used to assay for serum surrogate markers of liver and kidney function and general health.

4.3.6.2 Determination of blood glucose and triglyceride concentration

A drop of blood was drawn from blood in the heparinized blood collection tubes and used to determine fasting blood glucose concentration using a calibrated glucometer (Accu-Check® Active, Roche Diagnostics, Mannheim, Germany) as per the manufacturer's guidelines. Similarly, fasting blood triglyceride concentration was determined using a portable calibrated triglyceride meter (Cobas Accutrend Plus meter, Roche Diagnostics, Mannheim, Germany) as per the manufacturer's instructions.

4.3.6.3 Determination of packed cell volume

To determine the packed cell volume, blood collected into heparinised blood collection tubes was drawn out of the tubes using 70µL heparinised haematocrit micro-capillary tubes (Thermo Fisher Scientific, Pittsburgh, USA). Each of the haematocrit micro-capillary tubes containing heparinised blood was sealed with clay-type tube sealant (Thomas Scientific, Swedesbore, New Jersey) and then centrifuged at 9650 × g force for 5 minutes in an MSE Haemo Centrifuge (Sanyo, United Kingdom) at room temperature. The packed cell volume values were then read off a haematocrit reader (Hawksley, Lancing, Sussex).

4.3.6.4 Serum preparation and storage

The blood collected into plain blood collection tubes was centrifuged using a Thermo Sorvall® MC 12V centrifuge (Du Pont Instruments, Connecticut, USA) at room temperature (22ºC) for 15 minutes at a force of 13620 × g. A pipette (Thermo Fisher Scientific, Pittsburgh, USA) was used to decant the serum from the blood collecting tube into 1.5ml microtubes (Greiner Bio-One GmbH, Frickenhausen, Germany). Each serum sample was immediately stored at -20ºC pending assay for surrogate markers of liver and kidney function and the general health profile.

4.3.7 Slaughter, carcass processing and sample measurements

Immediately after blood collection, the lambs were transported from the animal house to the abattoir within the ARC-API campus, a distance of approximately 800 meters. They were then

rested in lairage with free access to drinking water for 1 hour following which they were humanely slaughtered. The slaughter was executed in a Grade D, low throughput experimental abattoir (ARC-API, Irene, Gauteng Province) according to standard South African procedures as described by Hoffman et al. (2003) with minor modifications. Briefly, each lamb was electrically stunned on the occipital region of the head using a standard scissor type electrical tongs (KZ-3 electric stunner, Kentmaster, Ohama, Nebraska, USA) with a direct current of 0.75 A, 300 Volts and 50 Hz frequency applied for 3seconds. Immediately following the stunning, each lamb was then exsanguinated by cutting the jugular vein, carotid arteries and oesophagus using a sharp knife and allowed to bleed for 8 minutes. Immediately thereafter, the carcass was hung on rolling hooks and then skinned. Viscera (abdominal and thoracic) were then carefully dissected out.

After the evisceration, each carcass was electrically stimulated for 30 seconds (400 V peak, 5ms pulses at 15 pulses per second) using Tender-Pulse high voltage stimulation power supply (Tender-Pulse, A.I.S Enterprises, Brisbane, Australia) in order to enhance meat quality traits (Adeyemi and Sazilihe, 2014). Each carcass was then weighed using an overhead digital scale (DIGI DS160 Industrial Scale, Toronto, Canada). The dressing percentage was calculated and expressed as a percentage of live weight as described by Bonvillani et al. (2010) using the equation: dressing percentage (%) = W_1/W_2 x 100; where W_1 is warm carcass weight and W_2 is lamb live weight.

The initial pH (pH_i) and temperature of each carcass was measured on the left *musculus longissimus lumborum* (*M. LL*) between the 2nd and 4th lumbar vertebra using a Cyberscan pH 300 digital pH meter (Eutech Instruments, Thermo Fisher Scientific, Vernon Hills, USA) fitted with a specially designed meat electrode (Eutech Instruments, Thermo Fisher Scientific, Vernon Hills, USA). The pH_i and warm carcass temperature were measured prior to chilling the carcass at 4ºC for 24 hours in preparation to determine cold carcass weight, ultimate pH (pH_u) and temperature and to take out samples for the determination of the physico-chemical traits of lamb.

4.3.7.1 Determination of rumen digesta pH and collection of rumen liquor

Following the dissecting out of the GIT viscera, the rumen was carefully opened using a knife and the pH of rumen digesta content was determined *in situ* using a 2-point calibrated portable

Cyberscan pH 310 digital pH meter (Eutech Instruments, Thermo Fisher Scientific, Vernon Hills, USA). Rumen digesta was manually squeezed (by hand) to extract rumen liquor. The extracted rumen liquor from each lamb was stored in a 90ml bottle to which 4ml of 25% phosphoric acid was added as a preservative. The bottles with the preserved rumen liquor were then stored at -20ºC pending assay for the volatile fatty acid content of the liquor.

4.3.7.2 Determination of viscera macro-morphometry

Following evisceration, the GIT viscera (rumen, reticulum, omasum, abomasum, small and large intestines and caecum) were separated and digesta gently removed from each of them. The weights of the GIT viscera were then measured using a digital balance (Kubota KA-10, Ang Mo Kio, Singapore) and the lengths of the small and large intestines were measured using a tape measure fixed on an abattoir preparation table. The weights of the other viscera (liver, kidneys and perirenal fat) were measured using a calibrated Precisa 310M electronic balance (Precisa Instruments AG, Precisa, Dietikon, Switzerland). Samples of the liver were stored in a freezer at -20°C pending the determination of hepatic glycogen and lipid content. The rumen health status, a description of the packing (density) and length of the rumen papillae, was determined using a 5-point scale [the lowest score of zero (0) denoting well developed papillae and the highest score of 4 denoting very short papillae with large areas of lesions] as described by Leeuw et al. (2009).

4.3.7.3 Determination of linear growth

Twenty-four hours post-slaughter, the tibia and femur from the right leg of each lamb's chilled carcass were dissected out and cleaned of all soft tissues. The cleaned tibia and femur were preserved in 0.09% saline using gauze wraps and stored at -20ºC pending the determination of linear growth. To determine linear growth, the frozen-stored femur and tibiae were allowed to thaw to room temperature before being dried in an oven (Labotec oven, Term-O-MAT, Johannesburg, South Africa) at 60ºC for 7 days to constant weight. After allowing the bones to cool to room temperature, the weight of each bone was measured using a digital platform weighing scale (Kubota KA-10, Ang Mo Kio, Singapore). The length of each tibia and femur was measured using a 0-200mm capacity digital caliper (Krekeler Sliding Caliper, Helmut Zepf Medizin Technik GmbH, Seitingen Oberflacht, Germany). In summary, the length of the femur

was determined by measuring the distance from the most proximal point on the femur head to the line connecting the two distal condyles (most distal point) of the femur. Tibia length was determined by measuring the distance from the centre of the lateral condylar surface to the centre of the distal articular surface (Lieberman et al., 2004). The Seedor index of the femur and tibia was determined as described by Seedor et al. (1991) using the equation: bone density (g/cm) = dry bone weight (g) / dry bone length (cm).

4.3.7.4 Determination of bone calcium, magnesium and phosphorus content

To determine bone calcium, magnesium and phosphorus content, the femoral diaphysis from each lamb was ashed in a muffle furnace (BDI 73 muffle furnace, Labotec, Sheffield, England) at 600ºC for 6 hours. The ash was allowed to cool to room temperature before being milled using a barrel mill (250 cc single pot barrel mill, Eriez Lab Equipment, Delta, Canada). The milled ash was then digested in concentrated nitric acid and perchloric acid at 200ºC to generate the digest solution (Zasoski and Burau, 1977). An aliquot from the digest solution was used for the inductively coupled optical emission spectrometric (ICP-OES) determination of calcium, magnesium and phosphorus on a Varian Liberty 200 spectrometer (Varian, Perth, Australia) as described by Huang and Schulte (1985).

4.3.7.5 Determination of rumen liquor volatile fatty acid content

The volatile fatty acid content of the rumen liquor was quantified as described by Webb (1994) using a gas chromatograph. In summary, after allowing each sample to thaw to room temperature, 2ml of each rumen liquor sample was diluted with 2.5ml of distilled water after which the sample was centrifuged at 10 000 × g for 15 minutes at 10ºC using a Beckman centrifuge (Avanti JE, Beckman Coulte, Inc, CA, Fullerton, USA). One millilitre (1ml) of the supernatant was then mixed with 3ml of distilled water to which 1ml of the internal standard (0.5g 3-methyl-n-valeric acid in 1L of 0.15mol/L oxalic acid) was added and centrifuged at 1400 × g for 4 minutes to remove the precipitate. Following centrifugation the mixture was filtered through a Whatman 0.45µm polyethersulphone membrane filter (Sigma-Aldrich, Dorset, UK) into a chromatographic vial. The VFA content of each sample was determined by injecting 1µL of the filtrate into a Varian gas chromatograph (Varian CP 3800, Massachusetts, USA) equipped

with a 25m × 0.53mm i.d. megabore column (coating CP-Wax 58 (FFAP) – CB (no. CP7614) (Varian, Middelburg, Netherlands).

4.3.7.6 Determination of hepatic lipid content

Prior to the determination of hepatic lipid content, each frozen (-20ºC) stored liver sample was allowed to stand at room temperature for 30 minutes in order to stabilise the moisture content. A one hundred gram (100g) sample was then weighed into a pre-weighed plastic container and was then freeze dried using a preconditioned freeze dryer (Specht Scientific Engineering (SSE), Cologne, Germany) connected to a vacuum pump (Sogevac SV28, Leybold GmbH, Cologne, Germany) over a 24-hour period with a conditioner temperature of -50ºC and shelf temperature of 26ºC. The freeze drying was carried out as per manufacturer's instructions. Each dried liver sample was then ground using a Retsch ZM 200 grinder (Retsch-Allee 1-5 42781, Haan, Germany) to pass through a 1mm sieve. The liver lipid content of the sample was determined using a Soxtec System HT 1043 Extraction Unit (Foss Analytical, HillerØd, Denmark) as described in Chapter 3 subsection 3.4.2.1.

4.3.7.7 Determination of hepatic glycogen content

Hepatic glycogen was determined using the indirect hydrolysis as described by Passonneau and Lauderdale (1974). In summary, 0.1g of each liver sample was placed in 1ml of 0.03 M hydrochloric acid and homogenised using an Ultraturrax homogeniser (Janke and Kunkel, Ika-Werk, Germany). Then 1ml of 1M hydrochloric acid was added prior to the mixture being boiled in a water bath for 2 hours. The sample mixture was then allowed to cool following which it was neutralised by adding 1ml of 1M sodium hydroxide. The glucose concentration of the hydrolysate was then determined using a glucometer (Accu-Chek® Active, Roche Diagnostics, Mannheim, Germany).

4.3.7.8 Determination of serum markers of health

The serum markers of liver function [total protein, albumin, globulin and total bilirubin concentration and alanine transaminase (ALT) and alkaline phosphatase (ALP) activity] and

kidney function [blood urea nitrogen (BUN) and creatinine concentration)] and other markers of general (inorganic phosphate, calcium, cholesterol and glucose concentration and amylase activity) health of each lamb was determined using a calibrated colorimetric-based clinical chemistry analyzer (IDEXX VetTest® Clinical Analyzer, IDEXX Laboratories Inc., USA) as per manufacturer's instructions. In summary, each of the stored serum samples was thawed and allowed to warm to room temperature and then gently inverted to mix the contents. The serum sample was placed into the analyser, which automatically drew up 150µL and dispensed 10µL of the sample onto each of the pre-loaded disks after which each sample was analysed and the print out of results provided.

4.4 Statistical analysis

Data are presented as mean weekly body weights. The General Linear Model (Proc GLM) of the Statistical Analysis System (SAS, 2003) was used to analyse the data using a repeated measures ANOVA. The statistical differences between means were compared using Fisher's Least Significance Difference (LSD) test. The level of significance was set at 5%. The model used for data analysis was:

$Y_{ijk} = \mu + T_i + W_j + TW_{ij} + e_{ijk;}$

where

Y_{ijk} = is the kth observation (body weight) of the ith dietary treatment of the jth week

μ = is the overall mean

T_i = is the fixed effect of the ith dietary treatment (i = 1, 2…5)

W_j = is the effect of the jth week of measurement (j = 1, 2… 3…..9)

TW_{ij} = is the interaction between dietary treatment and week

e_{ijk} = is the random residual error

Data on growth performance (body weight based indices and linear growth), feed utilisation efficiency, viscera macro-morphometry, circulating and stored metabolic substrates, rumen digesta characteristics and surrogate markers of liver and kidney function and other markers of health was analysed using Genstat statistical software (Genstat, 2000) by a one-way ANOVA.

Differences between means were determined using Fischer's Least Significance Difference test (LSD). The level of significance was set at 5%. The model used for data analysis was:

$Y_{ij} = \mu + T_i + e_{ij}$;

where

Y_{ij} = is dependent variable of interest (growth performance indices, rumen liquor pH and volatile fatty acid content, blood and stored metabolic substrates, viscera macro-morphometry and general health profile)

μ = is the overall mean common to all observations

T_i = is the fixed effect of the ith dietary treatment (i = 1, 2…5)

e_{ij} = is the random residual error.

4.5 Results

4.5.1 Weekly body weights

The mean weekly body weights of the growing-fattening male lambs on the different dietary treatments are shown in Figure 4.1.

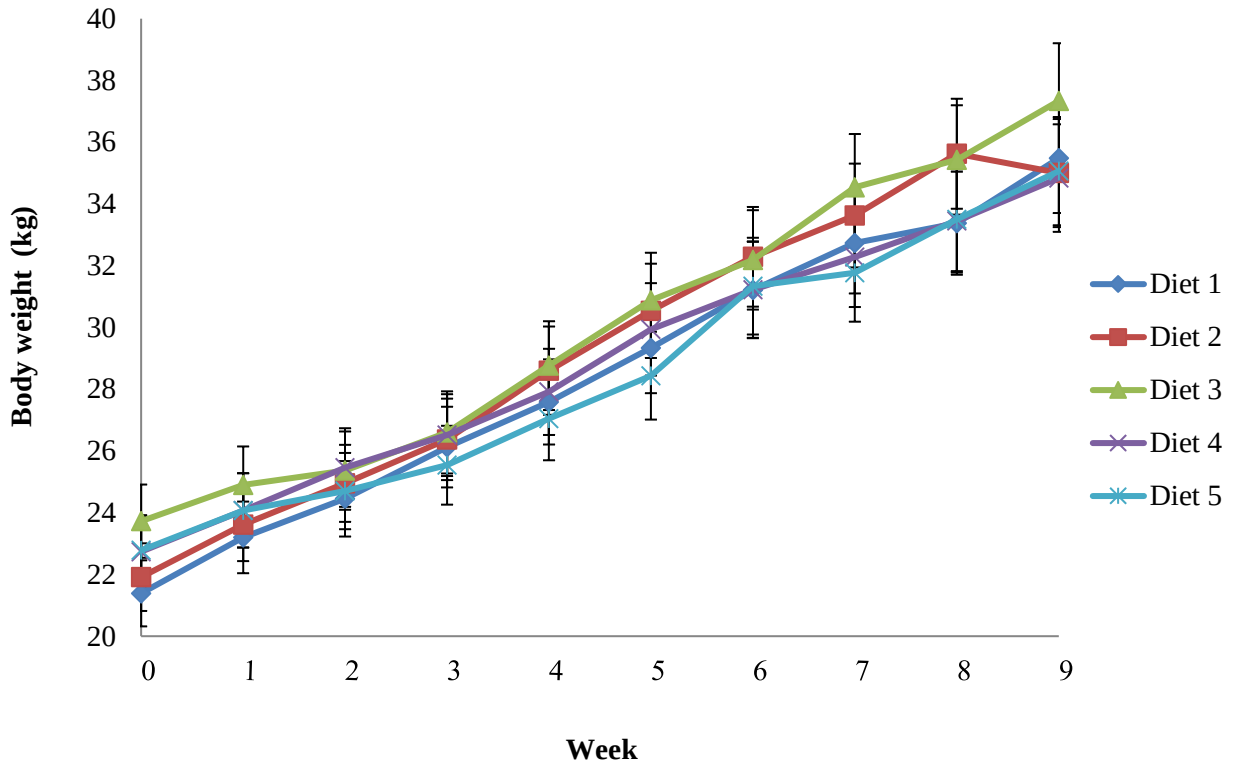

Figure 4.1: Effect of graded dietary substitution of soyabean meal with Marula nut meal on mean weekly body weights of the male Dorper lambs

The weekly mean body weights of the lambs across dietary treatments were similar ($P > 0.05$). Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; n = 8 lambs.

