EVALUATION OF OIL CAKES FROM AMARULA (*SCLEROCARYA BIRREA*), MACADAMIA (*INTEGRIFOLIA*) AND BAOBAB (*ADANSONIA DIGITATE L.*) AS PROTEIN SUPPLEMENTS FOR RUMINANT DIETS

by

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DECLARATION

I, Johannes Solomon Mogotsi Phenya declare that this dissertation, hereby submitted for the Master of Science degree in Agriculture at the University of South Africa is my own work and has not been submitted to any institution for a degree.

Signature... Date...

ABSTRACT

The current research was done to evaluate the nutritive values and the ruminal degradation of dry matter (DM) and crude protein (CP) from three non-conventional oil cakes, viz: amarula (*Sclerocarya birrea*) (AOC), macadamia (*Integrifolia*) (MOC) and baobab (*Adansonia digitate L.*) (BOC). The oil cakes were collected from biodiesel producers in Limpopo Province, transported to the ARC-Animal Production campus, where proximate and ruminal nutrient degradation analysis were conducted. Triplicates samples from each oil cake were analyzed for the nutritive values, mineral and amino acids contents. Three rumen cannulated mid-lactating (days in milk; DIM: 180±5) Holstein cows weighing 667±43 kg body weight were allocated to determine the *in situ* ruminal dry matter (DM) and crude protein (CP) degradation. The cows were offered a totally mixed ration (TMR) (60 concentrate: 40 forage ratio) that was compounded according to their daily nutrient requirements, and were milking was done twice per day at 12 hrs intervals. The three oil cake samples were ground using a 2-mm screen after which sub-samples (6.5 g) were put in 10 x 20 cm; 50 µm pore size polyester bags to achieve 15 mg/cm² (ratio of the sample size to surface area). The bags were then fistulated in each cow's rumen in triplicate for a period of 2, 4, 8, 16, 24, or 48 hrs. After being incubated, the bags were removed from the rumen and washed with cold (4°C) water in 20-L buckets. Following immersing in cold water, the bags were machine washed until clean water was obtained. The bags were then dried at 60 °C in an oven for 48 hrs. The dried bags were individually weighed, and the content of each bag were removed and stored into glass vial until analysis. The remaining two duplicate sets of each sample were rinsed using cold water in order to determine solubility at 0 hrs. The AOC had higher ($P<0.05$) ether extract (EE) and CP content than both BOC and MOC. Macadamia oilcake

(MOC) and BOC had higher ($P<0.05$) fractions of fibre (NDF, ADF and ADL) compared to the AOC. The AOC had greater ($P<0.05$) content of essential amino acids than in the BOC and MOC. Additionally, AOC had a high ($P<0.05$) phosphorus, but low calcium and potassium concentration. While AOC had high effective degradability of DM, it also had high water soluble as well as DM and CP rapidly degradable fractions. Effective degradation of CP was higher in AOC and BOC than in MOC. However, BOC had a high insoluble but degradable fraction of CP. Further work to determine the toxicology of these non-conventional oil cakes and animal feeding experiments is needed.

Keywords: degradability, energy, fats, fibre, ruminants, soybean meal

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LIST OF ABBREAVIATIONS

ADF – Acid detergent fibre

ADIN – Acid detergent insoluble nitrogen

ADL – Acid detergent lignin

ANTFs – Anti – nutritional factors

AOAC – Association of Official Analytical Chemistry

AOC – Amarula oil cake

BOC – Baobab oil cake

CP – Crude protein

D – Degradation

DAFF – Department of agriculture forestry and fishery

DM – Dry matter

DIM – Days in milk

DPW – Dried poultry waste

ED – Effective degradability

EE – Ether extract

FAO – Food and allied organisation

GIT – Gastrointestinal tract

IVOMD – *In vitro* dry matter digestibility

LSD – Least significant difference

MBM – Meat and bone meal

MOC – Macadamia oil cake

NDF – Neutral detergent fibre

NPN – Non-protein nitrogen

NRC – National Research Council

RUP – Rumen undegradable protein

SBM – Soybean meal

SEM – Standard error mean

TMR – Total mixed ration

WHO – World health organisation

CHAPTER 1

1.1 Introduction

Protein is a main nutrient, which is limiting in nutritionally inadequate forages that are mostly fed to ruminants by smallholder ruminant production in South Africa. Consequently, these farmers use wastes from cereal grains, oil seeds and animals (e.g. poultry litter) as sources of protein supplements to improve the quality of nutritionally inadequate forage based diets for feeding their ruminants (Ajila *et al.,* 2012). This supplementation helps to supply N to rumen microbes.

Soybean meal (SBM) is one of the widely preferred dietary source of protein in the South African animal feed production due to its rich crude protein (CP) content (Miller-Cushon *et al.,* 2014) and contains highly digestible amino acids (Kocher *et al.,* 2002). However, the demand for SBM by the South African livestock feed industry exceeds its production locally (Dlamini *et al.,* 2014) hence the importation of SBM (DAFF, 2012). The importation of SBM increased livestock feed costs, which thus negatively affects intensive animal production. Therefore, alternative protein sources should be found to cover the shortages of protein supplements for livestock production. Although the use of feed urea as a source of protein in ruminant diets is common, urea supplementation has its own limitations, which includes poisoning (Mentz *et al.,* 2015). In addition, the law (Act 36, 1947) in South Africa discourages the use of animal-derived protein sources. Fortunately, Silva *et al.* (2016) highlighted some studies that have proven that other by-products from the biodiesel production contain adequate quantity of protein and or energy that are essential for the nutrient requirements of animals. The by-products can thus be used to either complement or substitute conventional feedstuffs used in ruminant diets such as SBM.

Oil extraction from oilseeds in the biodiesel production plants results in the availability of oil cakes, which contain appreciable amounts of nutrients that can be beneficial to livestock production. Oil seeds such as amarula nuts, macadamia nuts and baobab seeds are used to produce oil in the biodiesel production industries in South Africa, which result in the availability of oil cakes. The oil cake from amarula nut (AOC) is reported to contain 39 % CP and a gross energy (GE) of 29 MJ/kg DM (Malebana *et al.,* 2018). Skenjana *et al.* (2006) reported macadamia oil cake (MOC) to have an *in vitro* dry matter digestibility (IVOMD) of 79.2 %, which makes it a valuable resource for animal nutrition. In addition, baobab oil cake (BOC) has been reported to contain 22.9 % CP (Chisoro *et al.,* 2018), which might contribute positively in the diets of ruminants.

1.2. Problem statement

The availability of nutrients in feed for ruminants is determined using various methods that includes *in situ*, *in vitro* and *in vivo*. However, the *in situ* (nylon bag technique) (Ørskov and McDonald, 1979) is the preferred method for studying the kinetics of nutrient degradation. Consequently, feed ingredients' degradability is essential to evaluate feed ingredients utilisation (Lei *et al.,* 2017). Malebana *et al.* (2018) compared the nutritive of AOC produced by either hydraulic filter press or cold press to that of soybean meal SBM but the nutrient degradability of the AOC was not tested. Chisoro *et al*. (2018) fed diets that contained degraded levels of BOC to broilers, and reported that 5 % inclusion level of dietary BOC improved growth performance of the broilers. Similarly, Acheampong-Boateng *et al*. (2017) reported that MOC should not be added at more than 5 % in diets of growing lambs. Data on the *in situ* degradation of nutrients from the three non-conventional oil cakes (i.e. AOC, BOC and MOC) is limited. In addition, the chemical composition of oil cakes varies widely, which might be attributed to the oil production methods (Malebana *et al.*, 2017), the structure of the seed/nut and the cultivar.

1.3. Justification of the study

Improvement of animal production is dependent on adequate supply of nutritious feedstuff. Protein and energy are essential nutrients whose supply in the South African feed industry is currently insufficient. Sources of the protein and energy are mainly from conventional oilcakes. Researches have been carried out to determine the potential use of non-conventional oilcakes as potential nutrient sources to complement the use of conventional oilcakes in feed. Amarula, macadamia and baobab oilcakes have been identified as such non-conventional oilcakes. Although several methods of analysis to evaluate the viability of amarula, macadamia and baobab oilcakes as nutrient sources have been conducted, the in *situ* degradability of nutrients from these three oilcakes has not been done. The *in situ* degradability of nutrients provide an insight on the degradability and digestibility of the nutrients by the animal. The release and availability of nutrients to the animal for maintenance, reproduction and production is affected by its ability to be degraded and digested. Hence, the *in situ* degradability of dry matter and crude protein from the amarula, macadamia and baobab oilcakes is imperative.