There were no significant differences in the weekly mean body weights of lambs across dietary treatments over the 9-week trial period. However, the lambs significantly grew with a body weight gain percentage range of 53.22 to 65.78% over the 9-week trial period.

4.5.2 Nutrient intake

The effect of graded dietary substitution of soyabean meal with Marula nut meal on nutrient intake by the growing-fattening intact male Dorper lambs is presented in Table 4.2.

Table 4.2: Effect of graded dietary substitution of soyabean meal with Marula nut meal on nutrient intake by the male Dorper lambs

	Diet							
Nutrient intake	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**LSD**	**SE**	***P***
Organic matter (g/day)	811.00[a]	845.00[a]	841.00[a]	781.00[a]	788.00[a]	69.30	67.70	0.231
Crude protein (g/day)	136.80[a]	143.10[a]	141.60[a]	134.30[a]	138.30[a]	11.76	11.48	0.553
Ether extract (g/day)	110.70[a]	116.30[a]	119.10[a]	123.40[a]	122.80[a]	9.79	9.56	0.076
Energy (MJ/kg DM/day)								
Gross energy	18.31[a]	19.78[a]	19.25[a]	18.36[a]	18.21[a]	1.59	1.55	0.199
Fibre fraction								
Acid detergent fibre (g/day)	131.30[a]	140.00[a]	136.40[a]	140.90[a]	145.80[a]	11.63	11.36	0.153
Neutral detergent fibre (g/day)	308.10[a]	308.40[a]	312.80[a]	292.60[a]	296.00[a]	25.95	25.34	0.446

[a] Within row means with the same superscripts are not significantly different (P > 0.05). The organic matter, crude protein, ether extract, gross energy, acid detergent fibre and neutral detergent fibre intake by the lambs were similar (P > 0.05) across dietary treatments. Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

The graded dietary substitution of SBM with MNM, on CP basis, had no significant effect ($P > 0.05$) on the nutrient intake by the Dorper lambs.

4.5.3 Growth performance

Table 4.3 shows the effect of graded dietary substitution of soyabean meal with Marula nut meal on the terminal body weight (TBW), body weight gain (BWG), average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR) of growing-fattening intact male Dorper lambs.

Table 4.3: Effect of graded dietary substitution of soyabean meal with Marula nut meal on growth performance of the male Dorper lambs

Parameter	Diet					LSD	SE	*P*
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5			
Induction body weight (kg)	21.39[a]	21.91[a]	23.92[a]	21.91[a]	21.39[a]	2.62	2.56	0.434
Terminal body weight (kg)	35.48[a]	35.00[a]	37.34[a]	34.84[a]	35.06[a]	2.94	2.87	0.407
Body weight gain (kg)	14.09[a]	13.09[a]	13.61[a]	12.10[a]	12.28[a]	2.27	2.22	0.344
Average daily gain (g/day)	223.60[a]	207.70[a]	216.10[a]	192.10[a]	194.90[a]	36.06	35.20	0.344
Feed intake (g/day)	931.00[a]	983.00[a]	973.00[a]	896.00[a]	911.00[a]	79.80	77.00	0.138
Feed conversion ratio (g/g)	4.23[a]	4.89[a]	4.67[a]	4.71[a]	4.65[a]	0.98	0.95	0.722

[a] Within row means with the same superscripts are not significantly different (P > 0.05). The terminal body weight, body weight gain, average daily gain, feed intake and feed conversion ratio of the lambs were similar (P > 0.05) across the dietary treatments. Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

The induction, terminal body weight, body weight gain, average daily gain, feed intake and feed conversion ratio of the growing-fattening intact male Dorper lambs that were fed the control diet (Diet 1) were similar to that of their counterparts fed test diets, that is, Diet 2 to Diet 5.

4.5.4 Linear growth

The effect of graded dietary substitution of SBM with MNM on linear growth (femur and tibia weight, length) and long bones Seedor ratio of growing-fattening intact male Dorper lambs is shown in Table 4.4.

Table 4.4: Effect of graded dietary substitution of soyabean meal with Marula nut meal on linear growth of the male Dorper lambs

	Diet							
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**LSD**	**SE**	***P***
Femur								
Dry weight (g)	179.88[a]	185.11[a]	183.23[a]	195.69[a]	181.06[a]	18.46	18.03	0.434
Length (cm)	17.09[a]	16.52[a]	16.66[a]	17.31[a]	16.86[a]	0.87	0.85	0.364
Seedor ratio (g/cm)	10.56[a]	11.20[a]	11.02[a]	11.33[a]	10.76[a]	1.27	1.24	0.729
Tibia								
Dry weight (g)	172.10[a]	176.63[a]	163.25[a]	162.71[a]	150.45[a]	22.40	21.87	0.179
Length (cm)	14.69[a]	14.66[b]	14.61[a]	15.16[a]	14.78[a]	0.58	0.57	0.326
Seedor ratio (g/cm)	11.72[a]	12.06[a]	11.19[a]	10.76[a]	10.76[a]	1.58	1.55	0.142

[a] Within row means with the same superscripts are not significantly different (P > 0.05). The weight, length and Seedor ratio of femur and tibia of lambs were similar (P > 0.05) in all dietary treatments. Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

Dietary MNM had no effect on linear growth (femur and tibia weight and length) and bone density (Seedor ratio) of the lambs.

4.5.5 Femora mineral content

Table 4.5 shows the effect of graded dietary substitution of SBM with MNM on the femora mineral composition of growing-fattening intact male Dorper lambs.

Table 4.5: Effect of graded dietary substitution of soyabean meal with Marula nut meal on mineral content of the femur of the male Dorper lambs

	Diet							
Mineral (%)	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**LSD**	**SE**	***P***
Calcium	37.74[a]	37.64[a]	38.49[a]	37.91[a]	38.09[a]	0.73	0.40	0.158
Magnesium	0.96[a]	0.92[a]	0.96[a]	0.96[a]	0.96[a]	0.14	0.08	0.917
Phosphorus	18.48[a]	18.41[a]	19.08[a]	18.89[a]	18.89[a]	0.80	0.44	0.452
Ca: P ratio	2.04[a]	2.04[a]	2.02[a]	2.04[a]	2.02[a]	0.05	0.03	0.535

[a] Within row means with the same superscripts are not significantly different (P > 0.05). The calcium, magnesium and phosphorus content and Ca: P ratio of the femora of lambs across dietary treatments were similar (P > 0.05). Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

The calcium, magnesium and phosphorus content and Ca: P ratio of the femora of lambs fed the control diet were similar to that of their counterparts fed the test diets.

4.5.6 Fasting blood metabolic substrate concentration.

The effect of graded dietary substitution of SBM with MNM on fasting blood glucose, triglycerides and cholesterol concentration of growing-fattening intact male Dorper lambs is shown in Table 4.6.

Table 4.6: Effect of graded dietary substitution of soyabean meal with Marula nut meal on fasting blood metabolic substrate concentration of the male Dorper lambs

	Diet							
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**LSD**	**SE**	***P***
Blood glucose (mmol/L)	4.29[a]	4.26[a]	4.49[a]	4.40[a]	4.38[a]	0.35	0.36	0.716
Blood triglycerides	nd	nd	nd	nd	nd	-	-	-
Cholesterol (mg/dL)	55.00[a]	62.56[a]	63.58[a]	59.39[a]	66.00[a]	13.36	13.16	0.510

[a] Within row means with the same superscripts are not significantly different (P > 0.05). Fasting blood glucose and cholesterol concentration of lambs were similar (P > 0.05) across dietary treatments. nd = not detectable (below the detection level). Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

The graded dietary substitution of SBM with MNM had no significant effect ($P > 0.05$) on the fasting blood glucose and cholesterol concentration of the lambs. The triglyceride concentration was below the detection level of the Accutrend GCT meter (minimum detection of 70 mg/dL).

4.5.7 Hepatic metabolic substrate content

The effect of graded dietary substitution of SBM with MNM on hepatic lipid and glycogen content of growing-fattening intact male Dorper lambs is shown in Table 4.7.

Table 4.7: Effect of graded dietary substitution of soyabean meal with Marula nut meal on hepatic metabolic substrate content of the male Dorper lambs

	Diet							
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**LSD**	**SE**	***P***
Liver lipid (%)	4.20[b]	4.25[b]	4.31[b]	4.72[a]	4.68[a]	0.31	0.17	0.008
Liver glycogen (mmol/L)	4.30[a]	3.73[a]	4.57[a]	4.57[a]	4.40[a]	0.76	0.42	0.172

[a, b] Within row means with different superscripts differ significantly at ($P \leq 0.05$). Liver lipid content of lambs fed diet 4 and 5 was significantly higher (P = 0.008) than that of lambs fed diets 1, 2 and 3. Liver glycogen content of lambs was similar (P > 0.05) across dietary treatments. Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

Dietary substitution of SBM with MNM significantly increased (P = 0.008) the liver lipid content of lambs fed diets 4 and 5 compared to the liver lipid content of their counterparts fed diets 1, 2 and 3. Dietary MNM had no significant effect (P = 0.172) on the liver glycogen content of the lambs across dietary treatments.

4.5.8 Packed cell volume

The packed cell volume of the growing-fattening male lambs on different dietary treatments is shown in Figure 4.2.

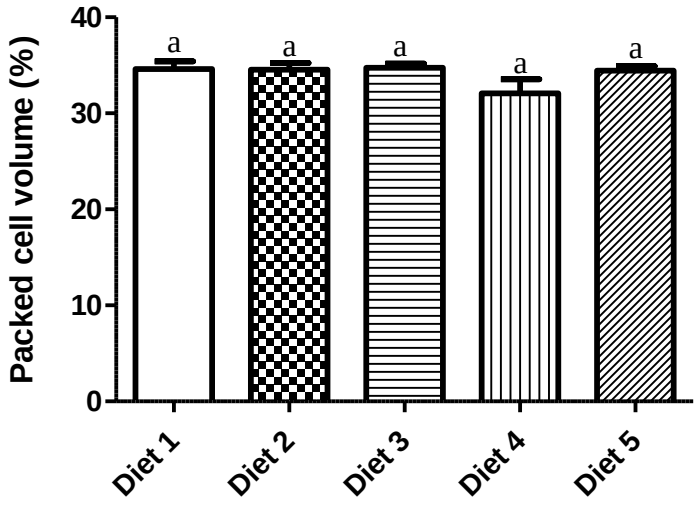

Figure 4.2: Effect of graded dietary substitution of soyabean meal with Marula nut meal on packed cell volume of the male Dorper lambs

[a] Bars with the same superscripts are not significantly different (P > 0.05). The packed cell volume of the lambs across dietary treatments was similar (P > 0.05). Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; n = 8 lambs.

The packed cell volume of the lambs fed the control diet (diet 1) was similar (P > 0.05) to that of their counterparts fed the test diets (diets 2 to 5).

4.5.9 Rumen digesta pH and rumen liquor volatile fatty acid content

The effect of graded dietary substitution of SBM with MNM on rumen digesta pH and the volatile fatty acid content of the rumen liquor from the lambs across dietary treatments is shown in Table 4.8.

Table 4.8: Effect of graded dietary substitution of soyabean meal with Marula nut meal on rumen digesta pH and rumen liquor volatile fatty acid content of the male Dorper lambs

	Diet							
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**LSD**	**SE**	***P***
Rumen digesta pH	6.87[a]	6.97[a]	6.80[a]	6.89[a]	6.90[a]	0.22	0.22	0.641
Volatile fatty acid (g/L)								
Acetic acid	2.79[c]	2.28[d]	4.67[a]	2.69[c]	3.11[b]	0.20	0.11	0.001
Butyric acid	0.27[bc]	0.26[bc]	0.55[a]	0.24[c]	0.29[b]	0.035	0.02	0.001
Iso-butyric acid	121.79[b]	114.26[c]	144.79[a]	94.63[e]	103.68[d]	4.82	2.65	0.001
Propionic acid	1.39[c]	1.17[d]	2.33[a]	1.32[c]	1.52[b]	0.09	0.05	0.001
Valeric acid	94.77[b]	73.05[d]	102.83[a]	61.30[e]	76.26[c]	3.19	1.75	0.001
Iso-valeric	458.30[b]	425.62[c]	526.92[a]	345.62[e]	393.42[d]	18.67	10.26	0.001

[a, b, c, d, e] Within row means with different superscripts differ significantly at $P < 0.05$. Rumen digesta pH of the lambs was similar ($P > 0.05$) across dietary treatments. Rumen liquor volatile fatty acid content differed significantly ($P < 0.05$) across dietary treatments. Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

While the pH of the rumen digesta (with a range of 6.87 to 6.97) from the lambs was similar ($P > 0.05$) across the dietary treatments, the volatile fatty acid (VFA) content of the rumen liquor was significantly different across the dietary treatments. The rumen liquor from the lambs fed diet 3 had a significantly higher ($P > 0.05$) VFA content compared to the VFA content from the rumen liquor of lambs fed diets 1, 2, 4 and 5. The rumen liquor from lambs fed diet 2 had the lowest ($P = 0.001$) content of acetic and propionic acid while that from lambs fed diet 4 had the lowest iso-

butyric, iso-valeric and valeric acid content. The butyric acid content was lowest (P = 0.001) in the rumen liquor from lambs fed diets 1, 2 and 5 compared to its content in the rumen liquor from lambs fed diet 3.

4.5.10 Viscera macro-morphometry

The effect of graded dietary substitution of SBM with MNM, on a CP basis, on the macro-morphometry of the GIT and other viscera of the growing-fattening intact male Dorper lambs is shown in Table 4.9.

Table 4.9: Effect of graded dietary substitution of soyabean meal with Marula nut meal on viscera macro-morphometry of the male Dorper lambs

	Diet							
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**LSD**	**SE**	***P***
Rumen weight (kg)	0.56[a]	0.63[a]	0.59[a]	0.57[a]	0.56[a]	0.08	0.08	0.402
Rumen papillae score	0.50[a]	1.17[a]	0.00[a]	0.83[a]	1.17[a]	0.92	0.88	0.074
Reticulum weight (kg)	0.09[a]	0.11[a]	0.09[a]	0.09[a]	0.09[a]	0.02	0.02	0.408
Omasum weight (kg)	0.09[a]	0.09[a]	0.10[a]	0.09[a]	0.10[a]	0.03	0.03	0.974
Abomasum weight (kg)	0.18[a]	0.16[a]	0.18[a]	0.17[a]	0.19[a]	0.03	0.03	0.545
Small intestine weight (kg)	0.85[a]	0.92[a]	0.87[a]	0.83[a]	0.89[a]	0.10	0.10	0.519
Small intestine length (m)	28.17[a]	27.37[a]	27.57[a]	27.56[a]	27.33[a]	2.81	2.74	0.974
Large intestine weight (kg)	0.38[a]	0.40[a]	0.37[a]	0.40[a]	0.40[a]	0.06	0.06	0.785
Large intestine length (m)	6.53[a]	6.25[a]	6.53[a]	6.52[a]	6.31[a]	0.53	0.52	0.691
Caecum weight (kg)	0.05[a]	0.05[a]	0.05[a]	0.05[a]	0.04[a]	0.10	0.97	0.417
Liver weight (kg)	0.55[a]	0.60[a]	0.58[a]	0.53[a]	0.55[a]	71.80	70.00	0.302
Kidney (pair) weight (kg)	0.11[a]	0.11[a]	0.11[a]	0.10[a]	0.10[a]	8.98	8.76	0.154
Visceral fat weight (kg)	0.20[a]	0.22[a]	0.23[a]	0.22[a]	0.22[a]	92.90	90.50	0.980

[a] Within row means with the same superscripts are not significantly different (P > 0.05). There were no significant differences (P > 0.05) in the macro-morphometry of the viscera and the rumen papillae score of lambs across dietary treatments. Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

The weight of the viscera and length, where relevant (rumen, reticulum, omasum, abomasum, small and large intestines, caecum, liver, kidney and visceral fat), as well as the rumen papillae score of the growing-fattening lambs, were similar ($P > 0.05$) across dietary treatments.

4.5.11 Surrogate markers of liver and kidney function and general health

The effect of graded dietary substitution of SBM with MNM, on CP basis, on serum surrogate markers of liver and kidney function and other markers of general health from the growing-fattening lambs is shown in Table 4.10.

Table 4.10: Effect of graded dietary substitution of soyabean meal with Marula nut meal on serum surrogate markers of liver and kidney function and other markers of general health from the male Dorper lambs

	Diet							
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**LSD**	**SE**	***P***
Total protein (g/dL)	6.85[a]	6.85[a]	6.73[a]	6.70[a]	6.71[a]	0.38	0.37	0.853
Albumin (g/dL)	2.85[a]	3.03[a]	3.05[a]	2.88[a]	2.84[a]	0.26	0.26	0.302
Globulin (g/dL)	4.00[a]	3.84[a]	6.19[a]	3.83[a]	3.86[a]	3.26	3.21	0.517
Total bilirubin (mg/dL)	0.45[a]	0.33[a]	0.50[a]	0.48[a]	0.34[a]	0.20	0.20	0.309
Alanine amino transferase U/L)	38.00[a]	30.59[a]	35.72[a]	33.76[a]	29.40[a]	12.79	12.58	0.639
Alkaline phosphatase (U/L)	161.00[a]	165.02[a]	176.00[a]	182.00[a]	194.00[a]	68.00	67.00	0.867
Creatinine (mg/dL)	1.01[a]	0.93[a]	0.93[a]	0.88[a]	0.95[a]	0.11	0.11	0.193
Blood urea nitrogen (mg/dL)	18.25[a]	18.12[a]	15.75[a]	13.62[a]	14.62[a]	4.18	4.24	0.122
Inorganic phosphate (mg/dL)	10.66[a]	11.68[a]	10.95[a]	11.32[a]	11.79[a]	1.79	1.77	0.685
Calcium (mg/dL)	9.93[a]	9.38[a]	10.03[a]	9.45[a]	9.45[a]	0.92	0.91	0.473
Amylase (U/L)	22.68[a]	27.78[a]	42.23[a]	33.00[a]	34.12[a]	23.39	23.04	0.537

[a] Within row means with the same superscripts are not significantly different (P > 0.05). There were no significant differences (P > 0.05) on the serum surrogate markers of liver and kidney function and other markers of general health from lambs across the diets. Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

Dietary substitution of SBM with MNM had no significant effect on the serum surrogate markers of liver function [(total protein, albumin, globulin, total bilirubin concentration) and alanine transaminase (ALT) and alkaline phosphatase (ALP) activities] and kidney function [blood urea nitrogen (BUN) and creatinine concentration] as well as other markers of general health (inorganic phosphate, calcium and cholesterol concentration and amylase activity) of growing-fattening lambs across the diets.

4.6 Discussion

The discussion for this chapter is partitioned into sections that focus on the effects of dietary substitution of SBM with MNM, on a CP basis, on the growth performance (feed intake, feed utilisation efficiency, body weight and long bone indices), viscera macro-morphometry, circulating and stored metabolic substrates, digesta and rumen liquor traits as well as on the health profile of the lambs.

4.6.1 Effect of dietary Marula nut meal on weekly growth profile

My results show similarities in the mean weekly body weights of the lambs across dietary treatments. These findings indicate that the lambs across the treatment diets had similar weekly growth rates suggesting that the substitution of SBM with MNM did not negatively impact on the lambs' weekly growth over the experimental period. The growth profile of the lambs depicted a consistent increase in body weight weekly over the 9-week feeding trial. The lambs used in this study were inducted to the feeding trial at 112-days old (average of 4 months). The study was terminated when the lambs were 175 days old (6.25 months old). The lambs' age at study termination fell within the pubertal age of 5 to 6.5 months for rams (Kridli et al., 2006) thus, during the duration of the study the lambs were in the pubertal growth stage which is characterised by rapid and consistent growth (Barbosa et al., 2013) hence the observed consistent weekly growth rate was in tandem with rapid and consistent growth characteristic of pubertal lambs. During this rapid growth phase, the demand for nutrients, in particular protein, for muscle accretion is high (Kumar et al., 2014; Cordova-Izquirdo, 2016). The observed constant increase in weekly body weight over the trial period suggests that as was the case with the control diet, the test diets met the high nutrient demand of the lambs during this rapid growth phase.

4.6.2 Effect of dietary Marula nut meal on feed intake and growth performance

The body weight based growth indices (TBW, BWG and ADG) are used to give an indication of and to compare growth performance between and or among animals under experimental and or practical feeding conditions (Kebede and Gebretsadik, 2010; Mahrose et al., 2015). Findings from the current study show that graded dietary substitution of SBM with MNM, on a CP basis,

had no significant effect on the lambs' TBW, BWG and ADG. This observation suggests that MNM, like SBM, can be utilised as a dietary protein source in lamb grower-fattening diets without compromising growth performance as measured by body weight based measures of growth.

Findings from the current study show similarities in the FI and FCR of the lambs across dietary treatments. The similarities in the FI and FCR suggest that the use of MNM did not affect the palatability of the feed and the feed utilisation efficiency by the lambs. When considered in view of the observed similarities in growth performance, these results further suggest that MNM could be utilised as a dietary protein source in place of SBM in lamb grower-fattening diets without compromising nutrient availability (protein and amino acid supply) to the lambs.

Cloete et al. (2000) reported that generally Dorper lambs weaned at 60 to 90 days gained from 240 to 280g/day on average. Schoeman et al. (1993) indicated that Dorper lambs weaned at 3 months 10 days gained approximately 180.00g/day from 100 to 200 days and 160.00g/day from 200 to 300 days. The lambs in this study were weaned at 90 days and inducted into the study at 112 days of age. They were then fed for 63 days, thus at study termination the lambs were 175 days old with live weights ranging from 34.00 to 37.00kg. In the current study, the lambs' ADG ranged between 192.10 to 223.60g/day, which was in between the ADG observed by Schoeman et al. (1993). The difference in ADG could be attributed to the fact that lambs in this study were grown under intensive feeding as opposed to veld rearing in the case of the study by Schoeman et al. (1993). Thus the observed higher ADG by the lambs in the current study could be ascribed to a higher plane of nutrition. Post weaning, lambs in this study had an overall average daily gain of 206.90 g/day. This suggests that the ADG was lower than the 240 to 280g/day reported by Cloete et al. (2000) for lambs of a similar age. Confinement, especially in metabolism crates, is known to cause stress (Bova et al., 2014). In the experiment by Cloete et al. (2000), the lambs were housed in feeding pens, which gave allowance for more space to move around, decreasing confinement stress. However, in the current study, each lamb was housed in a metabolism crate with limited space that is associated with increased stress (Chulayo and Muchenje, 2015). It is therefore reasonable to argue that the lower mean ADG of the lambs in the current study compared to that reported by Cloete et al. (2000) could be due to the confinement-induced stress as each lamb was housed in a confined space in the metabolic crate during the entire feeding trial

period. Animals react to confined environments with increased plasma cortisol (Eriksson et al., 2004). Marsden and Wood-Gush (1986) and Kapp et al. (1997) observed an increased plasma cortisol concentration in 4-months old lambs housed in metabolic crates compared to that in lambs that were free-ranging.

The National Research Council (NRC, 2007) points to the fact that the FCR for growing-fattening Dorper lambs ranges from 4.00 to 5.00g/g. In the current study, the FCR of the lambs across the dietary treatments ranged from 4.23 to 4.89g/g. These results are within the NRC recommended values. This suggests that MNM could potentially replace SBM as a dietary protein source in lamb grower-fattening diets without compromising feed utilisation efficiency. Feed intake, digestion and absorption are critical in the provision of nutrients that are necessary for supporting animal growth performance. Feed ingredient composition affects rumen fermentation hence the supply of energy (VFA) and protein in the form of microbial crude protein (MCP) to the host. Importantly, ingredient composition affects rumen residence time that in turn has regulatory effects on feed intake. Findings in this study show similarities in FI by lambs across the dietary treatments, which (similarities in FI) suggest that the use of MNM in place of SBM had no negative effect on rumen fermentation and digesta residence time in the rumen. Importantly, when tied to the observed similarities in weight based indices of growth performance, one can speculate that the MNM matched SBM in terms of VFA and MCP generation, hence nutrient supply to the lambs.

4.6.3 Effect of dietary Marula nut meal on linear growth

Unlike body weight based indices of growth performance that are influenced by the hydration status and gut fill of the animal (Pearson and Ouassat, 2000), long bone growth is a better measure of animal growth performance since the growth of long bones response to the stimulatory effects growth hormone in a dose-dependent manner (Cheek and Hill, 1974 as cited by De Lama et al., 2000). Long bone growth can be measured by determining the length, weight and Seedor ratio (Cruz et al., 2012) of the bone. The Seedor ratio is a measure of the bone density (Seedor et al., 1991. Fernandes et al. (2013) argued that the greater the value of Seedor ratio the greater the bone mineral density and thus, the denser the bone and greater the growth of bone. The results of the current study show similarities in tibia and femora weights, lengths and Seedor

ratio of the lambs was across the dietary treatments. The similarities in the long bone indices suggest that the use of MNM in place of SBM in lamb fattening diets did not compromise linear growth performance of the lambs.

In mammals bone mineral composition is affected by a complex set of mechanisms (Sarazin et al. 2000) regulated by endogenous and exogenous factors (Bruno, 2002). Among the critical endogenous regulatory factors are the hormones: growth hormone, IGF-I and II and dihydroxi-vitamin D3 (Watkins, 1999). Of the many exogenous (environmental) regulatory factors, nutrition is one of the major factors affecting bone size and mineral composition (Olguin et al., 2013). The mineral composition of the diet influences the calcium, phosphorus and magnesium composition of the bones (Palacios, 2006). Dietary deficiency of an individual mineral negatively influences the efficiency with which other minerals are utilised in the body (Teixeira et al., 2013). According NRC (1985) bone development and maintenance are dependent on the interrelationship between calcium and phosphorus metabolism. A deficiency or excess supply of one or both of the minerals interferes with homeostasis of the second mineral (Vitti and Kebreab, 2010), which results in reduced bone mineralisation (Hurwitzet al., 1995). A high dietary Ca: P ratio has been observed to result in increased bone turnover (Liesegang et al., 2013). A high bone turnover leads to impairment of the mineralisation phase of bone remodelling. According to Özcan et al. (2001), normal Ca: P ratio for long bones in male fattening lambs is 2:1. My findings show that the long bones Ca: P ratio of the male Dorper lambs was similar across dietary treatments. Importantly the ratio ranged from 2.02:1 to 2.04:1. These results suggest that the use of MNM in place of SBM on crude protein basis in lamb fattening diets did not negatively impact the Ca: P ratio of the long bones, which would have otherwise affected the bone turnover and thus, impaired the bone mineralisation. More so, results in my study show similarities in femora calcium, magnesium and phosphorus (major minerals found in bones) composition from the lambs across dietary treatments (Table 4.4). It therefore could be inferred that the use of MNM as a dietary protein source in lamb fattening diets did not negatively affect calcium, magnesium and phosphorus composition of the bones.

4.6.4 Effect of dietary Marula nut meal on circulating and stored metabolic substrates

Dietary fat, especially fat rich in lauric, myristic, and palmitic acid (fatty acids) is known to impact the *de novo* synthesis of cholesterol (Mensink et al., 2003). Metabolically, LDL-cholesterol is associated with atherosclerosis and coronary heart diseases (Carmena et al., 2004), while high-density lipoprotein cholesterol is associated with positive health outcomes (Kontush and Chapman, 2006). In the current study (Chapter 3, Table 3.4), the MNM-based diets, unlike the SBM-based diets had a significantly lower preponderance of lauric, myristic and palmitic acid, which could lead to increased *de novo* synthesis of cholesterol. In healthy growing lambs the normal serum cholesterol concentration ranges from 64.00 to 104.00mg/dL (Sitmo, 2014). In the current study the serum cholesterol concentration of the lambs ranged from 55.00 to 66.00mg/dL and was similar across dietary treatments. These findings only compare to the lower range of the cholesterol concentration in growing lambs as reported by Sitmo (2014). While a host of factors might have contributed to the lack of similarity in my findings and those of Sitmo (2014), what is striking is the similarities in the cholesterol concentration of lambs fed the control diet and those fed the MNM-based diets. These similarities suggest that the use of MNM in place of SBM did not alter the metabolism of cholesterol by the lambs and importantly its use (MNM) did not compromise the health of the lambs. However this assertion would carry more weight had I, in my study, partitioned the total cholesterol into low and high-density lipoprotein cholesterol.

During fasting, animals mobilise stored nutrients and convert them into energy to maintain homeostasis (Zhang et al., 2013) and the liver is known to play a key role in maintaining the blood glucose homeostasis by upregulating glycogenolysis. Upon depletion of glycogen, triglycerides, which are stored in adipose tissue are released into the circulation in the form of glycerol and free fatty acids (Izumida et al., 2013). Through gluconeogenesis hepatocytes convert the released glycerol to glucose while the free fatty acids are beta oxidised to produce energy (Cahill Jr, 2006). Results from the current study show similarities in the fasting blood glucose concentration and liver glycogen content in lambs across dietary treatments. The similarities in the fasting blood glucose concentration and liver glycogen content suggest that the graded substitution of SBM with MNM did not compromise the maintenance of blood glucose homeostasis or neither did it impact on glycogen metabolism by the liver.

The significantly higher lipid content (fasting) in the livers from the lambs fed diets 4 and 5 compared to the liver lipid content of their counterparts fed diets 1 through to diet 3; suggests that dietary substitution of SBM with MNM at 75% and 100% promoted hepatic lipid accretion more so when one considers the fact that diets 4 and 5 had a relatively high EE content compared to the rest (Table 4.1). Although the liver lipid content of the lambs fed diets 4 and 5 was higher compared to the rest, it is interesting to note that Radostists et al. (2007) assert that the normal liver lipid content of healthy sheep should be less than 10%. Findings from the current study show a liver lipid content that ranged from 4.20 to 4.72% (Table 4.7); values that are less than 10%. This observation suggests that despite the significantly higher liver lipid content in lambs fed diets 4 and 5, the substitution of SBM with MNM may not have elicited pathology of their livers since the lipid content was within the range expected in healthy sheep.

4.6.5 Effect of dietary Marula nut meal on visceral macro-morphometry

Dietary energy is essential for the development and growth performance of gastrointestinal tract (GIT) and viscera of animals at various stages of their growth periods (Zhang et al., 2017). Research show that the gastrointestinal tract of animals feeding on a high forage diet develops faster compared to that of animals feeding on a high concentrate diet owing to the higher intake of dry matter (Priolo et al., 2002; Cañeque et al., 2003). Feeding a high forage diet to animals also increases the viscera weight and mucosal architecture (villi height and crypt depth) of the intestines (Ngoc et al., 2012). In addition to the diet-induced viscera and mucosa variations, the presence of ANFs (known to interfere with feed digestion and nutrient absorption) in diets can reduce the availability of nutrients to the animal (Makkar et al., 2012), thus negatively affecting the GIT and other viscera morphometry. In the current study the macro-morphometry of the GIT viscera and other viscera of the lambs were similar across dietary treatments. The observed similarities in viscera macro-morphometry suggest that dietary MNM had no negative impact on the growth and development of the viscera.

4.6.6 Effect of dietary Marula nut meal on the rumen digesta pH, volatile fatty acid content and rumen papillae

Rumen digesta pH is an important variable in the assessment of fermentation in the rumen (Liu et al., 2012). The growth of fibre digesting bacteria is favoured when the rumen digesta content pH is between 6.00 and 6.80 (Karimizadeh et al., 2017). The proliferation of starch digesting bacteria growth is favoured when rumen digesta content pH ranges from 5.50 to 6.00 (Karimizadeh et al., 2017). In finishing lambs fed a high concentrate diet, a drop in rumen digesta content pH to below 5.60 to 5.80 is regarded as a threshold for rumen acidosis (Karimizadeh et al., 2017). In the current study, the rumen digesta content pH of the lambs ranged from 6.80 to 7.00 and was similar across dietary treatments. The similarities in the rumen digesta content pH suggest that the MNM-based diets did not affect the rumen fermentation negatively. Importantly, the rumen digesta content pH of lambs in this study was above the range (5.60 to 5.80) reported to cause diet-induced acidosis in lambs by Karimizadeh et al. (2017).