1.4 Research Aim

The aim of this study was:

To evaluate the nutritive value of amarula, macadamia and baobab oilcakes, and determine the DM and CP *in situ* degradability of the oilcakes.

1.5 Research Objectives

Objectives of this study were:

1.3.1. To determine nutritive values of the three non-conventional oilcakes.

1.3.2. To conduct an *in situ* degradability of DM and CP for the three non-conventional oilcakes.

1.6 Hypotheses of this study were that:

1.6.1 H_0: There are no differences in the nutritive value of amarula, macadamia and baobab oilcakes.

H_1: There are differences in the nutritive value of amarula, macadamia and baobab oilcakes.

1.6.2 H_0: There are no differences in the *in situ* degradability of DM and CP from amarula, macadamia and baobab oilcakes.

H_1: There are differences in the *in situ* degradability of DM and CP from amarula, macadamia and baobab oilcakes.

CHAPTER 2

LITERATURE REVIEW

2.1 Proteins

Proteins are macromolecules containing nitrogen and formed by amino acids linked by peptides (Hou *et al.*, 2017). Proteins function as major structural component of body tissues including muscles. They are utilised for production of hormones, haemoglobin and hormones. Although they are not the primary source of energy, they can be used as energy source (Hoffman and Falvo, 2004). As a condition for utilization by the body, proteins should be metabolized into simpler form of amino acids. To date, only twenty amino acids (AA) have been identified, which are required for development of body tissues and metabolic pathways in living organisms (Hoffman and Falvo, 2004). Of the twenty AA, twelve are non-essential (synthesized/produced by the body), while the remaining are the essential (unable to be synthesized by the body, hence derived from diets).

Proteins for animal feeding can be obtained from animal sources that include insect-based products (e.g. larvae and maggot meal), poultry by-products (e.g. feather meal), fish by-products (e.g. fishmeal), abattoir wastes (e.g. meat and bone meal and blood meal) as well as from plants like fodder trees, shrubs, legumes and oilseed cakes and meals (Ahmad *et al.*, 2017). Generally, dietary animal protein sources are referred to as balanced proteins since they possess all of the essential amino acids. According to Hoffman and Falvo (2004), proteins from plant resources are however regarded incomplete due to shortages of some of the essential amino acids. Plant-derived protein sources can be categorized into conventional and non-conventional protein sources. Majority of the conventional protein sources have been scientifically proven as potential nutrients for animal diet formulation. Although research on the utilization of non-conventional is ongoing, several studies have reported satisfactory results, which showed the significant role that the sources play in animal production (Lamba *et al.,* 2014).

In the animal production system, dietary proteins are significant macronutrients and costly constituents of animal feed (Raj *et al.,* 2016). Cost of protein account for 15% of feed cost in animal production (Singh, 1990; Banerjee, 1992). While the use of protein for feed in South Africa is expected to rise from 1.98 million tons in 2015 to 2.8 million tons by 2024, domestic supply of protein is expected to increase from 60 to 79% within the same years (de Jager, 2016). Hence, the need to research for cost effective and available alternative protein sources to contribute into the pool of providing high quality feeds to ruminants. However, the effectiveness of nutrients (e.g. protein and energy) in ruminant rations is accomplished through evaluation of its quality (Hoffman and Falvo, 2004).

Protein quality is critical for provision of nutritional benefits to animals (Hoffman and Falvo, 2004). The quality refers to the characteristics of protein source towards its capability to attain envisaged digestibility functions (Millward *et al.,* 2008). Consideration in terms of the ability of a ration to supply substrates needed for nitrogen synthesis and other biogenesis pathways, that is, suitable source of amino acids and nitrogen is included when assessing protein quality (Millward *et al.,* 2008). In the process of assessing protein quality, evaluation of amino acids composition, bioavailability of amino acids and digestibility (utilization of protein) are essential for predicting the metabolic function of the proteins in the animal (FAO/WHO, 1990). Several measurements and techniques are used to evaluate the quality of protein.

2.1.1 Role of proteins in animal nutrition

Requirement of protein by ruminants is provided by ruminal synthesis of microbial protein from degradable and undegradable dietary protein sources. The undegradable feed ingredients are not digested by rumen microorganisms but bypasses to the abomasum and small intestines (Şehu *et al.,* 2010). The influx and digestibility of protein from microbes determines the availability of metabolizable protein anticipated to be absorbed in the small intestines (NRC, 2001). Hence, the application of metabolizable protein for purposes of production depends on the evaluation of rumen microbial protein production, and the quantity and digestibility of undegradable protein source available in feedstuff/ingredients (Gao *et al.,* 2015). Some researchers (e.g. Chiou *et al.,* 1995) have shown that increased dietary protein content in livestock ration does not lead to improved animal performance. This can be attributed to the imbalances amongst fermentable carbohydrates (van Straalen *et al.,* 1997) and oversupply of ruminal degradable protein or amino acid absorption (Harstad and Prestløkken, 2001). Thus, more research is necessary to evaluate nutrient degradability, intestinal digestibility, amino acid composition, utilization of nitrogenous feedstuff from different protein sources (Gao *et al.,* 2015). Moreover, accurate data for the kinetics of ruminal degradation of protein is critical.

2.1.2 Different types of protein sources for animal nutrition

2.1.2.1 Animal-derived protein sources

2.1.2.1.1 By-products from fish industry

Fish processing by-products denote to the remaining tissues after much of the fish muscles have been removed (Bechtel, 2007). Wastes from processing of fish are utilised to harvest fishmeal and or fish oil. The primary use of the two commodities is as protein and energy sources in aquaculture, poultry and ruminant feed industries (Bechtel, 2007). Since the major nutrient component of fish is protein, fishmeal is considered as unconventional raw material for generation of high-protein ingredient in feed. This is attributed to the high content of proteins with good amino acid composition and digestible nutrients for the animals. When comparing fishmeal with other conventional feed ingredients (blood meal, by-products from poultry wastes, rendered meat meal and soybean meal), Cho and Kim (2011) found that the balanced content of amino acids in fishmeal, makes it a good ingredient for feed formulation.

2.1.2.1.2 Insect-based products

Studies on the potential of insects-based products like maggots, larvae meal, earthworms, termites and garden snails as nutrient supplements in animal ration have been conducted (Ugwumba and Ugwumba, 2003; Makinde, 2015; Mohanta *et al.,* 2016). Due to their small life cycle and large production biomass, these products are considered a viable option to explore as feed in animal production (Sheppard, 2002). Maggot meal has good nutritional value with a protein content range of 43 and 64% (Fasakin *et al.,* 2003; Awoniyi *et al.,* 2003). In additional to its nutritive value, maggot meal is cost effective and less tiresome to harvest compared to other protein sources from animal origin, hence can be utilised in feed formulation to replace the traditional protein sources. Moreover, maggot feed is generated from waste products, which would otherwise pollute the environment (Ogunji *et al.,* 2008). Dietary meal from larvae has been reported as a potential protein source to replace soybean meal (SBM) in monogastric diet mainly because of its balanced profile of amino acid consisting of high proportion of methionine and lysine (Leiber *et al.,* 2015).

2.1.2.1.3 Blood products and blood meal

Blood from monogastric animals is classified as animal by-products that may be utilised to produce both blood products and blood meal for purposes of animal feedstuffs (Jedrejek *et al.,* 2016). Blood products are generated from blood resulting from slaughterhouses that are fitted with separation systems, which allows the removal of blood from the animals that fail post-mortem examination (Jedrejek *et al.,* 2016). Blood meal on the other hand is sourced from animal by-product materials wherein no separation system in place is available, but heated to treat the blood from slaughtered warm-blooded animals. While products harvested from blood may be utilised as feed ingredients meant for monogastrics, blood meal can be used only for aquaculture feeding (Béné *et al.,* 2015). The two products have high content of protein with reasonable good balanced amino acids and heme iron (Wan *et al.,* 2002).

2.1.2.1.4 Meat and bone meal

Another feed ingredient from animal origin is meat and bone meal (MBM) which can be derived from slaughter wastes and be incorporated in poultry rations (Caires *et al.,* 2010). According to Mateos and Lazaro (2000), MBM could be incorporated in poultry rations up to 10% provided the nutrient variability is minimised. The MBM is known as an economical source of protein that can partially replace SBM in animal rations (Caires *et al.,* 2010). Moreover, MBM is being utilised in animal nutrition as a source of protein to replace protein-rich feed due to its essential amino acid content, vitamin B_{12} and minerals.