The VFA content in the ruminal fluid is affected by the feed composition, dry matter intake and digestibility of the feed (Moreira et al., 2014; Oliveira et al., 2015). In ruminants, feeding high concentrate diets results in an increase in substrates for microbial activity which translates to a high concentration of short chain fatty acids particularly propionic and butyric acid (Pedreira et al., 2013). However, the feeding of a high forage diet increases acetic acid concentration in the rumen (Beauchemin and McGinn, 2005). A high protein diet has been associated with an increase in branched-chain fatty acids (valeric and iso-butyric) concentration (Vargas et al., 2002). The propionic and butyric acids concentration promote the growth of rumen papillae, which (growth of rumen papillae) enhances nutrient absorption efficiency from the rumen (Hernández et al., 2014). In the current study, the acetic, propionic and butyric acid as well as the branched-chain fatty acid concentration were highest in rumen liquor of lambs fed diet 3 (Table 4.8). These observations suggest that replacement of 50% of SBM's dietary CP contribution with 50% dietary CP contribution from the MNM promoted rumen fermentation which resulted overall, in greater VFA production.

While various studies have reported that dietary lipid content higher than 5% and 6% in ruminant diets negatively affect feed intake (Weiss and Pinos-Rodriguez, 2009; Hess et al., 2008); Ueda et

al. (2003) and Harvatine and Allen (2006) reported that dietary lipid content higher than 3% can negatively affect ruminal fermentation efficiency. However, the assertion by Ueda et al. (2003) and Harvatine and Allen (2006) is at variance with that of Hristov et al. (2013) who reported that only at above 6 to 7% does dietary lipid content negatively impact rumen fermentation. In the current study, I formulated the diets to reduce the amount of the residual oil in MNM to be similar to that of SBM as indicated by similar ether extract content in Table 4.1. Importantly, the results in the current study show that feed intake and or concentration of volatile fatty acid did not decrease with an increasing inclusion level of MNM. The results suggest that MNM as a dietary protein source in lamb fattening diets did not affect the ruminal fermentation negatively thus feed intake.

Dietary lipids with different fatty acid composition can be used in ruminant diets (Duckett and Gillis, 2010), however, the degree of fatty acid unsaturation above the saturation capacity of rumen microbes can yield adverse effects on ruminal fermentation (Messana et al., 2012), thus growth performance. Compared to dietary lipids high in saturated fatty acids, those with a higher concentration of polyunsaturated fatty acids reduce feed intake (Ferlay et al., 1993), inhibit growth of ruminal microorganisms and reduce concentration of volatile fatty acids (Yang et al., 2009). Findings in this study show that while the MNMs had similar total saturated fatty acid concentration with SBM, the concentration of total polyunsaturated fatty acid was lower compared to that in SBM (Table 3.4). Importantly, the high concentration of volatile fatty acids in rumen liquor of lambs fed diet 3 suggest that replacement of SBM with 50% of MNM on CP bases in lamb fattening diets favoured the rumen microbial growth, but did not translate to improved growth performance.

The high propionic and butyric acid concentration in rumen liquor of lambs fed diet 3 did not induce any variations in the development of the rumen papillae. The rumen papillae score of the lambs in this study were similar across dietary treatments. Importantly the rumen papillae score ranged from 0 to 1, which indicate that the rumen had a mixture of long and short papillae, which were also well developed and tightly packed without any visible signs of inflammation or lesions (Leeuw et al., 2009). The results suggest that dietary substitution of SBM with MNM did not

elicit any negative impact on rumen health hence its capacity to absorb VFAs and supply them (VFAs) to the animal as an energy source for growth.

4.6.7 Effect of dietary Marula nut meal on health profile

4.6.7.1 Effect on packed cell volume

Among the many factors that influence PCV (for example, breed, age, sex, and disease), nutrition (diet and ANFs) is one of the critical factors (Etim et al., 2014). In healthy growing lambs the normal PCV ranges from 25 to 36% (Pampori, 2003). Higher than normal reference PCV values could be as a result of diarrhoea, congenital heart disease, polycythemia and or hypoxia (Thrall, 2004; Ndlovu et al., 2007). Lower than normal reference PCV values could be as a result of anaemia, haemorrhage or haemolysis, erythropoietin deficiency, copper and iron deficiency and or vitamins B_6 and B_{12} deficiency (Gernsten, 2009; Bunn, 2011). In the current study, the PCV of the lambs across dietary treatments ranged from 28.32 to 30.50% hence were within the normal PCV range of sheep as reported by Pampori (2003). This finding could be inferred to indicate that MNM can be used as a dietary protein source in lamb grower-fattening diets in place of SBM without triggering some physiological disorder that would negatively impact PCV.

4.6.7.2 Effect on liver function

Serum biochemistry is an important indicator of health and plays a major role in the diagnosis, treatment or prognosis of the effects of disease, toxins and ANFs in animals (Eze et al., 2010). A combination of serum surrogate biochemical markers of liver and kidney function are used to measure metabolites that directly reflect the health status of the liver and kidneys. Total serum protein concentration speaks to the synthetic capacity of the liver (Kubkomawa et al., 2015). In healthy growing lambs the normal serum total protein concentration ranges from 6 to 7. 9g/dL (Antunovic et al., 2011), while serum albumin concentration ranges from 2.4 to 3.0g/dL (Radostits et al., 2007) and serum globulin ranges from 1.16 to 6.19g/dL (Al-Hadithy et al., 2015). While dehydration, chronic infection and paraproteinaemia might result in higher than normal serum total protein concentration (Theodore, 2005), liver disease, poor nutrition and malabsorption, and glomerulonephropathies can cause lower than normal serum total protein

concentration (Rubino et al., 2006). In the current study the serum total protein, albumin and globulin concentration of the lambs were similar across dietary treatments. Importantly, findings from the current study show that the serum total protein, albumin and globulin concentration ranged from 6.70 to 6.85g/dL, 2.84 to 3.05g/dL and 3.84 to 6.91g/dL, respectively. These values are consistent with the reported serum concentration of these proteins (Antunovic et al., 2011; Radostits et al., 2007; Al-Hadithy et al., 2015), suggesting that dietary MNM did not impact on the liver's synthesis of these proteins amongst the various factors that influence serum protein concentrations.

Bilirubin, a catabolic product of hemoglobin, is produced in the reticulo-endothelial system and released in an unconjugated form in the liver (Mauro et al., 2006); where it is then conjugated via catalysis by uridine diphosphate glucuronyltransferase and excreted in bile (Mauro et al., 2006). Its serum concentration (total bilirubin) is used as a surrogate marker of liver function (Shanmugapriya et al., 2015). In healthy lambs the serum concentration of total bilirubin ranges from 0.0 to 0.6mg/dL (Sitmo, 2014). Higher than normal serum concentration of total bilirubin can cause jaundice, this is an indication of liver and bile duct damage (Gupta et al., 2005). My findings show that the serum bilirubin concentration of the lambs across the dietary treatments ranged from 0.33 to 0.50mg/dL and was within the reported normal serum bilirubin concentration of healthy growing lambs (Sitmo, 2014). This suggests that dietary MNM did not cause liver pathology that would have led to the development of jaundice.

Serum activities of ALT, AST and ALP have a bearing on the integrity of hepatocytes (Ambrojo et al., 2013). Injury to the hepatocytes can be detected by increased serum activities of ALT and AST while damage to bile duct cells and cells of the bile canaliculli can manifest with increased serum ALP activity (Kim et al., 2008; Nargis et al., 2014). Damage to hepatocytes results in the leakage of ALT and AST into the blood while damage to cells of the biliary system results in leakage of ALP. In the current study similarities in the serum ALT and ALP activities were observed (Table 4.10). Importantly, results of the current study recorded serum ALT and ALP activities ranges of 29.40 to 38.00 and 161.00 to 194.00 U/L, respectively. These ranges are in agreement with the ALT (30.00 to 47.00U/L) and ALP (17.00 to 1411.00UL) activities reported by Sitmo (2014) in healthy growing lambs. It is important to note that the enzymes (ALT and ALP) are non tissue specific and thus, not solely limited to the liver. The ALP can also be

released from bone, intestines and adipose tissue (Yang et al., 2012). Nevertheless, taking into account the findings with regards to surrogate markers of liver function, results from the current study point out that dietary MNM did not compromise the synthetic capacity, nor did it cause neither hepatocyte nor biliary cell damage in the lambs. It is important to note that due to the residual functional capacity of the liver, usually there needs to be greater than 50% loss of function before these markers are significantly elevated (Bergero and Nery, 2008).

4.6.7.3 Effect on kidney function

Among the many functions of the kidneys is the elimination of waste products of metabolism including those generated in the liver (Weiner et al., 2015). Creatinine, an end product of muscle metabolism is excreted through the kidneys (Issi et al., 2016). Its serum concentration is dependent on the glomerular filtration rate and is used as a surrogate marker of kidney function (Stevens and Levey, 2009). Impaired renal function due to lesions, obstruction of the lower urinary tract and decreased perfusion of the kidney causes increased serum creatinine concentration (Pandya et al., 2016). In healthy lambs, serum creatinine concentration ranges from 0.88 to 1.02mg/dL (Aruwayo et al., 2011). Results from the current study show similarities in the serum creatinine concentration of the lambs across dietary treatments (Table 4.10). Importantly, the observed serum creatinine concentration range of 0.88 to 1.01mg/dL) is in agreement with the serum creatinine concentration of healthy lambs (Aruwayo et al., 2011). This finding suggests that MNM could be used as dietary protein source (in lamb fattening diets) in place of SBM without perturbing the kidney's ability to filter creatinine.

Blood urea nitrogen (BUN), a waste metabolite of protein metabolism (Salazar, 2014), like creatinine, is also used as a surrogate marker of renal function (Lopez-Giacoman and Madero, 2015). Hydration status, diet type and the rate of protein synthesis influence serum BUN concentration (Lopez-Giacoman and Madero, 2015). Among the many possible causes, an increase in serum BUN concentration may be due to kidney disease or failure and or blockage of the urinary tract (Gowda et al., 2010). Low BUN concentration may result from excess body fluid, trauma and or malnutrition (Pagana and Pagana, 2013). In healthy lambs the serum BUN concentration ranges from 14.00 to 21.40 mg/dL (Aliyu et al., 2012). My results show similarities in the serum BUN concentration of the lambs fed the control and the test diets (Table 4.10).

Importantly, the BUN concentration of the lambs from the current study (range: 13.62 to 18.25mg/dL, Table 4.10) fall within the serum BUN range of healthy growing lambs as reported by Aliyu et al. (2012). When considering surrogate markers of kidney function results from the current study suggest that MNM could be used as a dietary protein source in place of SBM in lamb grower-fattening diets without compromising kidney function. As with the liver, the kidney also has a large residual functional capacity, therefore significant damage to the organ has to occur before elevations in the surrogate markers of function occur (Spasovski, 2013).

4.6.7.4 Effect on general health

Serum inorganic phosphate (Pi), calcium (Mellau et al., 2001) and amylase activity (Basnayake and Ratnam, 2015) are some of the markers used in the determination of the general health status of animals. Inorganic phosphate is a co-factor in kinases and phosphatases that catalyse the metabolism of carbohydrates, lipids and nucleic acids thus is vital for energy metabolism (Sapio and Naviglio, 2015). The serum Pi concentration is influenced by its absorption from the GIT, the exchange between the intracellular and bone storage pools, and renal tubular reabsorption (Moe, 2008). Kaneko et al. (2008) reported that in healthy growing lambs' serum Pi ranges from 5 to 73mg/dL. While high serum Pi concentration result from a multiplicity of diseases, for example, Paget's bone disease, Addison's disease, and leukemia (Calvo and Lamberg-Allardt, 2015); low serum Pi concentration can result from sepsis, malabsorption syndromes, hyperinsulinism and hyperparathyroidism (Singh et al., 2008; González-Parra et al., 2012). In this study, the serum Pi concentration from the lambs was similar across dietary treatments. This finding suggests that the substitution of SBM with MNM did not negatively impact on the availability of the mineral to the lambs. Importantly, the serum Pi concentration of lambs from the current study (range: 10.66 to 11.79mg/dL) fall within the serum Pi range of healthy growing lambs as reported by Kaneko et al. (2008). This suggests that the use of MNM in place of SBM did not alter the mineral's availability from the GIT and that it did not perturb its normal physiological regulation.

Calcium is an important mineral, which, in animals, exists in three forms: ionised, albumin-bound and anion-complexed (Burnett et al., 2000). The physiologically active ionised calcium is necessary for bone homeostasis (Fujita, 2006). It also mediates vascular contraction and vasodilation, nerve action potential transmission, secretion of ligands and muscle contraction

(Ross et al., 2011). Hypothyroidism and vitamin D deficiency (Jung et al., 2015), chronic renal failure (Singh et al., 2008), magnesium deficiency (Rude et al., 2009) and pancreatitis (Ahmed et al., 2016) are among the major causes of decreased serum calcium concentration. Elevated serum calcium concentration results from subacute ruminal acidosis (Brown et al., 2000). In healthy growing lambs serum calcium concentration was reported to range from 9.3 to 12.8mg/dL (Merck and Dohme, 2011). In the current study the concentration of serum calcium in lambs was similar across dietary treatments. Importantly, the serum calcium concentration of the lambs from the current study (range: 9.38 to 10.03mg/dL), Table 4.10) is within the calcium range of healthy growing lambs as reported by Merck and Dohme (2011). The results suggest that MNM could be used to substitute SBM, on crude protein basis, in fattening diets of growing lambs without the risk of triggering pathophysiology that could lead to altered serum calcium concentration in growing-fattening lambs.

The synthesis, storage and secretion of digestive enzymes by the exocrine pancreas are key to the digestion of the feed and subsequent absorption of nutrients in the small intestines (Li and Somerset, 2014). Alpha-amylase from the salivary gland (Sampaio et al., 2011) and or exocrine pancreatic gland (Zhang et al., 2016) is important in the digestion of carbohydrates hence energy supply to the animal. Among other factors salivary and exocrine pancreatic gland disorders (Meher et al., 2015; Köster et al., 2015) are the major causes of elevated serum amylase activity (Matull et al., 2006). The lack of differences in the serum alpha-amylase activity of lambs across dietary treatments suggests that MNM could substitute SBM as a dietary protein source in lamb fattening diets without eliciting exocrine pancreatic and salivary gland disorders.

4.7 Conclusion

Several key conclusions can be drawn from this *in vivo* study that evaluated the potential of MNM to substitute SBM as a dietary protein source in lambs fattening diets. The MNM-based diets, met the nutritional requirements of the lambs, were utilised efficiently and well tolerated by the lambs as demonstrated by the similarities in the growth performance, nutrient (dry matter) intake, feed utilisation efficiency of lambs fed the control, and those fed the test MNM-based diets. The substitution of 50% of the SBM's CP contribution to the diet with that from MNM

(50% SBM and 50% MNM) produced rumen liquor high in VFA content which is indicative of a well-balanced, highly fermentable diet with adequate nutrients (fibre and starch). The MNM-based diets did not cause acidosis in lambs. Importantly, the lambs remained healthy throughout the study as demonstrated by similarities in the serum surrogate markers of liver and kidney function and those (markers) of general health. At 75 and 100% substitution of the SBM CP with MNM CP, the lambs' livers accreted statistically significantly more liver lipid content but which nonetheless was less than 5% in all groups and regarded clinically as not being detrimental to the lambs' health. Having established that MNM has a potential to substitute SBM, on CP basis, in lamb grower-fattening diets without compromising their growth performance, feed utilisation efficiency, viscera macro-morphometry and the health of growing-fattening lambs, the next chapter (Chapter 5) interrogates the effect of utilising MNM (in place of SBM) on the product (meat) quality. Diet composition especially its lipid content and fatty acid profile are known to impact meat (animal product) quality. The residual lipid and fatty acid profile of the MNM (Chapter 3, Table 3.4) could potentially impact the meat quality parameters and its chemical (proximate, mineral and fatty acid profile) composition hence Chapter 5 interrogates these potential effects with the full knowledge that food (product) quality impacts consumer health.