2.1.2.1.5 By-products from poultry

By-products from poultry meals are wastes resulting from poultry production and processing industries such as poultry litter and skin, gizzard and heart (SGH) (Jayathilakan *et al.,* 2012). The animal by-products are essential sources of amino acid consisting high content of protein and energy content as well as total digestible dry matter comparable to that of fishmeal (Bureau *et al.,* 2000). Thus, by-product meals from poultry are considered feasible replacements in lieu of fishmeal (Soltan, 2009).

2.1.2.1.6 Advantages of animal-derived protein sources

The production system for the majority of animal by-products serves a binary purpose of providing a nutrient-rich source and a source of waste reduction and transformation, thus mitigating environmental pollution (Yones and Metwalli, 2016; Jayathilakan *et al.,* 2012; Kamruzzaman *et al.*, 2006). Apart from fishmeal, several studies have reported that animal by-products can be utilised in feed formulation since they are less costly. In addition, animal by-products possess significant levels of digestible nutrients that are necessary for animal performance (Jayathilakan *et al.,* 2012).

2.1.2.1.7 Disadvantages of animal-derived protein sources

The utilisation of animal by-products is however challenged by incidents associated with bovine spongiform encephalopathy (BSE) through animal feed. Hence, as part of safety measures the use of by-products derived from animals or mammalian tissue, as protein or nutrient source in feeds for animals has been banned (Momcilovic and Rasooly, 2000). South

Africa was not an exception in countries that were banned from using animal origin feed ingredients (Van Ryssen, 2001).

2.1.2.2 Plant-derived protein source

2.1.2.2.1 Fodder trees, shrubs and legumes

Until recently, shrubs and fodder trees have represented a great potential protein source aimed for ruminants (Devendra and Sevilla, 2002). Due to inadequate knowledge on numerous characteristics of their use and a shift of focus to more innovative systems of feeding, fodder trees and shrubs are no longer fully utilised (Devendra and Sevilla, 2002). Legume grains like pea, lupin and faba bean are easily cultivated, and as such can be utilised for feeding animals since they remain rich sources of nutrients, particularly minerals and amino acids (Ragab *et al.*, 2010; Facciolongo *et al.*, 2014). Legume grains contains high protein content, which is readily available and soluble, however, Hay and Probert (2013) reported that legume grains may not completely replace soybean meal in ruminant ration. Ruminants utilise protein in forage legumes poorly, due to a wide catabolism to non-protein nitrogen (NPN) composites throughout harvest and storage period, accompanied by more degradation in the rumen (NRC, 1989). While there are ongoing researches on the utilisation of legumes as sources of protein in animal nutrition, there is less attention on the utilisation of legume seed oil cakes/meals in ruminant feed (Okunade and Olafadehan, 2017).

2.1.2.2.2 Oilcakes and meals

Oilcakes and seed meals are by-products of seeds oil-extraction. Seed oilcakes and meals from soybean, sunflower, groundnut, cottonseed, canola and olive are considered as conventional sources of nutrients for animal feed (Ramachandran *et al.*, 2007). Non-conventional plant-derived protein sources include oilcakes and meals from baobab, macadamia, marula, castor, karanja, neem, jatropha, palm and rubber seeds (Alimon, 2004; Sharma *et al.,* 2014; Acheampong-Boateng *et al.,* 2017; Chisoro *et al.,* 2018). The non-conventional protein sources possess the nutritional potential of being utilised as nutrient (energy, protein, amino acids and minerals) sources for animal feed (Sudake *et al.,* 2013).

However, chemical composition of oil cakes and meals varies according to their edaphic conditions, quality of seeds and nuts, extraction methods (industrial processing methods) and storage parameters (Ramachandran *et al.,* 2007). Further to differences in the content of nutrients in non-conventional plant-derived source, they contain anti-nutritional factors (ANTFs) that can compromise animal performance when used as feed ingredients and thus their utilization is limited by the presence of ANTFs (Marghazani *et al.,* 2013). The ANTFs are substances produced naturally in feed ingredients by the regular metabolism of plants and interaction through diverse mechanisms (Marghazani *et al.*, 2013). Saponin, oxalic acid, gossypol, phytate, cyanogen, tannins, nitrates, protease inhibitors, alkaloids, aflatoxins and mycotoxin are some of the ANTFs that are found in plant-derived nutrient sources (Woyengo *et al.,* 2016; Tadele, 2015). By interacting with others components ANFTs can result in deactivation of nutrients, interfere the digestive system and interfere with feed metabolic utilization. Interference of metabolic feed utilization negatively impacts on animal performance by reducing nutrient absorption, digestion and performance (Akande *et al.,* 2010; Njidda and Ikhimioya, 2012). Nonetheless, different methods such as grinding/milling, heat treatment, pelleting, husking/hulling, alcohol/chemical extraction, fermentation, enzyme utilisation and germination have been employed to minimise the concentration of ANTFs in oilseeds (Ogbuewu *et al.,* 2010; Marghazani *et al.,* 2013). Reduction of the anti-nutritional concentrations in oilseeds (particularly the non-conventional) accelerates their potential to be explored as alternative protein sources in animal feed.

2.1.2.2.3 Conventional oilcakes and meals

Conventional plant-derived protein sources have ample nutrient value with a protein content range of between 15 and 50% (www.seaofindia.com, 2018) as cited by Ramachandran *et al.* (2007). Of the plant-derived conventional protein sources that are utilised in the livestock feed industry in South Africa, SBM is the commonly utilised source of protein (Acheampong-Boateng *et al.,* 2016). The SBM is highly preferred owing to its high protein content of 48 %, coupled with well-balanced content of amino acids and high digestibility of nutrients (Wickramasuriya *et al.,* 2015). However, utilisation of these sources of protein in animal feedstuff production is hindered by their limited supply due to seasonal availability and climatic variations (Acheampong-Boateng *et al.*, 2016). For instance, according to the Bureau for Food and Agricultural Policy (2018), South Africa will need to import approximately 1 million tons of soybean oilcake by 2019 in order to supplement the shortfall in domestic production. Inadequate availability of the protein sources leads to high pricing that translates to costly animal feeds (Malebana *et al.*, 2018). The high cost of SBM is further accelerated by its high demand in intensification of animal production meet the consumers demand for animal protein (Adeniji, 2008). This implicates smallholder farmers negatively by not being able to afford good quality feed and consequently compromising on animal performance (productivity and production).

2.1.2.2.4 Non-conventional oilcakes and meals

Due to their nutritive value (especially high crude protein and energy content), non-conventional protein sources are at times comparable to other conventional protein sources, and thus may be utilised as potential non-conventional sources of protein for formulating ruminant rations (Gowda and Sastry, 2000; Raj *et al.*, 2016). For an example, in a trial evaluating dietary effects of feeding castor seed cake in place of groundnut cake on protein source basis in lamb diets, no adverse effects were reported on the performance (growth), feed intake, nutrient digestibility and calcium, phosphorus as well as nitrogen balance. However, metabolizable energy was lower in rams that were fed on a ration that contained castor seed cake in comparison to those fed ration supplemented with groundnut cake (Nagalakshmi and Dhanalakshmi, 2015). Table 2.1 demonstrates the nutritive value (proximate analysis) of conventional as compared to that of non-conventional protein sources. This Table shows that non-conventional oilcake contain valuable nutrients that can contribute immensely in animal nutrition.

Table 2.1 Proximate analysis (%) of conventional and non-conventional feed resources.

Feed	DM	CP	Ash	EE	GE*	NDF	ADF	References
Conventional								
Soybean meal	89.2	46	8.4	1.4	-	9.5	6.1	Landero *et al.*, 2013
Groundnut meal	94	23	26	9.3	-	-	-	Mustafa *et al.*, 2001
Sunflower meal	-	27	4.8	31	-	-	-	Nkosi *et al.*, 2011
Cottonseed meal	91	45	7.1	1.6	-	-	17	NRC, 1984
Canola seed meal	94	26	6.1	19.5	-	-	-	NRC, 2001
Non-conventional								
Marula seed meal	90	47	5.6	39	-	19	13	Mlambo *et al.*, 2011b
Macadamia seed oilcake	94	20	2.8	10	-	52	42	Acheampong-Boateng *et al.*, 2008
Baobab seed oilcake	91	23	4.6	8	-	42	36	Chisoro *et al.*, 2018
Jatropha meal	-	36	9.4	9.3	18	4.3	-	Makkar and Becker, 1999
Castor bean cake	93	29	-	10.3	-	57	40	Alves *et al.*, 2016
Rubber seed meal	96	17.4	3.0	69	-	-	-	Eka *et al.*, 2010

*= MJ/kg DM, DM = dry matter, CP = crude protein, EE = ether extract, GE = gross energy, NDF = neutral detergent fibre, ADF = acid detergent fibre.