CHAPTER FIVE - EFFECT OF DIETARY MARULA NUT MEAL ON CARCASS CHARACTERISTICS AND LAMB QUALITY

5 INTRODUCTION

In the previous chapter, I interrogated the potential of MNM to substitute SBM as a dietary protein source in lamb fattening diets, specifically focusing on its effects on growth performance and feed utilisation efficiency, metabolic substrate (circulating and stored) concentration, viscera macro-morphometry and some surrogate markers of health in growing-fattening lambs. The results from the previous chapter revealed that the MNM (non-conventional dietary protein source) could indeed substitute SBM in lamb fattening diets with compromising growth performance, feed utilisation and lamb health.

Of the majority of factors that influence the quality of animal products, meat included, feed and diet composition are some of the most critical factors (Joo et al., 2013; Santos et al., 2013) that impact meat composition (Pighin et al., 2016). Dietary lipid profile affects the profile of lipid in meat, thus impact on the quality of the meat (Pighin et al., 2016). Fatty acid (saturated, monounsaturated and or polyunsaturated) composition of dietary lipids, through their potential to modify the fatty acid composition of lipids in meat, determine the firmness of adipose tissue and the oxidative stability of meat. Hence, affects meat flavour and colour (Trabalza-Marinucci et al., 2016). In addition to dietary lipid profile affecting the quality of meat, the presence of the secondary metabolites in plant-derived dietary protein sources has a bearing on the quality of meat through the inhibition of biohydrogenation of linoleic acid (Morales and Ungerfeld, 2015). The use of tannin rich ingredients in ruminant diets modifies the biohydrogenation of unsaturated fatty acid and thus, the profile of fatty acid outflowing the rumen, which influence the concentration of beneficial fatty acids such as linolenic acid, vaccenic acid and rumenic acid in meat (Morales and Ungerfeld, 2015). The compound effects of diets, feeds and secondary plant metabolites on meat quality have health implications on consumers (Van Cleef et al., 2017). In the process of characterising the MNMs, I found that the nut meal (MNM2) which was subsequently used in the formulation of the test diets (used for the *in vivo* experiment) over and above having a high CP content, also had a high residual oil content (Table 3.1, Chapter 3). The high residual oil had a preponderance of oleic acid that can impact on quality of lamb from growing-fattening lambs raised on MNM-based diets. Additionally, the MNM that was used to formulate the MNM-based test diets had some secondary plant metabolites such as tannin (Luciano et al., 2009) and saponin (Zhang et al., 2017) that could impact meat quality. Meat is

recognised as a major source of dietary nutrients, lipids included, in the human diet (Milićević et al., 2014) thus, the fatty acid profile of lipids in meat, as influenced by these exogenous factors (especially diet /feed composition) can impact human health (De Souza et al., 2015). It thus became pertinent for me to evaluate and unravel the effects of substituting SBM with MNM (with high residual oil characterised by an array of fatty acids) on carcass characteristics, meat physical attributes and chemical composition of the lamb from Dorper lambs.

5.1 Objectives of the study

The study sought to determine the effect of graded substitution of SBM as a dietary protein source, on a CP basis, with MNM in lamb fattening diets on the:

i. carcasses' warm and cold weight, dressing percentage, muscle pH and temperature from the lambs.
ii. lamb's (meat) drip loss, colour, moisture characteristics (thaw loss, total cooking loss, cooking drip loss and cooking evaporation loss) and tenderness.
iii. lamb's proximate composition, mineral content and fatty acids profile.

5.2 Hypotheses

The hypotheses of the current study were:

a. H_0: dietary substitution of SBM with MNM, on a CP basis, in lamb fattening diets does not affect the carcasses' warm and cold weight, dressing percentage, muscle pH and temperature from the lambs.

   H_1: dietary substitution of SBM with MNM, on a CP basis, in lamb fattening diets affects the carcasses' warm and cold weight, dressing percentage, muscle pH and temperature from the lambs.

b. H_0: dietary substitution of SBM with MNM, on a CP basis, in lamb fattening diets does not affect the meat drip loss, colour, moisture characteristics (thaw loss, total cooking loss, cooking drip loss and cooking evaporation loss) and tenderness.

   H_1: dietary substitution of SBM with MNM, on a CP basis, in lamb fattening diets affects meat drip loss, colour, moisture characteristics (thaw loss, total cooking loss, cooking drip loss and cooking evaporation loss) and tenderness.

c. H_0: dietary substitution of SBM with MNM, on a CP basis, in lamb fattening diets does not affect the meat proximate composition, mineral content and fatty acids profile.

   H_1: dietary substitution of SBM with MNM, on a CP basis, in lamb fattening diets affects the meat proximate composition, mineral content and fatty acids profile.

5.3 Materials and methods

5.3.1 Ethical approval and study site

Ethical approval for the study and a detailed description of the study site for this experiment are as described in Chapter 4; subheading 4.4.2.

5.3.2 Animals and animal management

The same animals discussed in Chapter 4 were used to collect data for this study. The source and management of the animals was described in Chapter 4; subheading 4.4.3.

5.3.3 Slaughter process

The slaughter process of lambs was described in Chapter 4; subheading 4.4.7.

5.3.4 Measurements

Immediately after evisceration, warm carcass weight was determined using an overhead digital scale (DIGI DS160 industrial scale, Toronto, Canada). Following determination of the warm carcass weight, warm muscle pH [initial pH (pH_i)] and temperature were measured on each of the carcasses' left *Musculus longissimus lumborum* (*M. LL*) 45 minutes after slaughter. Immediately following the determination of warm carcass weight, pH_i and warm carcass temperature, the dressing percentage expressed as a percentage of live weight was calculated as described by Bonvillani et al. (2010) using the equation: dressing percentage (%) = (W_1 / W_2) x100;where W_1 is the weight of each warm carcass and W_2 is the slaughter weight of each lamb.

Each carcass was then chilled in a cold room at 4ºC for 24 hours following which cold carcass weight was determined using an overhead digital scale (DIGI DS160 industrial scale, Toronto, Canada). Immediately after the determination of the cold carcass weight, ultimate pH (pHu) of the cold carcass and temperature were measured on the left *M.LL* between the 2nd and 4th lumbar vertebra using a Cyberscan pH 300 digital pH meter fitted with a specially designed meat electrode (Eutech Instruments, Thermo Fisher Scientific, Vernon Hills, USA).

5.3.5 Carcass processing, sample collection and storage.

A sample of the *M. LL* muscle from each carcass was then carefully dissected from the 12/13 region on both sides of the carcass. The sub-sample of the *M. LL* muscle from the left side of each carcass was cut into 25mm sub-samples that were allowed to bloom for 1 hour at 4ºC prior to the determination of meat colour and myoglobin redox forms while the other sub-sample was cut into 50g sub-samples and used for determination of drip loss. Two 30mm sub-samples were cut from the *M. LL* muscle sample harvested from the right side of each carcass. One of the sub-samples was aged for 1 day and the other for 7 days in vacuum bags at 2ºC and were then frozen stored at -20ºC until the determination of Warner Bratzler shear force. Two other 50g sub-samples were cut from the *M. LL* muscle sample from the right side of each carcass. One of the sub-samples was aged for 1 day and while the other was aged for 7 days both in vacuum bags at 2ºC. Following aging, each sample was frozen stored at -20ºC pending the determination of myofibrillar fragmentation length. The remaining samples of the *M. LL* muscle from both sides of

each carcass were then mixed and sub-sampled into approximately 100g and stored at -20ºC for later determination of proximate and mineral composition and fatty acid profile of the meat.

5.3.5.1 Determination of drip loss

Drip loss was measured as described by Strydom et al. (2008). In brief, each 50g sub-sample was sliced into 10 × 10× 20mm strips. Each of the strips was then weighed using a digital balance (Model GM-501, Lutron Electronic, Coopersburg, Pennsylvania, USA) to determine the initial weight of each strip. Each strip was then hung using a pin inside a 200ml sample bottle and stored at 4ºC for 72 hours. Following the 72-hour storage at 4ºC each strip was then re-weighed to determine the final weight. Drip loss was calculated as the difference between the initial weight and final weight of the strip and expressed as a percentage of initial weight using the equation: drip loss percentage (%) = $W_1 – W_2 / W_1 \times 100$; where W_1 is the initial weight of the sub-sample and W_2 is the final weight of the sub-sample.

5.3.5.2 Determination of colour

Meat colour was measured using a Minolta meter (Chroma Meter CR-200, Minolta Co., Ltd., Osaka, Japan) as described by Krzywicki (1979). The three fundamental colour components L*, a* and b* were determined. The L* indicates lightness on a scale of 0 (all light absorbed) to 100 (all light reflected), a* indicates red to green colour and b* indicates yellow to blue colour. Briefly, following 1 hour of blooming the fresh sub-sample at 4ºC to allow for sufficient oxygenation, three colour measurements were taken on each sample at three different locations. Values from the colour measurements were used to calculate chroma (C*: colour saturation) and hue [(H*: spectral colour)] of the meat as described by Young et al. (1999) using the equations: $\sqrt{a^{*2} + b^{*2}}$ and $\tan^{-1}(b^*/a^*)$, respectively.

5.3.5.2.1 Determination of myoglobin redox reaction forms

The sub-samples that were used to determine meat colour were also used to determine the myoglobin redox reaction forms (oxymyoglobin, metmyoglobin and deoxymyoglobin) of the meat as described by the American Meat Science Association (AMSA, 2012). In summary, the

reflectance of each sub-sample was measured using a Minolta CM-2002 spectrophotometer (MiniScan EZ, HunterLab, Reston, Virginia, USA) at wavelengths of 572, 525, 473 and 730 nm. The reflectance percentage of each sub-sample was converted to K/S values using the equation: $K/S = (1 - R)^2 / (2R)$; where R is the reflectance percentage (%) expressed as a decimal, K is the absorption coefficient and S is the scattering coefficient. The K/S values were then used to compute the proportion (in %) of the metmyoglobin (MMb) and deoxymyoglobin (DMb) as described by Krzywicki (1979), while the oxymyoglobin (OMb) percentage was obtained using the equation %OMb = 100 − (%MMb + %DMb) as described by Mancini et al. (2003).

5.3.5.3 Determination of moisture characteristics

The determination of the thaw, total cooking, cooking drip and cooking evaporation losses was done as a prerequisite for the determination of the Warner Bratzler shear force.

5.3.5.3.1 Thaw loss

Following freezing the two 30mm sub-samples at -20ºC each sub-sample was weighed using a digital balance (Model GM-501, Lutron Electronic, Coopersburg, Pennsylvania, USA) to determine the initial weight of the sub-sample. Immediately after determination of the initial weight, the sub-sample was thawed at 4ºC for 24 hours after which the final weight was similarly measured using the digital balance. Thaw loss was calculated as the difference between the weights of the frozen and thawed sub-sample and expressed as a percentage using the equation: thaw loss percentage (%) = $W_1 - W_2 / W_1 \times 100$ (Jama et al., 2008);
where W_1 is the weight of frozen the sub-sample and W_2 is the weight of thawed the sub-sample.

5.3.5.3.2 Total cooking loss

Immediately after the determination of thaw loss each thawed sub-sample was broiled in a Mielé electric oven (Model H217, Mielé & Cie, Gütersloh, Germany) as described by AMSA (1995). The oven was set at 260ºC for 20 minutes prior to broiling of sub-samples. Each sub-sample was broiled until an internal temperature (recorded by a hand-held digital probe: Model Kane-May 1012, Kane International Ltd, Hertfordshire, United Kingdom) of 70ºC was reached. Immediately

after broiling, the final weight of each broiled sub-sample was determined. Total cooking loss was then calculated as the difference between the weights of thawed raw sub-sample and broiled sub-sample and expressed as a percentage using the equation: total cooking loss percentage (%) = $W_1 – W_2 / W_1 \times 100$ (Hwang et al., 2004); where W_1 is the weight of thawed raw sub-sample and W_2 is the weight of the broiled sub-sample.

5.3.5.3.3 Cooking drip loss

Following the determination of total cooking loss, cooking drip loss for each sub-sample was computed as the difference between the weights of each sub-sample's drip loss and thawed raw sub-samples, and expressed as a percentage using the equation: cooking drip loss percentage (%) = $W_1 / W_2 \times 100$ (Jama et al., 2008); where W_1 is the weight of drip (purge and melted fat) and W_2 is the weight of thawed raw sub-sample.

5.3.5.3.4 Cooking evaporation loss

After the determination of cooking drip loss, cooking evaporation loss for each sub-sample was calculated as the difference between the weights of drip loss and thawed raw sub-samples and expressed as a percentage using the equation: cooking evaporation loss percentage (%) = $100 – W_1 / W_2 \times 100$ (Jama et al., 2008);
where W_1 is the weight of broiled sub-sample and W_2 is the weight of thawed raw sub-sample.

5.3.6 Determination of Warner Bratzler shear force

The Warner Bratzler shear force [(WBSF): a measure of meat tenderness] of each sub-sample was determined as described by Raharjo et al. (1992). In brief, following broiling, each sub-sample was allowed to cool to room temperature for at least 2 to 3 hours after which 6 cylindrical round cores (12.5 mm core diameter) were cored parallel to the grain of each sub-sample using a coring device. Each of the six cores was sheared using a Warner Bratzler shear device mounted on an Instron Universal Testing Machine (Model 4301, Instron Ltd, Buckinghamshire, England). Each core was sheared once through the centre at a crosshead speed of 200 mm/min, test speed

with a 1kN load cell perpendicular to the muscle fibre. The average shear force from the six cores per sub-sample was recorded.

5.3.7 Determination of myofibrillar fragmentation length

The myofibrillar fragmentation length (MFL) of each muscle sub-sample was measured as described by Culler et al. (1978). In summary, each 50g frozen (20°C) sub-sample was thawed at room temperature following which connective tissue and fat were trimmed. Each trimmed sub-sample was then cut into 3g pieces. Each of the 3g sub-sample pieces was then placed into 50ml Bühler round bottom glass which contained 30ml of 0.02M potassium phosphate extraction buffer (constituted from 100mM potassium chloride, 1mM magnesium chloride, 1mM ethylenediaminetetraacetic acid and 1mM sodium azide) and homogenised for 30 seconds using a Bühler HO_4 homogeniser (Type H0-4 NR 3012, Labortechnik Admund Bühler, Wehingen, Germany). Immediately after homogenising, each mixture was centrifuged for 10 minutes at 2987 × g at 4ºC using a Hermle centrifuge (Hermle Labortechnik GmbH Z 36 HK, Wehingen, Germany). The supernatant was discarded and the pellet resuspended in 30ml extraction buffer and centrifuged for the second time for 10 minutes at 2987 × g at 4ºC. The supernatant was discarded and the pellet resuspended in 10ml extraction buffer and centrifuged for the third time for 10 minutes at 2987 × g at 4ºC. Immediately after centrifuging, the suspension was then filtered under vacuum using 1000µm polyethylene strainer (Krackeler Scientific, Alany, New York). The resultant filtrate was then filtered under vacuum using 250µm polyethylene strainer (Krackeler Scientific, Alany, New York). To measure the MFL of the meat, a drop (50µL) of the filtrate was placed on a microscopic slide and then covered with a coverslip and viewed at a 400x magnification using an Olympus BX40 system microscope (Olympus, Tokyo, Japan). The MFL was determined as the average length of 100 single myofibril fragments per sub-sample.

5.3.8 Determination of the chemical composition of lamb

5.3.8.1 Determination of proximate composition

The proximate [moisture, organic matter (OM), crude protein (CP) and ether extract (EE)] content of lamb from carcasses of male Dorper lambs was determined using the standard AOAC analytical procedures as described in Chapter 3; subheading 3.3.2.1.

5.3.8.2 Determination of mineral composition

The mineral composition of lamb from carcasses of male Dorper lambs was determined as described by Giron (1973). The analytic procedures have been described earlier in Chapter 3; subheading 3.3.2.5.

5.3.8.3 Determination of fatty acid profile

The fatty acid profile of lamb from carcasses of male Dorper lambs was determined as described by Christopherson and Glass (1969). See chapter 3 of this thesis, subheading 3.3.2.7 for the description.

5.4 Statistical analysis

Data are presented as carcass characteristics, lamb (meat) physical attributes and chemical composition. Genstat statistical software (Genstat, 2000) was used to analyse data using a one-way ANOVA. Means were separated using Fisher's Least Significant Difference (LSD) test. The level of significance was set at 5%.The model used for data analysis was:

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

where

Y_{ij} = is the individual observation of the ith dietary treatment and the jth replicate,

μ = is the overall mean

T_i = is the fixed effect of the ith dietary treatment (i = 1, 2…5)

e_{ij} = is the random residual error

5.5 Results

5.5.1 Effect of dietary Marula nut meal on carcass traits, muscle pH and temperature

Table 5.1 below shows the effect of graded dietary substitution of SBM with MNM on carcass traits as well as the pH and temperature of the *M. LL.*

Table 5.1: Effect of graded dietary substitution of soyabean meal with Marula nut meal on carcass characteristics, pH and temperature of muscle from Dorper lambs

	Diet							
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**LSD**	**SE**	***P***
Warm carcass weight (kg)	16.58[a]	17.69[a]	17.31[a]	16.04[a]	15.98[a]	1.90	1.86	0.277
Cold carcass weight (kg)	16.00[a]	17.17[a]	16.85[a]	15.58[a]	15.51[a]	1.83	1.79	0.256
Dressing percentage (%)	46.46[a]	48.55[a]	46.29[a]	46.12[a]	46.61[a]	4.32	4.22	0.776
Warm muscle pH_i	5.93[a]	5.01[a]	5.87[a]	6.10[a]	5.88[a]	0.18	0.17	0.075
Cold muscle pH_u	5.63[a]	5.66[a]	5.66[a]	5.70[a]	5.58[a]	0.11	0.11	0.293
Warm muscle temperature ºC)	33.26[a]	34.40[a]	35.19[a]	34.12[a]	34.7[a]	1.79	1.74	0.274
Cold muscle temperature (ºC)	5.38[a]	4.98[a]	3.69[a]	4.25[a]	2.45[a]	2.89	2.82	0.282

[a] Within row means with the same superscripts are not significantly different (P > 0.05). Warm and cold carcass weights of the lambs across dietary treatments were similar (P > 0.05). Carcass dressing percentage was similar (P > 0.05) across dietary treatments. The initial and ultimate pH of the *M. LL* from carcasses of lambs across dietary treatments were similar (P > 0.05). The warm and cold temperature of the *M. LL* from carcasses across dietary treatments were similar (P > 0.05). pH_i = initial pH, pH_u = ultimate pH, Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein

source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

There were no significant difference ($P > 0.05$) in warm and cold carcass weight as well as the dressing percentage of carcasses from lambs that were fed the control and the test diets. Dietary MNM had no significant effect ($P > 0.05$) on the pHi and pHu, and the warm and cold temperature of the *M. LL* from carcasses of the male Dorper lambs across dietary treatments.

5.5.2 Effect of dietary Marula nut meal on physical traits of lamb

5.5.2.1 Drip loss and colour

Table 5.2 shows the effect of graded dietary substitution of SBM with MNM on the drip loss, colour and myoglobin redox reaction forms of lamb from carcasses of male Dorper lambs.

Table 5.2: Effect of graded dietary substitution of soyabean meal with Marula nut meal on the drip loss, colour and myoglobin redox reaction forms of lamb from male Dorper lambs

	Diet							
Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**LSD**	**SE**	***P***
Drip loss (%)	1.59[a]	1.56[a]	1.45[a]	1.35[a]	1.54[a]	0.25	0.24	0.296
L* (D_{65})	36.75[a]	36.53[a]	35.35[a]	36.47[a]	36.64[a]	2.25	2.22	0.715
a* (D_{65})	11.19[a]	11.18[a]	11.47[a]	10.50[a]	11.06[a]	1.39	1.37	0.712
b* (D_{65})	12.02[a]	12.47[a]	12.06[a]	11.86[a]	12.53[a]	1.09	1.08	0.668
C* (D_{65})	16.47[a]	16.77[a]	16.65[a]	15.88[a]	16.75[a]	1.47	1.45	0.719
H* (D_{65})	47.05[a]	48.13[a]	46.47[a]	48.73[a]	48.79[a]	3.44	3.39	0.568
Myoglobin redox reaction (%)								
Metmyoglobin	18.62[a]	17.08[a]	18.00[a]	17.43[a]	18.12[a]	1.76	1.73	0.431
Deoxymyoglobin	54.60[a]	56.00[a]	56.00[a]	58.00[a]	54.80[a]	7.71	7.59	0.889
Oxymyoglobin	26.88[a]	26.86[a]	27.20[a]	24.86[a]	26.88[a]	6.14	6.31	0.949

[a] Within row means with the same superscripts are not significantly different (P > 0.05). Drip loss, colour components (L*, a*, b*, C* and H*) and myoglobin redox reaction forms (metmyoglobin, deoxymyoglobin and oxymyoglobin) of lamb from carcasses of lambs were similar (P > 0.05) across dietary treatments. Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein

source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

The drip loss, colour and myoglobin redox reaction forms of lamb from carcasses of lambs fed the control diet (diet 1) did not differ significantly ($P > 0.05$) with those from carcasses of lambs fed the test MNM-based diets.

5.5.2.2 Moisture characteristics and shear force

Table 5.3 shows the effect of graded dietary substitution of SBM with MNM on thaw, total cooking, cooking drip and cooking evaporation losses and shear force of lamb from carcasses of growing-fattening male Dorper lambs.

Table 5.3: Effect of graded dietary substitution of soyabean meal with Marula nut meal on moisture characteristics and shear force of lamb from carcasses of male Dorper lambs

Parameter	Diet					LSD	SE	*P*
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5			
1-day aged lamb								
Thaw loss (%)	0.78[a]	0.47[a]	0.62[a]	0.72[a]	0.32[a]	0.39	0.39	0.134
Total cooking loss (%)	14.08[a]	13.27[a]	12.61[a]	13.07[a]	12.96[a]	2.45	2.41	0.799
Cooking drip loss (%)	2.35[a]	2.38[a]	1.94[a]	2.31[a]	2.16[a]	1.51	1.49	0.975
Cooking evaporation loss (%)	11.73[a]	10.89[a]	10.68[a]	10.77[a]	10.80[a]	1.72	1.69	0.722
WBSF (kg)	5.79[a]	4.53[a]	5.08[a]	5.33[a]	5.00[a]	1.27	1.25	0.373
7- days aged lamb								
Thaw loss (%)	0.31[a]	0.23[a]	0.32[a]	0.33[a]	0.35[a]	0.24	0.23	0.853
Cooking loss (%)	13.65[a]	13.93[a]	14.41[a]	13.73[a]	14.80[a]	2.63	2.59	0.887
Cooking drip loss (%)	2.44[a]	3.34[a]	3.17[a]	2.92[a]	3.50[a]	1.75	1.72	0.762
Cooking evaporation loss (%)	11.21[a]	10.59[a]	11.24[a]	10.81[a]	11.30[a]	1.58	1.55	0.863
WBSF (kg)	3.36[a]	2.87[a]	3.08[a]	2.93[a]	3.02[a]	0.99	0.97	0.870

[a] Within row means with the same superscripts are not significantly different (P > 0.05). Thaw, total cooking, cooking drip and cooking evaporation losses and shear force of lamb from carcasses of lambs were similar (P > 0.05) across dietary treatments. The Warner Bratzler Shear Force (WBSF) of lamb aged for 1 and 7 days from carcasses of lambs was similar (P > 0.05) across dietary treatments. Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

There were no significant differences in the thaw, total cooking, cooking drip and cooking evaporation losses and shear force of lamb from carcasses of lambs across dietary treatments.

5.5.2.3 Myofibrillar fragmentation length

Table 5.4 below shows the effect of graded dietary substitution of SBM with MNM, on a CP basis, on myofibrillar fragmentation lengths of lamb from carcasses of growing-fattening male Dorper lambs.

Table 5.4: Effect of graded dietary substitution of soyabean meal with Marula nut meal on myofibrillar fragmentation lengths of lamb from carcasses of male Dorper lambs

	Diet							
Parameters	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**LSD**	**SE**	***P***
1-day aged lamb								
Myofibrillar fragmentation length (µm)	40.55[a]	40.97[a]	40.68[a]	42.34[a]	40.20[a]	5.33	5.22	0.936
7-days aged lamb								
Myofibrillar fragmentation length (µm)	25.37[a]	26.62[a]	25.85[a]	23.61[a]	24.65[a]	5.03	4.95	0.783

[a] Within row means with the same superscripts are not significantly different (P > 0.05). Myofibrillar fragmentation lengths of lamb from carcasses of lambs across dietary treatments were similar (P > 0.05). Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

The myofibrillar fragmentation lengths of lamb from carcasses of lambs fed the control diet were similar to that of lamb from carcasses of lambs fed diets 2 to 5.

5.5.3 Effect of dietary Marula nut meal on chemical composition of lamb

5.5.3.1 Proximate composition

Table 5.5 below shows the effect of graded dietary substitution of SBM with MNM, on CP basis, on the proximate content of lamb from carcasses of growing-fattening male Dorper lambs.

Table 5.5: Effect of graded dietary substitution of soyabean meal with Marula nut meal on the proximate composition of lamb from carcasses of male Dorper lambs

Parameter (% DM)	Diet					LSD	SE	*P*
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5			
Dry matter	25.82^{d}	27.7^{a}	26.32^{c}	26.55^{b}	27.83^{a}	0.14	0.07	0.001
Moisture	74.24^{a}	72.18^{d}	73.70^{b}	73.36^{c}	72.06^{d}	0.14	0.07	0.001
Ash	3.51^{a}	3.13^{c}	3.50^{a}	3.33^{b}	3.31^{b}	0.14	0.08	0.001
Organic matter	22.21^{d}	24.66^{a}	22.02^{c}	23.26^{b}	24.57^{a}	0.14	0.07	0.0001
Crude protein	77.87^{a}	72.99^{b}	74.16^{ab}	74.08^{ab}	69.78^{c}	1.79	0.99	0.001
Ether extract	16.41^{c}	22.48^{a}	17.43^{c}	18.95^{b}	22.57^{a}	1.43	0.78	0.001

$^{a, b, c, d}$ Within row means with different superscripts differ significantly at ($P < 0.05$). Dry matter, moisture, ash, organic matter, crude protein and ether extract content of lamb from carcasses of lambs were significantly different ($P < 0.05$) across dietary treatments. Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

Lamb from carcasses of lambs fed diets 2 and 5 had the highest ($P < 0.05$) dry matter content, compared to lamb from carcasses of lambs fed diets 1, 3 and 4. Dietary MNM significantly increased the ash content of lamb from carcasses of lambs fed diet 1 and 3 compared to lamb from carcasses of lambs fed diets 2, 4 and 5. The organic matter content of lamb from carcasses of lambs fed diets 2 and 5 was significantly higher ($P < 0.05$) compared to that of lamb from carcasses of their counterparts fed diets 1, 3 and 4. Compared to lamb from carcasses of lambs fed diets 2 and 5, lamb from carcasses of lambs fed diets 1, 3 and 4 had a significantly higher ($P < 0.05$) crude protein content. Dietary substitution of SBM with MNM significantly increased ($P = 0.001$) the ether extract of lamb from carcasses of lambs fed diets 2 and 5 than that of lamb from carcasses of lambs fed diets 1, 3 and 4.

5.5.3.2 Mineral composition

Table 5.6 shows the effect of graded dietary substitution of SBM with MNM on the mineral composition of lamb from carcasses of growing-fattening male Dorper lambs.

Table 5.6: Effect of graded dietary substitution of soyabean meal with Marula nut meal on the mineral composition of lamb from carcasses of male Dorper lambs

	Diet							
Mineral	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**LSD**	**SE**	***P***
Macro-mineral (mg/kg DM)								
Calcium	69.39[a]	76.85[a]	69.16[a]	74.74[a]	62.37[a]	22.89	12.58	0.669
Phosphorus	2061.00[a]	1969.00[a]	2000.00[a]	2063.00[a]	2069.00[a]	176.80	97.20	0.640
Magnesium	248.24[a]	236.46[a]	240.14[a]	251.55[a]	253.30[a]	20.84	11.46	0.358
Potassium	3406.00[a]	3152.00[b]	3100.00[b]	3069.00[b]	3042.00[b]	233.43	128.30	0.036
Sodium	712.00[a]	756.00[a]	666.00[a]	648.00[a]	776.00[a]	126.20	69.30	0.188
Nickel	0.08[a]	0.09[a]	0.06[a]	0.06[a]	0.05[a]	0.04	0.02	0.415
Micro-mineral (mg/kg DM)								
Copper	0.83[a]	0.82[a]	0.87[a]	0.85[a]	0.90[a]	0.07	0.04	0.149
Iron	14.96[a]	16.71[a]	12.09[a]	15.54[a]	14.45[a]	2.96	1.63	0.059
Manganese	0.02[b]	0.02[b]	0.02[b]	0.04[a]	0.04[a]	0.01	0.01	0.001
Zinc	19.24[a]	18.75[a]	18.94[a]	24.12[a]	21.67[a]	4.68	2.48	0.114
Molybdenum	0.01[a]	0.01[a]	0.00[b]	0.01[a]	0.00[b]	0.01	0.03	0.002
Selenium	0.01[c]	0.04[b]	0.05[b]	0.02[c]	0.07[a]	0.01	0.01	0.001
Chromium	0.01[b]	0.02[b]	0.02[b]	0.03[a]	0.02[b]	0.01	0.01	0.008

[a, b, c] Within row means with different superscripts differ significantly ($P < 0.05$). Macro-mineral composition of lamb from carcasses of lambs across dietary treatments was similar ($P > 0.05$) except for potassium which was higher ($P = 0.036$) in lamb from carcasses of lambs fed the control diet. Copper, iron and zinc concentration in lamb from carcasses across dietary treatments were similar ($P > 0.05$). Manganese, molybdenum, selenium and chromium concentration in lamb from carcasses of lambs were significantly different ($P < 0.05$) across dietary treatments. Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source,

Diet 5 = 100% MNM as protein source; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

Except for potassium, the macro-mineral (copper, iron and zinc) concentration in lamb from carcasses of lambs fed the control diet was similar (P > 0.05) to that in lamb from carcasses of lambs fed the test diets. While the concentration of copper, iron and zinc in lamb from carcasses of lambs across dietary treatments did not differ significantly (P > 0.05), the concentration of manganese molybdenum, selenium and chromium in lamb from carcasses of lambs differed significantly (P < 0.05). While the concentration of manganese in lamb increased with increasing dietary MNM, the concentration of selenium was higher (P = 0.001) in lamb from lambs fed diet 5 compared to that (lamb) from lambs fed diet 1 and 4. The molybdenum and chromium concentration were higher (P < 0.05) in lamb from carcasses of lambs fed diet 4 compared to lamb from carcasses of lambs fed diets 3 and 5.

5.5.3.3 Fatty acid profile

Table 5.7 below shows the effect of graded dietary substitution of SBM with MNM, on a CP basis, on the fatty acid profile of lamb from carcasses of growing-fattening male Dorper lambs.