2.1.2.3 Non-protein nitrogen

Non-protein nitrogen (NPN), a nitrogen source utilised in animal nutrition and includes components such as urea and other compounds that are none proteins but can be transformed to proteins by rumen microorganisms (Tadele and Amha, 2015). The NPN can also be converted to urea by generating ruminal ammonia that is transported to the liver and converts to urea (Tadele and Amha, 2015). Urea is commercially, a source of NPN for utilisation in animal feeds. It contains 47% of nitrogen as compared to the 16% that is contained in other proteins (Tadele and Amha, 2015). In comparison to other conventional sources of protein, which include oilcakes and meals from soybeans, groundnuts and cottonseeds, urea is less costly hence preferred to be used by smallholder farmers. Urea in commonly used to formulate rations for dairy cattle, sheep and beef cattle. In study using sorghum as a nutrient source to feed cattle, supplementation of urea improved the calves weight at birth, live weight and growth rate, improved milk yield and reduced mortality of neonatal (Ryley, 1961). Beames (1963) attested that addition of urea in animal ration enables animals to survive in less favourable dry conditions. According to Harris and Mitchell (1941), feeding lambs ration that contain nitrogen at 40 to 65% in the form of urea led to improved body weight and retention of body nitrogen.

2.1.2.3.1 Advantages of non-protein nitrogen

Feeding supplements containing low levels (1 to 1.5%) of urea (NPN compound) to ruminants daily improves its utilization as opposed to abrupt or periodic intake. Continuous intake of urea containing supplements in ration improves microbial activity thus growth performance (Pandey *et al.*, 2011a).

Dried poultry waste (DPW) is a compound that contains high quantity of NPN in the form of uric acid, which is rumen degradable (Lanyasunya *et al.,* 2006). Uric acid is used by rumen microbes for production of protein. Due its difficulty to dissolve in rumen fluid, ammonia is slowly released making it more efficient to be utilised (Lanyasunya *et al.,* 2006). Hence, DPW may be utilised as a CP supplement in the diets for growing and finishing lambs (Bierman *et al.,* 1996).

2.1.2.3.2 Disadvantages of non-protein nitrogen

While replacement and supplementation of low nutritive value with urea is advisable for feeding ruminants, levels of urea above 3% is associated with toxicity and can lead to mortality (Tadele and Amha, 2015). Urea toxicity is associated with high intake of diets that contain urea and directly feeding urea supplemented diets that lack source of fermentable carbohydrates (Uzatici, 2012). Common cause of urea toxicity include inaccurate formulation of ration, poor feed mixing, short adaptation period, low water intake, feeding urea with poor-quality fibre-based diet that might increase ruminal pH (Pandey *et al.,* 2011b).

2.1.2.4 Amino acids

Amino acids (AA) are organic substances that consist of building blocks (amino and acid groups) for proteins, and are found in cells for synthesis of polypeptides (Hou *et al.,* 2017; Wu, 2009). The carbon skeletons of the following amino acids are not made from non-amino acids particles in animal cells (Wu, 2013): cysteine, lysine, isoleucine, histidine, leucine, methionine, threonine, phenylalanine, tyrosine, tryptophan and valine. These amino acids are categorised as essential amino acids and should be incorporated in the diets for Monogastric animals. Inclusion of the essential amino acids in monogastric diets maintains the biological and physical functions of tissues, cells and body system (Baker, 2009). The non-essential AA however, are sufficiently made in the body to meet the body's optimum health and growth. The non-essential amino acids have critical regulatory functions in nutrient metabolism to favour reduction of white adipocytes and growth of lean tissue (Dai *et al.,* 2012). For achievement of optimum growth and performance, animals require nutrients for both essential AA and non-essential AA (Wang *et al.,* 2013). Animals require AA in two classifications namely: qualitative and quantitative (Wu, 2013). Qualitative requirements pertain to amino acids essential for maintenance, performance (growth, reproduction and lactation) and optimal health resistance to infections, stoppage of chronic metabolic disorders and prompt recovery from sickness. The quantity of AA that is vital for maintenance, performance and optimal animal health on the other side is referred to as quantitative animal requirements. Oilseeds are good sources of essential AA namely, arginine, glycine, alanine, methionine and leucine; and other amino acids are present in moderate quantities. Serine is one of the amino acids that are lacking in oilseeds (Ingale and Shrivastava, 2011). Experiments have been carried out to figure out both the former and the latter and found that by measuring the nitrogen losses in animals fed a nitrogen diet free in amino acids through gas, urine and faeces, minimum requirements of amino acids by animals can be estimated (Kim *et al.,* 2004; Wu, 2013).

2.2 Evaluation of protein quality

The value of protein in ruminant feed is extensively affected by the degree of degradation in the rumen (Sharma and Singh, 1997). There is a need to understand feed protein degradation as a prominent way of evaluating feed protein and determining protein requirements of ruminants (NRC, 1985). Comprehension of the degradability of feed protein necessitate suitable database that permits the use of protein evaluation systems in practise to formulate ruminant diets as well as predict animal response (Sharma and Singh, 1997). Accurate estimation of ruminal protein degradability provides effective formulation of ruminant diets, thus improve ruminant performance (Sharma and Singh, 1997). Feed nutrient availability may be evaluated through different methods, which includes *in vitro*, *in sacco* and *in vivo* digestibility/degradability (Habib *et al.,* 2013).

2.2.1 *In vitro* (two-stage technique)

In vitro (two-stage technique) technique mimics the gastrointestinal tract conditions on how feed ingredients are digested and absorbed (Cheli *et al.,* 2012). The technique does not require the use of cannulated animals and hence not labour intensive and is cost effective (Aufrère and Guérin, 1996). Due to the use of enzymes when carrying out the method, the *in vitro* technique is ethically accepted than other chemical methods employed when evaluating nutrient quality (Tilley and Terry, 1963). The enzymatic method predicts feed value better and generates estimates closer to values produced by *in vivo* digestibility trials (Aufrère and Guérin, 1996).

2.2.2 *In sacco* (*in situ* or nylon bag technique)

In sacco (*in situ* or nylon bag technique) is a widely used method that is effective in estimating potential degradability of feed ingredients and or nutrient for ruminants (Homolka *et al.,* 2002; Şehu *et al.,* 2010). The technique is regarded as the most reliable method for protein evaluation (NRC, 2001), and a reference method to evaluate ruminal degradation dynamics of feed and or nutrients including protein (Habib *et al.,* 2013). The procedure for the *in sacco* technique is based on the use of synthetic fibre bags that contain nutrients under evaluation, and incubated within the rumen. The rate at which nitrogen is lost from the bags is thus utilised to evaluate the degree and extend to which the nutrients are degraded (Mehrez and Ørskov, 1977). While the *in sacco* evaluates the disappearance rate from the bag instead of the actual rate of degradation, and that nutrients are confined in a bag and not chewed or ruminated by the animal, Ha and Kenelly (1984) advised that data acquired from *in sacco* technique ought to be cautiously analysed and be compared with data from *in vivo*. Şehu *et al*. (2010) reported that *in sacco* is an ideal technique to use when determining supreme feedstuff to formulate rations for ruminants.

In a study comparing the degradation of DM and CP of seed meals from sesame, hemp, poppy, hemp and canola using the *in sacco* technique, Şehu *et al*. (2010) found that canola seed meal had slower DM degradability at incubation time of 16 h, followed by the hemp seed meal with the slowest DM degradability at 16 and 48 h of incubation time; compared to other seed meals. Compared to other seed meals, poppy seed meal had the lowest degradability of CP at 24 h of incubation time whilst sesame seed meal had the highest CP degradability at 48 of incubation. Table 2.2 and 2.3 show the comparable degradation of DM and CP from conventional and non-conventional sources of proteins.

Table 2.2 Dry matter degradation (%) of conventional and non-conventional feed resources.

Feed	Degradation kinetics				References
	a	b	a+b	c	
Conventional					
Soybean meal	32	65	97	0.064	Lei *et al.*, 2018
Rapeseed meal	38	42	80	0.076	Lei *et al.*, 2018
Ground nut meal	29.1	55.7	61.1	0.102	Sehu *et al.*, 2010
Non-conventional					
Poppy seed meal	36.7	46.0	56.4	0.05	Sehu *et al.*, 2010
Avocado oil cake	32.3	34.9	67.1	0.07	Skenjana *et al.*, 2006
Macadamia oil cake	47.6	36.7	84.3	0.004	Skenjana *et al.*, 2006
Amarula seed cake	0.1	85.6	85.7	0.05	Mlambo *et al.*, 2011a

a = water soluble and rapidly degradable fraction;

b = insoluble but degradable fraction;

$a+b$=extent of degradation

c = degradation rate of fraction

Table 2.3 Crude protein degradability (%) of conventional and non-conventional feed resources.

Feed	Degradation kinetics				References
	a	b	a+b	c	
Conventional					
Soybean meal	12	84	96	0.078	Lei *et al.,* 2018
Rapeseed meal	36	46.8	82.7	0.087	Lei *et al.,* 2018
Sunflower oil cake	37.4	54.3	-	0.247	Habib *et al.,* 2013
Cotton seed cake	40.2	41.8	-	0.112	Habib *et al.,* 2013
Canola meal	42.7	43.3	68.2	0.087	Sehu *et al.,* 2010
Ground nut meal	35.3	55.5	64.7	0.135	Sehu *et al.,* 2010
Non-conventional					
Coconut cake	23.3	62.0	-	0.073	Marghazani *et al.,* 2013
Avocado oil cake	23.9	33.5	61.7	0.09	Skenjana *et al.,* 2006
Macadamia oil cake	73.5	18.7	92.2	0.019	Skenjana *et al.,* 2006
Amarula seed cake	2.1	96.3	96.7	0.05	Mlambo *et al*, 2011a
Guar meal	22.7	72.5	-	0.152	Marghazani *et al.,* 2013
Palm kernel cake	14.6	75	-	0.086	Marghazani *et al.,* 2013
Sesame oil cake	58.1	38.3	-	0.187	Marghazani *et al.,* 2013
Watermelon seed cake	82.2	17.2	99.4	0.20	Mustafa and Alamin, 2012

a = water soluble and rapidly degradable fraction;

b = insoluble but degradable fraction;

a+b=extent of degradation

c = degradation rate of fraction

2.2.3 *In vivo*

The *in vivo* digestibility/degradability is another method that can be utilised to determine protein in feedstuffs (Stern and Satter, 1984). The method involves using animals that are cannulated in the rumen and duodenum (Stern *et al.*, 1983). It gives indication of the association between nutrient content and nutrient availability to the animals as the nutrients are more visible to the biological pathways in the rumen for metabolic processes (Safwat *et al.,* 2015). Hence, a relation among the animal and feed nutrients (Khan *et al.,* 2003). One disadvantage of using *in vivo* digestibility is labour intensiveness, time consumption and subjectivity to considerable errors in terms of separation of microbial and dietary protein (Stern *et al.*, 1983).

2.3 Animal feeding experiments: replacement of conventional protein source with non-conventional protein source

2.3.1 Effects of animal growth on performance

According to a study by Alves et al. (2016), replacement of SBM with castor bean cake as a source of protein in lamb diets showed no differences in nutrient intake and average daily gain. Similarly, in a study using castor seed cake to replace groundnut meal in lamb diets, the intakes of DM and CP did not differ across the treatments except for metabolizable energy intake, which was lower for lambs fed castor seed cake supplemented diets as source of protein. Digestibility of nutrients and N balance of lambs that were fed diets supplemented with castor seed cake was not different from that of lambs fed on diets that contained groundnut meal as a dietary protein source (Nagalakshmi and Dhanalakshmi, 2015).

2.3.2 Effects on milk production and quality

When SBM was replaced with detoxified neem (51.6%) and karanj (37.9%) meals in dairy cows, the yield of milk and total proteins in milk were increased in cows that were fed on detoxified neem as source of protein compared to the control (soybean meal-based diet). The average milk fat was reduced in milk from cows fed the karanj supplemented diets in relation to milk of cows that consumed the SBM based diet (Raj *et al.,* 2016). According to Raj et al. (2016), detoxified neem and karanj meals could be included in total mixed rations of dairy cattle to replace SBM as source of protein with no adverse effect to the milk production efficiency and composition. Replacement of sunflower oilcake with rapeseed oilcake on crude protein basis in dairy sheep resulted in similar milk fat content. Nutritionally significant fatty acid (vaccenic, trans-11, CLA isomer C18:2 cis-9, oleic and total unsaturated fatty acid) was increased in the milk of ewes that were fed diets that were added with rapeseed oilcake than that from ewes fed diets added with sunflower cake (Amore *et al.,* 2014).

2.3.3 Effects on carcass quality

In their study, Nagalakshmi and Dhanalakshmi (2015) substituted groundnut meal with castor seed cake as a source of protein in lamb diets, and reported no differences on the carcass characteristics (meat proportion, dressing characteristics, organ weights, fat and bone); and chemical content of the muscle (*longissimus dorsi*) from lambs fed the groundnut meal supplemented diets and those fed the castor seed cake-based diets. The study indicated that no adverse effects emerged on the carcass traits and nutritional value of the lamb muscles from feeding lambs castor seed cake in place of groundnut meal as a source of protein. Replacement of SBM with castor bean cake (on protein basis) in lamb diets did not affect hot carcass yield and organ weights negatively (Alves *et al.*, 2016). These researchers recommended that castor seed cake can be utilised as a replacer of SBM in the diets of lambs.

2.4 Conclusion

The global shortage of protein sources in animal feeds, particularly in developing countries of Africa, has compelled the research on less costly animal feed resources that contain sufficient proteins with a balanced profile of amino. While the shortage of animal protein sources is accelerated by the exchange rate due to importation of the commodities as well as restriction on the use of by-products from animal origin for animal feeding, the use of conventional protein sources from plant origin is limited by their seasonality and thus high prices. Additionally, the use of non-conventional protein sources is restricted by the existence of secondary metabolites; however, these feedstuffs/ingredients can be processed or treated to reduce the ANFTs concentration, thus making the ingredients potential protein sources for animal feeds. Utilization of non-conventional plant-derived protein sources for possible incorporation into animal feeds as a replacement and or complement for the expensive conventional sources is inevitably necessary. Several researches have reported that non-conventional protein sources (e.g. castor seed cake, etc) can be incorporated in animal diets without adverse impacts on the animal growth performance, milk composition, characteristics of carcass and meat quality. Hence, evaluation of the potential of baobab, macadamia and amarula seed meal as non-conventional protein sources in animal feed is worth investigating.

CHAPTER 3

MATERIAL AND METHODS

3.1. Collection of non-conventional oil cakes

3.1.1. Collection and proximate analyses of non-conventional oil cakes

Batches of oil cakes (Amarula, Macadamia and Baobab) were collected from the oil production factories in the Limpopo Province, South Africa. Amarula oil cake (AOC) was collected from Phalaborwa Foundation (corner Calvin Ngobeni & Tambo streets, Namakgale, 1391), macadamia oil cake (MOC) was acquired from the Royal Macadamia factory (portion 38, Schoonuitzicht, Rotambo, Levubu, 0929) and baobab oil cake (BOC) was collected from Eco Products (Prognos Medical center, 119 Krogh street, Makhado, Louis Trichardt, 0920). Triplicates samples from each oil cake were analyzed for the nutritive values, mineral and amino acids contents.

3.1.2. Ruminal degradation of dry matter and protein

Three (3) rumen cannulated mid-lactating (days in milk; DIM: 180±5) Holstein cows weighing 667±43 kg were assigned to test the *in situ* rumen dry matter (DM) degradation. Milking of cows was done twice a day at 12 hr intervals and cows were offered a diet that contained 60 concentrate: 40 forage ratio *ad lib* in the morning (8:00). The diet was compounded to meet the cows` daily nutrient demands (NRC, 2001). Cows were managed as per approval of the Animal Ethics Committee of the ARC-Animal Production, Irene. The experimental treatments were three grounded non-conventional oil cakes viz: amarula, macadamia and baobab. The oil cake samples were sieved using a 2-mm screen and 6.5 g sub-samples placed in polyester bags (10 x 20 cm; 50 µm pore size) (Ankom, Fairport NY, USA) to achieve 15 mg/cm^2 (sample size to surface area ratio). The bags were then fistulated simultaneously in the rumen of each Holstein cow in triplicate for a period 2, 4, 8, 16, 24, or 48 hrs. Two duplicate sets of each sample that were non-incubated in the rumen, were splashed in water (cold) to assess solubility at 0 hrs. Immediately following incubation, bags were collected from the rumen and put in 20-L buckets with water (cold: 4°C) before washing in a machine and washed until clean water was obtained. The bags were then dried in an oven (60 °C) for a period of 48 hrs. Dried bags were individually weighed, the content of each bag removed and stored into glass vial until analysis.

3.1.2.1 Calculations

Disappearance of DM and CP as well as the effective degradability (ED) with time were calculated using the model of Ørskov and McDonald (1979) as:

$D = a + b \times (1 - e{-}c \times t)$ and $ED = a + [b \times c /(c + k)] \times e{-}k \times t$,

where D and ED are the degradation and effective degradability after t hours of rumen incubation; a is the water-soluble and rapidly degradable fraction; b is the insoluble but degradable fraction; c is the degradation rate of fraction b. The nonlinear procedure (PROC NLIN) of SAS (SAS Institute, 2009) was assigned to analyse the degradation data. A model producing the least sum of squares was considered as the best fit to the data set. Passage rate was calculated at 0.05 $h{-}1$ from the equation developed by NRC (2001) for concentrates.

3.2.3. Chemical analysis

3.2.3.1 Proximate analysis

The dry matter (DM) of the three non-conventional oil cakes was assessed by drying the samples using an oven (60 ℃). After achieving a constant weight form drying the samples, they were sieved through a 1-mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) for crude protein (CP), ether extract (EE), fibre (aNDF, ADF and ADL) analyses. The crude protein (ID 968.06) and the EE (ID 963.15) were determined as described by the procedure of AOAC (1990). The neutral detergent fibre (aNDF), acid detergent fibre (ADF) and the acid detergent lignin (ADL) were evaluated according to the procedures of Van Soest *et al.* (1991). The aNDF was assessed using heat stable alpha-amylase (Sigma-Aldrich Co. LTD., Gillingham, UK, no. A-1278) with sodium sulfite, and ADF was evaluated using the Fibertec TM System equipment (Tecator LTD., Thornbury, Bristol, UK). The ADL was determined using concentrated sulphuric acid and boiling at 700º C in a Tecator Fiber System (Tecator LTD., Thornbury, Bristol, UK).

3.2.3.2 Mineral and amino acid analyses

The mineral and amino acid from the three non-conventional oil cakes were analysed as outlined in the study of Malebana *et al.* (2018).

3.3. Statistical analysis

Genstat (2011) was used to analyse data on the nutritive value and amino acid profiles of the three non-conventional oil cakes in a completely randomized design by ANOVA.

Differences within means of treatments were separated using the least significant difference (LSD). Significance was set at the probability level of 0.05% probability (Snedecor and Cochran, 1980). Data was fitted to the following model:

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

Where Y_{ij} is the individual observations of the ith treatment and the jth replicate, μ is the general mean; τ_i is the main effect with error ε_ij as the random variation or experimental error. Predicted values for a, b, c, and ED, the percentage of DM disappearance at different incubation times were analysed by a completely randomized block design assigned to the MIXED procedure of SAS (SAS Institute, 2009). The following model was used for the analysis:

$Yij = \mu + Fi + cj + eij$,

Where Yij is the value of the variable studied on the ith diet for the jth cow; μ is the overall mean; Fi is the fixed effects of ith diet; cj is the random effect of jth cow; and eij is random error. Means were separated by Tukey test.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Nutritive values of non-conventional oil cakes

Data on the nutritive values for the three non-conventional oil cakes is shown in Table 4.1. The CP content in amarula oil cake (AOC) was greater (P<0.05) in relation to other oil cakes (Table 4.1). Malebana *et al*. (2018) carried out a research that compared the nutrient composition of AOC to that of SBM. The results showed that AOC had lower (391 g/kg DM) content of CP in comparison with SBM (512 g/kg DM). The CP content of AOC in the present study corroborates with that of Malebana *et al*. (2018).

Table 4.1 Nutritive values (% DM) of non-conventional oilcakes (n=3).

	Oilcakes				
Parameter	AOC	MOC	BOC	SEM	*P* -value
DM %	96.6^{a}	92.0^{b}	91.4^{b}	93.3	0.0001
CP, % DM	36.0^{a}	13.6^{c}	25.4^{b}	1.31	0.0001
EE, % DM	63.2^{a}	16.4$^{\mathbf{b}}$	10.9^{b}	6.38	0.002
aNDF, % DM	33.8^{c}	49.9^{a}	41.8^{b}	2.12	0.005
ADF, % DM	16.8^{b}	39.4^{a}	37.8^{a}	2.27	0.001
ADL, % DM	9.44^{b}	3.00^{c}	19.72^{a}	1.475	0.001
ADIN, % DM	0.33	0.49	0.60	0.051	0.0267

^{abc} Means of the same row with different superscripts differ significantly ($P < 0.05$).

AOC, amarula oil cake; MOC, macadamia oil cake; BOC, baobab oil cake; DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; ADIN, acid detergent insoluble nitrogen.

Tiwari and Jha (2017) reported 26 % CP in MOC, which is double of 14 % CP reported for MOC in the present research. However, Skenjana *et al.* (2006) recorded 13 % in MOC, which is similar to CP of MOC in Table 4.1. The lower CP content of MOC in this study in comparison with that of Tiwari and Jha (2017), might be attributed to the presence of nuts and hulls in the MOC. The content of CP in AOC was higher than that found in most conventional protein sources like sunflower oil cake (34 % CP) and canola oil cake (34% CP) used in livestock feeds. The CP content of the BOC was higher than that of 17 % CP in BOC indicated by Madzimure *et al*. (2011) as well as that of 22 % CP recorded by Chisoro *et al.* (2018) and Nkafamiya *et al*. (2007) in BOC. Makkar *et al.* (2008) reported 24.4 % CP in Jatropha curcas seed cake, which is comparable to 25.4 % CP recorded in BOC of the present study. According to van Soest (1994), forages that contain CP lower than 7% may not adequately support microbial activity in the rumen. Therefore, the CP contents of the AOC, MOC and BOC in the present study suggest that they will support microbial activities in the rumen.

The AOC had higher (P<0.05) content of EE in comparison to the other two cakes. The differences in the EE content amongst these cakes might be attributed to differences in the methods used to produce the cakes (Navarro and Rodrigues, 2016). Malebana *et al*. (2018) reported higher EE in AOC produced by hydraulic filter press compared to that produced by cold press method. The EE content of AOC in the current study is higher than 49.8 % recorded in AOC by Malebana *et al*. (2018). However, the EE content of AOC is quite high relative to SBM (1.98 % EE, Malebana *et al.,* 2018) and cotton seed meal (7.7 % Gao *et al.,* 2015). The EE content reported in MOC by Tiwari and Jha (2017) was 11.9 %, lower than 16.4 % in the present study. Kinman and Stark (1954) contended that for every 1 % rise in CP, accounts for 0.85 % reduction in oil content, which might be the reason for the variation in the CP content of MOC between these studies. Further, Madzimure *et al.* (2011) reported 5.3 % EE in BOC, which was lower than 10.9 % EE recorded in BOC in the current study. However, the EE in BOC in the present study is comparable to 8.3 % EE reported by Chisoro *et al.* (2018).

It is well known that lipids are energy dense compared to proteins and carbohydrates (McDonald *et al.,* 2010). Although too much dietary fats (> 5 %) have been reported to negatively affect ruminal microbial balance by suppressing the rumen bacteria that is responsible in methane production (Hills and West 1991), growing ruminants still need high energy diets to support their body maintenance and the development of body tissues. This is the case with intensive fattening systems (e.g. feedlot) since it relies on high energy rations for animals to develop intramuscular fat which will improve product quality and taste. Nevertheless, the high residual fat in AOC means that it has high energy content, which makes it a supplement for both CP and energy. However, the residual fat content in oil cakes may affect its use in the diet of ruminants, since it will depress ruminal microbial functioning especially if it exceeds 7 % of the diet.

Neutral detergent fibre has been reported to adversely affect the intake of DM by ruminants, especially when dietary NDF represents higher than 15 % DM of the total diet (Lippke, 1986). This means that the more dietary NDF is in the diet, the more the dietary intake will be suppressed. As structural carbohydrates, the fibre fractions (NDF and ADF) have some benefits to the ruminants, which include the prevention of acidosis, and the protection of rumen function by regulation of pH via stimulation of rumination and generation of energy (van Soest, 1994). The MOC and BOC had more (P<0.05) fibre fractions compared to AOC (Table 4.1). However, Malebana *et al. (*2017) reported higher fibre fractions in AOC when compared to SBM. The MOC and BOC can be good sources of dietary fibre compared to AOC due to their high NDF and ADF content. MOC has been reported to contain 35.8 % NDF and 28 % ADF by Tiwari and Jha (2017), which is lower compared to that recorded in MOC in the current study. Chisoro *et al.* (2018) reported 42.3 % NDF and 35.9 % ADF in BOC, which are comparable with those reported in BOC of the present study. Skenjana *et al.* (2006) reported MOC to contain 40 % NDF and 33 % ADF, which is comparable to the NDF and ADF values recorded in MOC in this study. Maize, a common energy source that is used to formulate animal feeds, contains NDF and ADF of 29.9 % and 5 %, respectively (Nkosi *et al.*, 2010), which is lower than that recorded in the three non-conventional oil cakes in the present study. Lucerne hay, a common dietary fibre supplement in most South African dairy rations, and represents up to 40 % in the diets of cattle. Scholtz *et al*. (2009) analysed 168 lucerne hay samples and reported 44.1 % NDF and 33.2 % ADF, which is higher than that of AOC but comparable to those of MOC and AOC.

Data on the amino acid profiles of the three non-conventional oil cakes is shown in Table 4.2. The AOC had higher ($P<0.05$) proportion of essential amino acids profile in comparison with the other two oil cakes. This suggests that AOC can be a good supplier of amino acids when formulating animal diets than with BOC and MOC. Malebana *et al.* (2018) reported methionine levels that range between 0.6 to 0.75 % in AOC, which was lower than 1.0 % for SBM. The methionine level in AOC in the current study is similar to those of Malebana *et al.* (2018) and Mthiyane and Mhlanga (2017). Tiwari and Jha (2017) reported 0.7 % and 0.34 % of lysine and methionine in MOC, respectively, while Chisoro *et al.* (2018) reported 1.04 % lysine and 0.26 % methionine in BOC, respectively, which is in agreement with the results of the current study. In order to avoid nutritional deficiencies when using these oil cakes in animal diets, it is advisable to supply monogastric animals with lysine and young ruminants with methionine (Malebana *et al.,* 2018), since their nutritional values are below those that are required for the respective animals.

According to Visagie (2010) methionine is the critical amino acid in the microbial protein synthesis for lambs, hence supplementing methionine to lambs during their growth stage is important. SBM, a conventional protein supplement, contains 2.88 – 4.19 % lysine and

0.56 - 0.72% methionine (Singhal and Mudgal, 1984; Ravindan *et al.* 2014), which are more than those recorded in the three non-conventional oil cakes of the present study. However, the methionine in the AOC is within the range of that of SBM. The lysine content (0.43%) in mustard cake, a non-conventional oilcake (Singhal and Mudgal, 1984), is lower than that of those reported in the three non-conventional oil cakes in the present study. However, mustard cake contains 6.3% methionine (Singhal and Mudgal, 1984), higher than that of the three non-conventional oil cakes in the current study. Ghosh and Mandal (2015) reported 1.69 % lysine and 0.53% methionine in ground nut oil cake, which is comparable to those reported in the three non-conventional oil cakes of the present study.

Table 4.2 Amino acids profile (%) of the non-conventional oilcake (n=3)

Parameters	Oilcakes			SEM	*P*-value
	AOC	MOC	BOC		
Essential amino acids					
Tryptophan	0.67^{a}	0.20^{b}	0.35^{b}	0.081	0.017
Threonine	0.93^{a}	0.38^{b}	0.74^{a}	0.065	0.003
Methionine	0.66^{a}	0.14^{c}	0.46^{b}	0.0527	0.001
Valine	1.67^{a}	0.62^{c}	1.19^{b}	0.068	0.0001
Phenylalanine	1.59^{a}	0.48^{c}	1.22^{b}	0.078	0.0002
Isoleucine	1.43^{a}	0.49^{c}	0.99^{b}	0.088	0.0008
Leucine	2.14^{a}	0.87^{b}	1.96^{a}	0.255	0.025
Histidine	1.32^{a}	0.35^{c}	0.82^{b}	0.1328	0.006
Lysine	1.01^{b}	0.92^{b}	1.50^{a}	0.0812	0.005
Non Essential amino acids					
Cysteine	1.45	0.95	1.43	0.404	0.638
Arginine	5.39^{a}	1.19^{c}	2.86^{b}	0.144	<.0001
Serine	1.54^{a}	0.60^{c}	1.19^{b}	0.0650	0.0001
Aspartic Acid	2.33^{a}	1.08^{b}	1.98^{a}	0.186	0.008
Glutamic Acid	7.10^{a}	1.76^{c}	4.78^{b}	0.299	<.0001
Glycine	1.64^{a}	0.82^{c}	1.08^{b}	0.057	0.0001
Alanine	1.11^{a}	0.43^{b}	1.01^{a}	0.099	0.005
Tyrosine	1.72^{a}	0.80^{b}	0.71^{b}	0.239	0.045
Proline	1.07^{a}	0.62^{a}	0.87^{a}	0.0730	0.013
HO- Proline	0.06^{c}	0.36^{a}	0.13^{b}	0.0164	<.0001

^{abc} Means in the same row with different superscripts differ significantly ($P < 0.05$).

AOC, amarula oil cake; MOC, macadamia oil cake; BOC, baobab oil cake

The content of minerals except fluoride in the three non-conventional oil cakes varies widely (Table 4.3). The higher contents of magnesium, phosphorus and potassium in the AOC in comparison with those in MOC and BOC will be of great benefit when used to supplement minerals in animal feeds. Further, the high content of phosphorus, could be beneficial in the breeding stock of ruminants since it is a key element for fertility in ruminants. Magnesium, a critical mineral that drives metabolic processes in animals, can be supplemented by using either AOC and or BOC in the diet. Calcium is one of the essential minerals which is responsible for the flow of nerve impulses and for the contractile properties of muscles (McDonald *et al.*, 2010). The MOC contains higher ($P<0.05$) calcium in relation to the other two non-conventional oil cakes of the present study. Malebana *et al*. (2018) reported calcium of a range of 110 – 143 mg in AOC, which is agrees to the calcium content of AOC in the current research (Table 4.3). Van Ryssen *et al.* (2014) recorded 160 mg for calcium and 230 mg phosphorus in MOC, which the calcium is lesser to that of the MOC in the current research. However, Chisoro *et al.* (2018) reported calcium of 240 mg and phosphorus of 660 mg in BOC, which is comparable to the calcium and phosphorus in BOC of the present study.

When compared with conventional oil cakes, Malebana *et al.* (2018) reported 273 mg calcium and 743 mg phosphorus in SBM. The calcium in SBM is higher than that of AOC but lower than that of MOC and BOC in the present study. However, the phosphorus in AOC is higher compared to the SBM of Malebana *et al*. (2018). When compared with other non-conventional oil cakes, Thanaseelaan (2013) reported that rapeseed meal contains 1080 mg calcium, which is higher than that of the three cakes in the present study. However, rapeseed cake contains 680 mg phosphorus and 550 mg magnesium (Thanaseelaan, 2013), which are comparable to those recorded in the three cakes of the present study. Further, sesame oil cake is reported to contain 561 mg calcium and 117 mg potassium (Sunil *et al.*, 2015), with the latter lower than that of the three non-conventional oil cakes of the present study.

Table 4.3 Mineral composition of non-conventional oilcakes (n=3).

Parameter (mg/100g)	Oilcakes			SEM	*P*-value
	AOC	MOC	BOC		
Calcium	111^{c}	418^{a}	317^{b}	20.5	0.0001
Magnesium	453^{a}	244^{b}	479^{a}	17.8	0.0002
Phosphorus	927^{a}	225^{c}	731^{b}	35.8	<.0001
Potassium	497^{b}	1013^{a}	901^{a}	51.5	0.0009
Sodium	13.9^{b}	36.2^{a}	18.8^{b}	2.97	0.0043
Fluoride	1.07^{a}	1.45^{a}	1.05^{a}	0.126	0.1161

^{abc} Values in the same row with a different superscript are different ($P < 0.05$).

AOC, amarula oil cake; MOC, macadamia oil cake; BOC, baobab oil cake.

4.2 *In situ* dry matter and protein degradation

Data on the DM and CP degradation of the three non-conventional oil cakes is illustrated in Table 4.4. The AOC had higher (P<0.05) soluble fraction (a) of DM compared to the BOC and MOC, but the insoluble fraction and the degradation rates of DM were similar (P>0.05) amongst the oil cakes. However, the effective degradability of DM was higher (P<0.05) in the AOC and BOC compared to MOC. The ruminal DM degradability increased with increased time of incubation (Figure 4.1). After 48 hr of incubation, AOC had 90 % disappearance of DM, but those of BOC and MOC were lower than this value.

Table 4.4 Rumen degradation parameters (%) and effective degradability of DM and CP for rumen degradation parameters and effective degradability of DM and CP for the three non-conventional oil cakes (n=8).

Parameter (%)	**Oil cakes**			**SEM**	***P*- value**
	AOC	**BOC**	**MOC**		
Dry matter					
a	43.3^{a}	31.5^{b}	31.9^{b}	2.83	0.001
b	50.1	49.1	60.3	6.31	0.005
c	0.10	0.08	0.04	0.13	0.189
ED1	87.0^{a}	66.8^{b}	68.0^{b}	1.81	0.049
ED2	81.8^{a}	57.0^{b}	56.3^{b}	0.97	0.001
ED3	78.1^{a}	52.3^{b}	50.2^{b}	1.67	0.007
Crude protein					
a	42.7^{a}	29.2^{b}	26.1^{b}	2.37	0.001
b	54.0^{b}	66.0^{a}	57.8^{b}	1.18	0.004
c	0.23	0.20	0.17	0.08	0.088
ED1	89.4^{a}	89.5^{a}	77.3^{b}	0.38	0.001
ED2	82.9^{a}	82.8^{a}	69.7^{b}	1.20	0.001
ED3	78.6^{a}	77.5^{a}	64.1^{b}	1.84	0.006
RUP	11.2	12.0	14.9	3.08	0.072

^{ab} Values in the same row with a different superscript are different ($P < 0.05$)

AOC= amarula oil cake, BOC= baobab oil cake, MOC= macadamia oil cake

a = water soluble and rapidly degradable fraction;

b = insoluble but degradable fraction;

c = degradation rate of fraction;

ED1 = effective degradability calculated with a passage rate of 0.03 h^{-1};

ED2 = effective degradability calculated with a passage rate of 0.05 h^{-1}.

ED3 = effective degradability calculated with a passage rate of 0.08 h^{-1};

RUP = $b[k/(k + c)]$

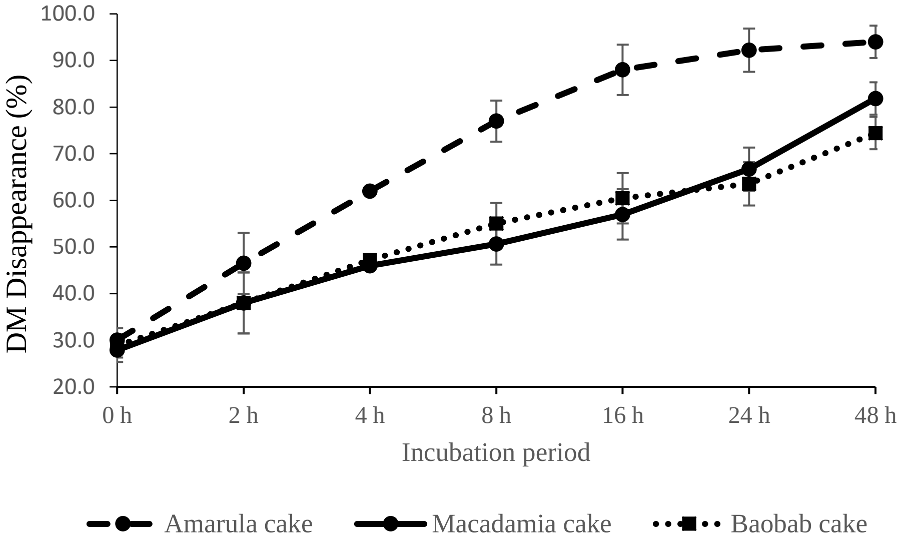

Figure 4.1. *In vitro* dry matter disappearance of amarula, baobab and macadamia cake over 48 h incubation.

Skenjana *et al.* (2006) conducted an *in situ* study that determined the degradation of DM and CP in MOC. The MOC was reported to have 47.6 % soluble fraction of DM, 36.7 % potentially degradable fraction of DM and 0.04 % degradation rate of fraction DM. The water soluble fraction and rapidly degradable fraction of DM (31.9%) in MOC in the current research, is lesser than that reported in the study by Skenjana *et al.* (2006). However, the insoluble fraction of DM (60.3%) in the present study was higher than the 36.7 % reported by Skenjana *et al.* (2006). These researchers reported MOC to have 73.5 % soluble fraction of CP, higher than the 26.1 % recorded in the present study (Table 4.4).

The rapidly degradable fraction (a) of CP was greater (P<0.05) in the AOC compared to the other oil cakes (Table 4.4) and has reached 90 % disappearance in 48 hrs of incubation (Figure 4.2). The BOC had more (P<0.05) insoluble fraction of CP in comparison with the other two oil cakes, but the degradability rate fraction of CP were similar (P>0.05) amongst the oil cakes. The effective degradability of CP was lower (P<0.05) in the MOC in comparison with the other oil cakes. The rumen undegradable protein (RUP) did not differ amongst the three oil cakes.

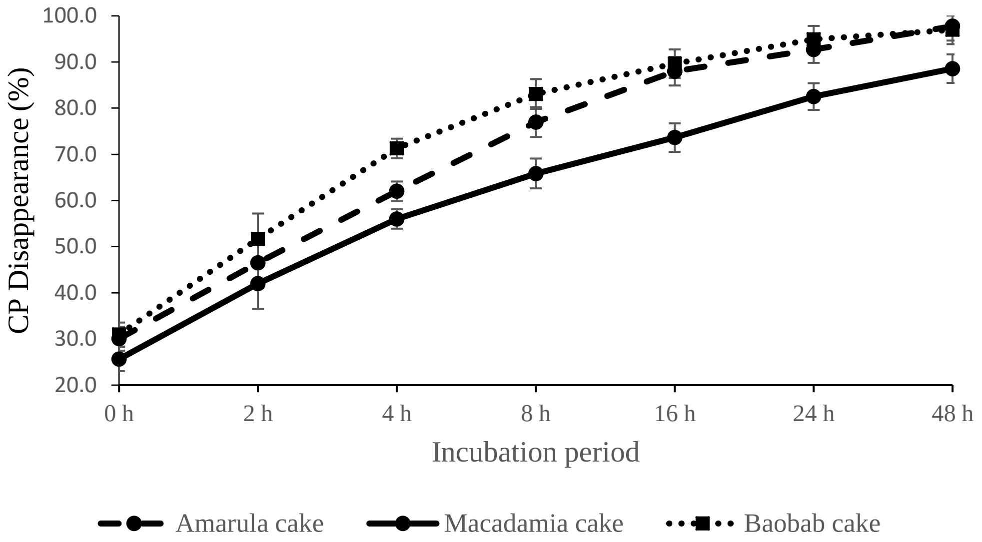

Figure 4.2. *In vitro* crude protein disappearance of amarula, baobab and macadamia cake over 48 h incubation

Soybean meal (SBM), cottonseed cake and sunflower cake are some of the conventional oil cakes that are widely used as CP and energy supplements in commercial animal operations worldwide. Lei *et al.* (2018) reported SBM to have 32 % soluble fraction of DM, 65 % insoluble but potentially fermentable DM and 97 % degradation rate of DM. They further reported rapeseed meal to have 38 % soluble fraction of DM, 42 % insoluble fraction and 80 % degradation rate of DM. When comparing with conventional oil cakes, Lei *et al.* (2018) reported SBM to contain 12 % soluble degradable fraction of CP, 84 % insoluble but potentially fermentable of CP and 96 % potentially degradable fraction of CP. This 12 % soluble degradable fraction of CP in SBM is lower than that recorded in the three non-conventional oil cakes of the present study. However, sunflower oil cake has been reported to contain 37.4 % soluble degradable fraction of CP (Habib *et al.*, 2013), which is comparable to that of the three non-conventional oil cakes of the present study. Gao *et al.* (2015) reported cotton seed meal to have 3.4 % soluble degradable CP fraction, which is lower than those reported in the three oil cakes of the current research. Mlambo *et al*. (2011a) reported AOC to contain 0.1% soluble degradable fraction of CP and 48.2% effective degradability of CP, which is quite different to

AOC of the present study (Table 4.4). The reason for this difference in these two studies is not known.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

The high CP and EE in the AOC make it a good dietary supplement for both protein and energy. Further, AOC contains appreciable amounts of essential amino acids and its use in ruminant nutrition will help to supplement ruminants with amino acids. The MOC and BOC are high in fibre fractions, making them good supplements for fibre in the diets for ruminants. In addition, MOC and BOC are good sources of minerals (calcium and potassium) while AOC is rich in phosphorus. The ruminal degradation study shows that AOC is high in the effective degradability of DM but contain higher water soluble and rapidly degradable fractions of both DM and CP. However, BOC have a high insoluble but degradable fraction of CP, while both AOC and BOC have higher effective degradation of CP.

The results of the nutritive values and the rumen degradability of these three non-conventional oil cakes suggest that they can be incorporated in ruminant diets and partially replace some of the conventional oil cakes. However, the toxicology of these three non-conventional oil cakes should be determined to avoid poisoning and metabolic disorders in ruminants when fed diets containing these oil cakes. Further, studies that evaluate growth performance, nutrient digestion and carcass characteristics in ruminants fed rations that are supplemented with these non-conventional oil cakes, should be done.

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