Table 5.7: Effect of graded dietary substitution of soyabean meal with Marula nut meal on the fatty acid profile of lamb from carcasses of male Dorper lambs

Fatty acid (% of total lipid)	Diet					LSD	SE	*P*
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5			
Saturated								
C10:0 (Capric acid)	0.01^{d}	0.12^{b}	0.15^{a}	0.04^{c}	0.00^{d}	0.02	0.01	0.001
C12:0 (Lauric acid)	0.15^{bc}	0.18^{ab}	0.21^{a}	0.12^{cd}	0.11^{d}	0.04	0.02	0.001
C13:0 (Tridecylic acid)	0.01^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.01^{a}	0.01	0.01	0.078
C14:0 (Myristic acid)	2.49^{b}	2.72^{a}	2.54^{b}	2.20^{c}	2.09^{c}	0.17	0.09	0.001
C15:0 (Pentadecyclic acid)	0.38^{b}	0.41^{a}	0.37^{bc}	0.36^{bc}	0.35^{c}	0.02	0.01	0.001
C16:0 (Palmitic acid)	19.88^{c}	21.50^{a}	21.76^{a}	20.46^{bc}	20.67^{b}	0.76	0.42	0.001
C17:0 (Margaric acid)	1.07^{a}	0.99^{b}	0.97^{c}	0.93^{d}	0.92^{d}	0.02	0.01	0.001
C18:0 Stearic acid	22.45^{c}	22.68^{c}	22.71^{c}	24.86^{a}	24.11^{b}	0.57	0.31	0.001
C20:0 (Eicosanoic acid)	0.17^{a}	0.16^{a}	0.24^{a}	0.19^{a}	0.19^{a}	0.08	0.04	0.253
C22:0 (Behenic acid)	0.20^{a}	0.14^{a}	0.52^{a}	0.15^{a}	0.63^{a}	0.62	0.34	0.324
C23:0 (Tricosylic acid)	0.00^{a}	0.02^{a}	0.28^{a}	0.01^{a}	0.01^{a}	0.401	0.220	0.469
TSFA	**46.90^{b}**	**49.01^{a}**	**49.68^{a}**	**49.32^{a}**	**49.09^{a}**	**0.75**	**0.41**	**0.001**
Monounsaturated								
C14:1 (Myristoleic acid)	0.08^{a}	0.09^{a}	0.08^{a}	0.06^{a}	0.07^{a}	0.02	0.02	0.063
C16:1 (Palmitoleic acid)	1.16^{cd}	1.27^{b}	1.36^{a}	1.13^{d}	1.23^{bc}	0.08	0.05	0.001
C17:1 (Heptadecanoic acid)	0.39^{a}	0.37^{b}	0.34^{c}	0.32^{c}	0.33^{c}	0.02	0.01	0.001
C20:1 (Paullinic acid)	0.10^{a}	0.03^{a}	0.01^{a}	0.00^{a}	0.00^{a}	0.14	0.08	0.539
C24:1 (Nervonic acid)	0.01^{a}	0.06^{a}	0.09^{a}	0.07^{a}	0.02^{a}	0.05	0.03	0.150
C18:1n9c (Oleic acid)	37.46^{b}	37.56^{b}	38.15^{b}	37.68^{b}	38.88^{a}	0.73	0.40	0.008
C18:1n9t (Elaidic acid)	7.29^{a}	7.39^{a}	6.20^{c}	7.01^{b}	7.00^{b}	0.18	0.10	0.001

C22:1n9 (Erucic acid)	0.00^{b}	0.03^{a}	0.02^{ab}	0.03^{a}	0.02^{a}	0.02	0.01	0.045
TMUFA	**39.44^{b}**	**39.64^{b}**	**40.14^{ab}**	**39.45^{b}**	**40.71^{a}**	**0.86**	**0.47**	**0.034**
Polyunsaturated								
C18:2n6c (Linoleic acid)	3.72^{a}	2.86^{b}	2.89^{b}	2.91^{b}	2.30^{c}	0.12	0.07	0.001
C18:2n6t (Linolelaidic acid)	0.53^{a}	0.37^{a}	0.28^{a}	0.27^{a}	0.24^{a}	0.25	0.14	0.135
C20:2 (Eicosadienoic acid)	0.13^{a}	0.08^{a}	0.09^{a}	0.12^{a}	0.14^{a}	0.07	0.04	0.246
C18:3n3 (α- linolenic acid)	0.46^{a}	0.34^{b}	0.26^{c}	0.22^{c}	0.15^{d}	0.05	0.03	0.001
C18:3n6 (γ-linolenic acid)	0.06^{a}	0.08^{a}	0.04^{a}	0.08^{a}	0.10^{a}	0.09	0.05	0.622
C20:3n3 (Eicosatrienoic acid)	0.06^{a}	0.01^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.08	0.04	0.496
C20:3n6 (Dihomo-γ-linolenic acid)	0.04^{ab}	0.07^{a}	0.03^{bc}	0.07^{a}	0.00^{c}	0.04	0.02	0.009
C20:4n6 (Arachidonic acid)	0.44^{a}	0.22^{c}	0.40^{ab}	0.35^{b}	0.38^{b}	0.07	0.04	0.001
C20:5n3 (Eicosapentaenoic acid)	0.04^{a}	0.04^{a}	0.09^{a}	0.03^{a}	0.02^{a}	0.05	0.03	0.072
TPUFA	**5.91^{a}**	**3.70^{b}**	**3.84^{b}**	**3.78^{b}**	**3.04^{b}**	**1.17**	**0.64**	**0.003**
Trans fatty acid	7.82^{a}	7.76^{a}	6.48^{c}	7.28^{b}	7.24^{b}	0.16	0.09	0.001
Cis fatty acid	41.19^{a}	40.47^{a}	41.03^{a}	40.60^{a}	41.18^{a}	0.84	0.46	0.251
Omega 3	0.56^{a}	0.39^{b}	0.36^{b}	0.25^{bc}	0.17^{c}	0.15	0.08	0.002
Omega 6	4.79^{a}	3.69^{b}	3.63^{b}	3.59^{b}	2.98^{c}	0.14	0.08	0.001
Omega 3:6 ratio	0.12^{a}	0.11^{a}	0.10^{ab}	0.07^{bc}	0.06^{c}	0.03	0.02	0.006
Omega 9	44.77^{b}	45.03^{ab}	44.36^{b}	44.73^{b}	45.91^{a}	0.90	0.50	0.031
Eicosapentaenoic acid	0.04^{d}	0.03^{d}	0.09^{d}	0.03^{d}	0.02^{d}	0.05	0.03	0.084

[a, b, c, d] Within row means with different superscripts differ significantly ($P < 0.05$). TSFA concentration was highest in lamb from lambs fed diets the test diets. TMUFA concentration of lamb was highest in lambs fed diets 3 and 5. TPUFA concentration was higher ($P < 0.05$) in lambs fed the control diet than those fed the test diets. Diet 1 = 100% SBM as protein source, Diet 2 = 75% SBM + 25% MNM as protein source, Diet 3 = 50% SBM + 50% MNM as protein source, Diet 4 = 75% SBM + 25% MNM as protein source, Diet 5 = 100% MNM as protein source; TSFA = total saturated fatty acid; TMUFA = total monounsaturated fatty acid; TPUFA = total polyunsaturated fatty acid; LSD = least significant difference; SE = standard error; *P* = probability value; n = 8 lambs.

Lamb from carcasses of lambs fed the test diets had a higher TSFA content compared to that from carcasses of lambs fed the control diet. Compared to lamb from carcasses of lambs fed diets 1, 2 and 4, lamb from carcasses of lambs fed diet 3 and 5 had a higher concentration of TMUFA. The TPUFA concentration in lamb from carcasses of lambs fed the test diets was significantly lower ($P < 0.05$) compared to that in lamb from carcasses of lambs fed the control diet. Stearic, palmitic and myristic acids were the most dominant saturated fatty acids in lamb from carcasses of lambs across dietary treatments. Oleic acid concentration was highest in lamb from carcasses of lambs fed diet 5. Lamb from carcasses of lambs fed the control diet had a significantly higher concentration ($P= 0.0001$) of linoleic acid compared to lamb from lambs fed the test diets. While there were no significant differences in the omega-9 and cis fat concentration of lamb from carcasses of lambs across dietary treatments, omega-3 and omega-6 concentration were significantly higher ($P < 0.05$) in lamb from carcasses of lambs fed the control diet compared to that in lamb from carcasses of lambs fed the test diets. The omega 3:6 ratio in lamb from carcasses of lambs fed dietary treatments decreased with increasing dietary MNM.

5.6 Discussion

This discussion focuses on the effects of dietary MNM on carcass traits of male Dorper lambs and physico-chemical properties of lamb.

5.6.1 Effect of dietary MNM on carcass traits, muscle pH and temperature.

Research has amply demonstrated that heavier animals at slaughter yield carcasses of higher weight characterised by higher intramuscular fat (Esterhuizen et al., 2008). Shivambu et al. (2012) observed that in Dorper lambs feeding concentrate diets resulted in increased slaughter weight and dressing percentage. However, the observed increased slaughter weight and dressing percentage did not translate to increased carcass fat (Shivambu et al., 2012). These observations thus show dichotomy in regard to research findings on the relationship between slaughter weight and carcass fat. In the current study, the slaughter weight and dressing percentage are similar across dietary treatments (Table 5.1). This observation suggests that the use of MNM as a dietary protein source in place of SBM in lamb fattening diets did not compromise these parameters. The determination of the intramuscular fat content of the carcasses would have assisted to draw better insights on the effect of MNM on carcass quality. The distribution and fatty acid content of intramuscular fat in muscle (meat) have an effect on the drip loss, flavour, juiciness and tenderness of meat (Przybylski et al., 2010) hence it (intramuscular fat content) is a key factor in the quality of meat (Albrecht et al., 2006). Although I measured the visceral fat content of the lambs (Chapter 4, Table 4.9; page 101), visceral fat does not necessarily give an indication of intramuscular adiposity.

Ocak et al. (2016) reported that high concentrate diet fed male Dorper lambs slaughtered at approximately 6 months of age are expected to have on average a warm and cold carcass weight of 14.7 and 14.5kg , respectively as well as a mean dressing percentage of 44.9%. Results of this study show a higher warm and cold carcass weight as well as higher mean dressing percentages (Table 5.1, page 129) compared to those reported by Ocak et al. (2016). This observed difference could be due to the differences in diet composition. The similarity in the carcass traits and the higher carcass weights of the lambs in the current study compared to those reported by Ocak et

al. (2016) suggest that the use of MNM as a dietary protein source in place of SBM in lamb fattening diet did not compromise carcass yield.

According to Thompson (2002) muscle pH_i of less than 6 at temperatures between 35 and 40ºC in the warm carcass muscle ~~o~~ leads to heat muscle shortening. While Thompson (2002) asserts that warm muscle temperatures (between 35 and 40ºC) coupled with a warm muscle pH of less than 6 cause heat muscle shortening~~,~~ Hoffman et al. (2009) argue that a decrease in warm muscle pH at temperatures above 15ºC results in heat muscle shortening. Findings in this study show similarities in pH_i and warm muscle temperature of the carcasses from growing-fattening Dorper lambs across dietary treatments. My results for the warm carcass muscle show a pH_i range of 5.01 to 6.10 and an initial temperature range of 33.26 to 35.19ºC. Although not exactly below a pH of 6, the pH_i range is very close to the warm muscle pH (pH_i) reported by Thompson (2002). Importantly, when considering the expected warm muscle temperature as reported by Thompson (2002) and Hoffman et al. (2009), one can argue that the warm muscle temperature of the carcasses from lambs across dietary treatments fell below the range that Thompson (2002) argued would result in heat muscle shortening. My results, therefore suggest that MNM can be used as a dietary protein source in place of SBM without causing heat shortening of the muscles from carcasses of male Dorper lambs.

The ultimate pH (pH_u) values of above 6 at temperatures between 5 and 10ºC in cold carcass muscle results in cold muscle shortening (Hoffman et al., 2009). The recommended pH_u for the meat of acceptable quality is 5.8 or less at temperatures below 15ºC (Tejeda et al., 2008). The pH_u and ultimate temperature of the cold carcass muscles of the male Dorper lambs in the current study were similar across dietary treatments. While Hoffman et al. (2009) attest that an increase in ultimate pH at temperature between 5 and 10ºC results in cold muscle shortening, my results show that the ultimate pH (5.58 to 5.70) and temperature (2.25 to 5.38ºC) ranges of cold muscles from carcasses of male Dorper lambs across dietary treatments were below a pH of 5.8 at temperatures below 15ºC reported by Tejeda et al. (2008). These results suggest that the use of MNM as a dietary protein source in lamb fattening diets did not result in cold shortening of the muscles from carcasses of male Dorper lambs.

5.6.2 Effect of diet on meat quality

5.6.2.1 Drip loss and colour

Diet influences muscle colour by affecting the glycogen and antioxidant of muscle (Priolo et al., 2002; Joy et al., 2008). Bee (2002) reported that the feeding of high concentrate diet to animals results in higher glycolytic potential at slaughter. The resultant higher glycolytic potential leads to an increase in meat drip loss from meat/muscle (Hamilton et al., 2003; Ruusunen et al., 2007). Meat drip loss results from an increase in the shrinkage of muscle cell due to increased degradation of proteins (actin and myosin) that bind myofibrils together and myofibrils to the muscle cell (Huff-lonergan and Lonergan, 2005). According to Ingólfsson and Dransfield (1991), good quality lamb has drip loss range between 1 and 5%. Findings from the current study show the lamb from the carcasses of the Dorper lambs to have a drip loss that ranged from 1.35 to 1.59% which is in agreement with the acceptable drip loss of quality lamb (Ingólfsson and Dransfield, 1991). Importantly, results on drip loss show similarities in the drip loss of lamb from the carcasses of lambs fed the control and test diets (Table 5.2). In the context of the recommendation by Ingólfsson and Dransfield (1991) and the observed similarities in drip loss of lamb from lambs fed the control and MNM-based (test) diets; results of the current study suggest that MNM can be used in lamb fattening diets in place of SBM without negatively impacting on post mortem shrinkage of actin and myosin fibres, hence on drip loss.

Feeding animals high concentrate diets results in high accumulation of glycogen in muscle, which (glycogen) is necessary for the development of normal pH_u in the muscle (Daly et al., 1999). The development of normal pH_u results in meat of acceptable red colour (Daly et al., 1999). Animals fed high forage diets (typified by having a high dietary tocopherol content) have been observed to have better meat colour stability compared to animals fed high concentrate diets (Wiklund et al., 2010). Forages contain tocopherols that have antioxidant activities and are known to inhibit autoxidation of oxymyoglobin to metmyoglobin and thus, promoting colour stability of the meat (Wiklund et al., 2010). Additionally, the stability of meat colour is also influenced by myoglobin (Hernández et al., 2015), which occurs in three redox reaction forms: oxymyoglobin, metmyoglobin and deoxymyoglobin (Mancini and Hunt, 2005). Oxymyoglobin occurs in the presence of oxygen and results in a cherry red colour in meat (Mancini and Hunt,

2005). Metmyoglobin, which occurs in muscle with limited oxygen supply, results in meat with a brown colour (Mancini and Hunt, 2005). Deoxymyoglobin occurs in the absence of oxygen, for example, in vacuum sealed meat and gives the meat a purple-red colour (Mancini and Hunt, 2005). Over and above the effect of the various oxidation states of myoglobin, the light emitted from the Milton meter bulb, through its diffusion, also affects the amount of light that is reflected from the meat due to differences in refractive index at the margins between the light reflecting particles (Anadón, 2002). In the current study meat colour was similar across dietary treatments. Across dietary treatments, lamb had a high concentration of deoxymyoglobin, which could mean that the myoglobin content in the lamb might not have been efficiently exposed to oxygen due to competition between myoglobin and mitochondria for oxygen (AMSA, 2012). There is also a high possibility that insufficient oxygenation in the cold room (4ºC) where the meat was left to bloom for 1 hour might have influenced the observed meat colour. That said, the observed similarities in meat colour from carcasses of lambs across dietary treatments suggest that substitution of SBM, as a dietary protein source in lamb fattening diet with MNM did not impact negatively on the colour of the meat. Ideally, the percentages of the three myoglobin pigments [redox reaction forms (OMb, MMb and DMb)] add to 100% (Hernández et al., 2015). In my results the summation of the individual redox forms of the meat added to 100% thus, it can be inferred that dietary MNM as a protein source did not compromise the oxidative stability of the meat.

5.6.2.2 Moisture characteristics

Fluid loss during thawing and cooking are critical processing traits used to evaluate meat eating quality (Koohmaraie et al., 2002). Lambs reared on concentrate diets have greater carcass fat compared to those reared on forage diets (Jacques et al., 2011). Importantly, meat from carcasses with more fat has been observed to have lower thaw and cooking losses than meat from leaner carcasses (Abdullah and Qudsieh, 2009). High thaw loss has been reported to be associated with high cooking loss (Jeong et al., 2011; and Leygonie et al., 2012). High cooking loss produces tough meat (Gregory, 2007), while low cooking loss yields juicy meat (Ameha, 2006). Additionally high cooking drip loss results in loss of meat texture, appearance and nutritional value (Barbosa-Filho et al., 2017), while high cooking evaporation loss leads to low meat yield and firmness (Yu et al., 2005). Findings in the current study show similarities in the thaw, total

cooking, cooking drip and evaporation losses of lamb (aged for 1 and 7 days) from carcasses of lambs across dietary treatments (Table 5.3). Interestingly, results of the current study on thaw, total cooking, cooking drip and cooking evaporation losses of the lamb are within the normal thaw, total cooking, cooking drip and cooking evaporation losses (0.2 to 0.3, 11.9 to 17.1, 1.9 to 7.8 and 9.4 to 10.0%, respectively) reported by Van Heerden et al. (2007) of lamb from carcasses of Dorper lambs fed a high concentrate diet. Importantly, the similarities in thaw, total cooking, cooking drip and cooking evaporation losses of the lamb from the Dorper lambs in the current study suggest that MNM can potentially be utilised as a dietary protein source in lamb fattening diets without eliciting excessive fluid loss and hence moisture characteristics of lamb.

5.6.2.3 Shear force and myofibrillar fragmentation length

The degree of tenderness is a major determinant of the eating quality of meat. According to Cavitt et al. (2004), the most common mechanical appraisal of meat tenderness is by determining its (meat) Warner Bratzler shear force (WBSF). Meat from animals fed forage diets tends to have higher WBSF compared to that from animals fed concentrate diets (Razminowicz et al., 2006). On forage diets animals take longer to finish thus attain slaughter weight at an older age by which time their muscles would have accumulated more collagen (Miller, 2005) of decreased (collagen) solubility (Lepetit, 2007). Meat with a shear force value above 9kg is considered tough and unacceptable (Yarali et al., 2014). Results from the current study show that the WBSF of lamb aged for 1 and or 7 days, from carcasses of lambs across dietary treatments were similar (Table 5.3). Importantly, the 7-day aged lamb from carcasses across dietary treatments had a WBSF range from 2.93 to 3.36kg, which is below the 9kg shear force reported for unacceptable meat (Yarali et al., 2014). Findings of the current study therefore suggest that MNM could be used to replace SBM in lamb fattening diets without compromising the tenderness of lamb.

Gallo et al. (2009) contend that high forage diets result in muscles with short myofibrillar fragmentation length (MFL) and Muchenje et al. (2008) report that short MFL results in tough meat. Compared to muscles from animals fed high forage diets, muscles from animals fed high concentrate diets have higher activity of proteolytic enzymes that improve muscle tenderness (Lehnert et al., 2006). During muscle ageing, calpains (proteolytic enzymes) cleave proteins that are involved in inter- and intra-myofibrillar linkages and those connecting myofibrils to the

sarcolemma (Pearce et al., 2011). By cleaving the proteins, the calpains degrade and disrupt the organised lattice of the myofibrillar structure thus improving tenderness (Lian et al., 2013). Findings in this study show that the MFL of lamb aged for 1- and or 7-days, from carcasses of lambs across the dietary treatments was similar (Table 5.4) suggesting that dietary MNM did not affect MFL of the meat. This can be inferred to suggest that it (dietary MNM) can be exploited in lamb fattening diets without negatively impacting the activity of calpains and hence tenderness of meat.

5.6.3 Effect of dietary MNM on the chemical nutrient composition of lamb

5.6.3.1 Proximate content

Using the loin muscle from carcasses of Dorper lambs, research has shown that the dry matter, crude protein, ether extract and ash content of lamb range from 24.8 to 29.2%, 17.8 to 21.0%, 2.2 to 11.25% and 1.2 to 2.88% , respectively (Van Heerden et al., 2007; Schönfeldt et al., 2011; Burger, 2015). Findings of my study show that the proximate content of lamb from carcasses of male Dorper lambs was different across the dietary treatments (Table 5.5). Except for lamb from carcasses of lambs fed diet 3, results of the current study show that an increase in dietary MNM resulted in an increase in the lipid (ether extract) of the meat with lamb from carcasses of lambs fed diet 5 having the highest lipid content. The general increase in the meat's lipid content with an increase in dietary MNM could be explained by the higher residual oil (ether extract) in the MNM compared to that in SBM. Although the CP content of lamb across dietary treatments in my study were higher than that reported for lamb by other researchers [Van Heerden et al. (2007); Schönfeldt et al. (2011); Burger (2015)], the higher lipid content in the lamb (range: 16.41 to 22.57%, Table 5.5) which is higher than the 9.01% recommended by Van Heerden et al. (2007) might negatively impact consumer health.

5.6.3.2 Mineral composition

The mineral content of meat affects meat colour, flavour, odour, tenderness and oxidation (Osorio et al., 2007) thus it (mineral content) is one of the determinants of meat quality. Iron, zinc, copper, manganese and nickel are components of antioxidant enzyme system [superoxide

dismutase (SOD)] that catalyse the dismutation of superoxide radical anions that cause cellular damage (Abreu and Cabelli, 2010). The dismutation of superoxide radicals delays oxidation reactions in animal tissues (Descalzo and Sancho, 2008). Selenium serves as a component of glutathione peroxidase that catalyses the removal and decomposition of hydrogen peroxide and lipid peroxide in meat (Delles et al., 2014). The antioxidant activities of the superoxide dismutase and glutathione peroxidase inhibit the oxidative deterioration of meat, which would affect quality (Lametsch et al., 2008; Narciso-Gayatán et al., 2010). The magnesium, potassium, sodium, iron and zinc of the loin muscle from lambs fed a high concentrate diet were observed to range from 20.1 to 22.7, 291 to 323, 83.4 to 101, 0.96 to 0.99 and 2.25 to 2.71mg/100g, respectively (Van Heerden et al., 2007) while the copper content of lamb was noted to range from 0.09 to 0.14mg/100g (William, 2007). Findings in this study show that lamb from carcasses of lambs fed the control and test diets had a similar macro-mineral content except for potassium, which was highest in lamb from the carcasses of lambs, fed the control diet (Table 5.6). The higher potassium concentration in the lamb from the carcasses of lambs fed the control diet could be due to the higher dietary concentration of potassium in SBM than in MNM2 (Table 3.3). While the copper, iron and zinc (micro-minerals) concentration was similar in lamb across dietary treatments, lamb from lambs fed diet 5 (substitution of SBM with 100% MNM) had higher manganese and selenium concentration than lamb from carcasses of lambs fed the control diet. The higher concentration of manganese and selenium could not be attributed to the concentration of the minerals in MNM2, since manganese concentration was higher in SBM than in MNMs and that of selenium was undetected in the MNMs and SBM. These could be attributed to the other dietary constituents. Lamb from carcasses of lambs fed diet 1 had higher molybdenum concentration compared to that from carcasses fed diet 5. The higher concentration of molybdenum in the lamb from the carcasses of lambs fed the control diet could be as a result of the higher dietary concentration of molybdenum in SBM than in MNM2. The potassium, magnesium, sodium, iron, zinc and copper concentration in lamb across dietary treatments in the current study is higher than those previously reported (Van Heerden et al., 2007; William, 2007). Based on these findings, it could be inferred that MNM can be used in place of SBM as a dietary protein source in lamb grower-fattening diets without the risk of depleting the mineral content of lamb, particularly minerals that are components of the antioxidant system.

5.6.3.3 Fatty acid profile of lamb

Dietary lipid composition affects the quantity and quality of the fat of meat (Beriain et al., 2000; Lopes et al., 2014; Costa et al., 2015). Recent studies have shown that feeding high concentrate diets to lambs increases the concentration of saturated fatty acids (SFAs) such as myristic, palmitic and stearic acids and monounsaturated fatty acids (MUFAs) particularly oleic acid but decreases the polyunsaturated fatty acid (PUFAs viz omega-3 fatty acid) content of lamb (Lanza et al., 2011; Scerra et al., 2011). Lamb, like other ruminant meat, has been reported to have a high concentration of monounsaturated and saturated fatty acids and low polyunsaturated fatty acids concentration (Costa et al., 2012). The fatty acid composition of loin from lambs fed a high concentrate diet ranged from 48.39 to 50.03, 44.25 to 46.80, 4.92 to 6.16% and 12.68 to 25.10 for SFAs, MUFAs and PUFA, respectively (Costa et al., 2012). Results in my study conform to lamb being known to have a high TSFA and low TMUFA content as reported by Costa et al. (2012). Despite this general conformity with regards to TSFA and TMUFA content, lamb from lambs fed the MNM-based diets had a higher TSFA and lower TPUFA content; compared to that from lambs fed the SBM-based (Table 5.7). The SFAs, MUFAs and PUFAs profile of the lamb from the current study followed the same pattern as reported by Costa et al. (2012). The lamb n-3: n-6 ratio in my study ranged from 0.06 to 0.12, which is in agreement with the recommended ratio in meat (Scollan et al., 2014). A high PUFA concentration in meat is associated with an increased risk of peroxidation, which compromises meat quality (de Silva et al., 2000). It can be argued, therefore, that the low TPUFA concentration in lamb from carcasses of lambs fed the MNM-based diets could lead to an increase in the shelf life of the lamb due to a possible decrease in peroxidation. A high dietary intake of SFA increases the risk of consumers developing low density lipoprotein cholesterol mediated cardiovascular diseases (Arbex et al., 2015). It is therefore important to take caution when using MNM as a dietary protein source in lamb grower and fattening diets since in the current study dietary MNM has been observed to increased TSFA content of lamb. Despite the high TSFA and lower TPUFA content of lamb from carcasses of lambs fed MNM-based diets, its (lamb from lamb fed MNM-based diets) high oleic acid dominated TMUFA content is beneficial. Oleic acid (OA) is known to have health beneficial effects through its cholesterol-lowering, stroke reducing, systolic, and diastolic blood pressure decreasing activity (Samieri et al., 2011). Thus one could argue that the possible negative health

outcomes that could be stimulated by consuming the lamb with the high SFA content (lamb from MNM-based) could mitigated by the high OA in the lamb.

5.7 Conclusion

The aim of this study was to investigate whether the substitution of SBM in lamb grower-fattening diets with MNM, on crude protein basis, would have any effect on the carcass traits and meat quality. My findings show that MNM could be utilised as a dietary protein source in lamb grower-fattening diets without compromising meat yield as determined by the warm and cold carcass weight and dressing percentage. Dietary MNM also did not affect the warm and cold muscle pH and temperature. Additionally, it (dietary MNM) could be used to replace SBM as a dietary protein source in lamb grower-fattening diets without the risk of negatively affecting the physical quality traits (colour, drip loss, moisture characteristics and tenderness) of lamb. Based on the results, dietary MNM could be used to increase the OA content in lamb. However caution needs to be taken as the use of MNM as a dietary protein source in lamb grower-fattening diets caused a significant increase in the TSFA content of lamb which (increased TSFA) could negatively influence the consumer perception of the product. Overall, MNM could potentially be utilised as a partial or complete substitute of SBM in lamb grower-fattening diets without compromising the carcass and physical properties of lamb.

Having interrogated the chemical potential of MNM to supply dietary protein (Chapter 3), the effects of its substituting of SBM on the growth performance and health profile of lambs (Chapter 4) and on its effects of dietary MNM on the carcass characteristics and meat quality (Chapter 5), the next chapter gives a summary of the main study findings (conclusions), limitations and recommendations for future research.

CHAPTER SIX - CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The chemical nutrient and ANFs composition of Marula nut meals (MNMs) were determined with the aim of ascertaining the meals' potential as a dietary protein source in livestock feeds, with a particular target being sheep fattening diets. Despite their lower CP content compared to that of SBM, the CP content of the MNMs was within and at times higher than the reported CP content of other plant-derived dietary protein sources in livestock feeds. The MNMs had a relatively poor essential amino acid content compared to SBM, thus for use in monogastric feeds, there might be a need to supplement MNM with amino acids in order to meet the essential amino acid requirements.

The MNMs had a high residual oil component which was found to be beneficial in that it negated the need for supplementary oil as an energy source. With an increasing use of SBM diet, there was need to supplement them with canola oil (additional feed formulation cost) to meet dietary energy requirements for growing fattening lambs. The residual oil in MNMs had a fatty acid profile which was beneficial for health.

The graded dietary substitution of SBM with MNM had no negative effect on nutrient intake, growth performance, rumen function, viscera macro-morphometry, circulating and stored metabolic substrates. In livestock production, the focus should not only be on the products, but the health of the animals should also feature prominently. The surrogate markers of liver and kidney function and the general health profile of male Dorper lambs were not negatively impacted by the dietary MNM.

Dietary MNM did not compromise carcass yield, dressing percentage and the physical attributes of the lamb. While the partial replacement of SBM with MNM in lamb fattening diets produced lean meat with a high protein and a low fat content, complete replacement of SBM with MNM yielded meat rich in oleic acid.

My study has shown that the biomass of MNM, which is currently being underexploited should be used as a locally produced non-conventional dietary protein and energy source in fattening lamb feeds, thus adding to the value chain of Marula and cutting the import bill for SBM.

6.2 LIMITATIONS AND RECOMMENDATIONS

In the current study, I did not carry out an *in vivo* digestibility trial prior to determining the dietary effect of substituting SBM with MNM on the growth performance of male Dorper lambs. An *in vivo* digestibility trial would have helped to generate valuable data in regard of both total tract digestibility of nutrients and gastrointestinal tract segment specific nutrient digestibility, for example, ileal digestibility of protein and or specific amino acids.

For purposes of increasing the accuracy of data collection during this study, each of the lambs was confined in a metabolic crate. However, some parameters might have been affected as a result of stress induced due to the confinement in metabolic crates. Future studies should measure markers of stress (e.g. corticosteroids) and undertake the feeding trials under conventional housing of lambs.

With regards to the evaluation of the quality of the lamb (Chapter 5), I did not determine the intramuscular fat which could have given further insight on the effect of dietary MNM on meat quality since intramuscular fat is a major determinant of eating (flavour, aroma, and taste) quality of meat. While a complete replacement of SBM's dietary CP contribution with CP from MNM yielded relatively low protein content in meat, an evaluation of the amino acid composition of the meat would have provided nutrition information regarding the protein quality of the meat.

Although clinical biochemistry assays can be used as surrogate markers of health, more sensitive assessments such as vital organ histology and assays for tissue specific molecular markers of function should be considered in future studies.

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APPENDICES

Appendix 1: Animal ethics clearance certificates

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2014/50/B

APPLICANT: Ms IMM Malebana

SCHOOL: Physiology

LOCATION: Faculty of Health Sciences

PROJECT TITLE: *Dietary effects of Sclerocarya birrea caffra seed meal in growing-fattening male Doper sheerDoper sheep*

Number and Species

56 Doper Lambs

Approval was given for to the use of animals for the project described above at an AESC meeting held on 26 August 2014. This approval remains valid until 25 August 2016.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

None.

Signed: _____ (Chairperson, AESC) Date: 12th SEPTEMBER 2014

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ (Registered Veterinarian) Date: 12TH SEPTEMBER 2014

cc: Supervisor: Prof K Erlwanger & Dr E Chivandi
Director: CAS

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ARC • LNR
Excellence in Research and Development

Date: 19th December 2014

Dear IMM Malebana,

Re: Ethical evaluation of the project entitled "Dietary effects of *Sclerocarya birrea caffra* seed meal in growing-fattening male Dorper sheep".

Your application for the ethical evaluation of the project entitled "Dietary effects of Sclerocarya birrea caffra seed meal in growing fattening male Dorper lambs" has been finalized and approved. Its Ref no is **APIEC14/019.**

I would like to inform you that the project was evaluated and found to be ethically acceptable. Please note that should any amendments or changes be made to the protocol, you are obliged to submit an application to the Ethics committee and that the protocol should be resubmitted for review annually.

Regards

Dr. C. M. Pilane (Ph.D.)
Interim-Chairperson ARC-API Ethics Committee
Tel. No. -012-672-9337
Fax/Faks (012) 665-1604
E-mail/E pos: cyril@arc.agric.za

Appendix 2: Animal transportation certificate and identification document

Vervoer verwyderings sertifikaat.

Wet op veediefstal 57/1959 ART/SEC 6 Dokument van identifikasie

ART/ SEC 8 Verwydering Sertifikaat

VERKOOPER SE NAAM EN ADRES: Pos Bus 12689. Kiewiet Straat. Onderstepoort. 0110.

NAAM VAN TRANSPORTEERDER TELEFOON NOMMER: Mrs. Lize-Marie de Klerk. +2772 380 0846

VOERTUIG REG: FP 52 ZN GP MAAK: Toyota Hilux Bakkie.

ROETE: N1 Gauteng

NA: Agricultural Research Council, Irene

GETAL	RAS OF SOORT	GESLAG	KLEUR	BRAND OF IDENTIFIKASIE
40	Dorper lammers	Mans	Swart wit	Oor gemerk

Hiermee sertifiseeer ek Lize-Marie de Klerk dat ek die wetige eienaar van bogenoemde vee is/ dat ek behoorlik deur die eienaar gemagtig is om die vee te vervoer.

Datum uitgereik: 23 August 2015

Handteking Eienaar/ Koper:

Handteking Bestuurder: