EFFICIENT UTILIZATION OF MACADAMIA OIL CAKE BASED DIETS IN RUMINANTS

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A dissertation submitted in fulfilment of the requirements for the degree of Master of Science in Agriculture (Animal Science)

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DECLARATION

I, **Mkhonto Zinhle Debra** (Student number: 14007373), hereby declare that this dissertation submitted in fulfilment of the requirements for the Master of Science in Agriculture (MSCANS) in the Department of Animal Science, Faculty of Science, Engineering and Agriculture, University of Venda has not been submitted previously for any degree at this or another university. It is original in design and execution, and all reference material contained therein has been duly acknowledged.

Signature: ____________________ **Date:** 19th January 2023

ZINHLE DEBRA MKHONTO

DEDICATION

In memory of my father Thabiso Mkhonto, who could not live to see me get this far. May the Lord's arms continue to hold his soul in peace.

This work is also dedicated to my mother Thobile Sibiya, my sister Zama Magagula and my niece Keabetswe Magagula, thank you for being supportive financially, morally, spiritually and socially. To my daughter Hlelolwenkosi Zoya Mkhonto, all this is for you.

ACKNOWLEDGEMENTS

I am humbly giving thanks to God my creator for carrying me throughout the study, "for I can do all things through Him who strengthens me" (Philippians 4:13).

I honestly express my heartfelt gratitude to my supervisor, Prof J.J Baloyi, for his constant lift, supervision, immense knowledge, moreover for the constructive criticism. There could have never been not a better supervisor for my Masters. My humble thanks also goes to my co-supervisor, Dr M.S Mikasi, for his support and wise counsel throughout the study.

I confer my honour to the National Research Foundation (NRF) for the financial backing (Grant No: 141165). I also express many thanks to Dr F Fushai for the provision of funds from his research project, support, and assistance with feed formulation during the study. The laboratory technician, Mr M.E Nyathi for his untiring efforts to assist with the setting up of the study site and laboratory analysis. I greatly appreciate Dr E Bhebhe whose door has always been open for assistance on statistical models and data analysis.

All thanks to Moalamedi Masechaba, Muleya Thomas, Sekonyane Mmatlou, Maemu Rambau, Innocent Tshikovhi and Khangwelo Selapyane for cooperation during data collection, ideas and loyalty.

Finally, I extend my deepest gratitude to my family and friends, more importantly, the Mondlane family for looking after my daughter as I work on my study, I value your support and prayers.

ABSTRACT

Ruminants and rumen bacteria form a symbiotic relationship. However, because of nitrogen (N) inefficiency, this interdependent relationship loses energy and protein. Strategic feeding of ruminants with diets containing non-conventional feedstuffs appears to be a promising way to enhance proficiency of protein and energy utilization in ruminants. Macadamia Oil Cake (MOC) can be classified among the non-conventional ingredients that can be included in animal diets, without resulting in any negative effects. The present study evaluated the utilization of MOC-based diets in ruminants. Eight Boer and eight Pedi bucks initially weighing an average of 34 ± 8.39 kg and aged 18 to 24 months were housed individually in metabolic cages (1.8 m × 0.58 m × 1.33 m) to measure nutrient intake, apparent digestibility, nitrogen (N) retention and microbial protein yield. The goats were balanced for breed and allocated in a completely randomized design (CRD) to four dietary treatments containing 0, 10, 15, and 20% MOC inclusion. Microbial protein yield was assessed using the urinary excretion of purine derivative (PD) allantoin method to estimate the efficient utilization of nitrogen in the MOC-based diets. The study was carried out for 21 days, beginning with 14 days for acclimatization and seven days for sample collection. MOC inclusion significantly affected ($P<0.05$) the intake of ether extract (EE), neutral detergent fibre (NDF) and acid detergent fibre (ADF). However, the intake of dry matter (DM) , organic matter (OM) and crude protein (CP) were not significantly affected ($P>0.05$) by MOC supplementation. Significant differences ($P<0.05$) were observed for OM and EE apparent digestibility of MOC-based diets by goats. However, No significant differences ($P>0.05$) were found in the apparent digestibilities of DM, CP, NDF and ADF. Nitrogen (N) retention was not significantly affected ($P>0.05$). All goats had positive N-retention values. Urinary allantoin excretion differed significantly ($P<0.05$) within treatments and affected ($P<0.05$) microbial protein yield. To conclude, the results attained in this study imply that MOC does not affect apparent digestibility of DM, CP and N-retention but enhanced microbial protein yield. Therefore, MOC showed to be a promising protein source to be utilized in goat diets without significantly causing any adverse effects.

Keywords: Allantoin, apparent digestibility, goats, Macadamia Oil Cake, microbial protein yield, nitrogen retention, utilization.

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LIST OF ABBREVIATIONS

%	Percentage
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
CP	Crude Protein
CRD	Completely Randomized Design
CSM	Cottonseed meal
DAFF	Department of Agriculture, Forestry and Fisheries
DM	Dry Matter
DMI	Dry Matter Intake
DP	Purine Derivatives
EMNS	Efficiency of microbial nitrogen synthesis
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
GLM	General linear model
Kg	Kilograms
g/kg DM	Grams per Kilogram Dry Matter
MOC	Macadamia Oil Cake
N	Nitrogen
NDF	Neutral Detergent Fibre
NRC	National Research Council
OM	Organic matter
SEM	Standard Error Mean
SBM	Soyabean meal
SFM	Sunflower meal

CHAPTER 1

INTRODUCTION

1.1 Background

Ruminants need consistent nutrient intake throughout their lives to sustain high production standards (Yacout, 2016). The availability of natural forage and its nutritive value decrease in winter, resulting in nutrient deficits in ruminants, particularly protein (Mnisi & Mlambo, 2017). This problem affects mostly small-scale farmers in the rural societies who rely on natural pastures for feeding their livestock. Small-scale farmers cannot afford protein supplements feedstuff since they are often too expensive or not readily available (Nkosi *et al*., 2011). Therefore, there is a demand for alternative or cheaper non-conventional feedstuff to replace the expensive conventional feedstuff (Cherdthong *et al*., 2011; Rufino *et al*., 2013). Many developing countries have used agro-industrial by-products as protein source supplements for improving animal feeding (Xianjun *et al.,* 2012).

Soyabean meal (SBM) is widely utilized as a source of protein in concentrates (Silva *et al*., 2016). Although SBM has high crude protein (CP) content of approximately 46% as fed (Palmieri *et al.,* 2016), the cost of SBM as an ingredient is high. As a result, alternative feedstuff must be sought to supplement or replace SBM in concentrate diets to increase nitrogen (N) supply to rumen microbes. Supplementing low quality tropical grass with agro-industrial by products has shown to have an effect in improving the roughages intake and digestibility (Nurfeta, 2010).

Among these agro-industrial by-products, Macadamia Oil Cake (MOC) is obtained by extracting oil from the cracked, insect-damaged or poorly developed Macadamia nuts using Soyabean husk as a foundation to keep the nuts from sticking to the sides of the oil extraction equipment (Mikasi *et al*., 2018). Macadamia Oil Cake (MOC) has a crude protein (CP) content of approximately 20% (Acheampong-Boateng *et al.,* 2008). Skenjana *et al*., (2002) indicated that MOC can be utilized in ruminants' diet as a source of protein and has a highly relative influence on *in vitro* digestibility. This is consistent with the study of Mikasi *et al.,* (2018) which indicated that MOC has the potential to be included in the diet of livestock as a protein supplement, due to its high degradation (33.75 g/kg DM) in the rumen making it a perfect feedstuff for ruminants.

However, research on the effects of MOC inclusion in goat diets is currently sparse and as a result it is not effectively used for feeding by small scale farmers due to the lack of information. Therefore, the current study evaluated the efficiency of utilization of MOC by determining the apparent digestibility, nitrogen (N) retention and microbial protein yield in goats.

1.2 Problem statement

The feed quantity and quality for ruminants within seasons and between years are usually the main factors limiting production (Gulwa ,2017). In terms of quality, protein is typically the limiting nutrient (Yacout, 2016). Ruminants mostly rely on natural pastures, which are higher in fibre and deficient in protein, and require high and adequate nutritional intake to sustain optimal production, maintenance, and reproduction (Yacout, 2016). During the winter season in South Africa, conditions restrict fodder availability and nutrient quality for ruminants, resulting in reduced nutrient intake due to low quality forage. Under these conditions, the crude protein (CP) content is inadequate for rumen microbial protein synthesis. Being an essential nutrient element nitrogen (N) is important for ruminant growth, maintenance and productivity. However, ruminants utilize N of feed inefficiently (Hristov *et al*., 2011). Therefore, understanding the metabolism of N and strategies that can advance the efficient utilization of N for productive purposes should be a main priority in ruminant nutrition (Broderick, 2015).

A major challenge in South Africa for ruminant production is the scarcity and high cost of protein feed sources (Nkosi *et al*., 2011), which account for about 60 - 70 % of the total cost of production (Lawrence *et al*., 2008). Non-conventional feed materials such as Macadamia Oil Cake (MOC) which may be locally available have a potential to alleviate the problem of high protein costs (Mikasi *et al*., 2018). Information on the efficiency of utilization of MOC by goats is limited.

1.3 Justification

Ruminants continue to make essential contributions to global food supply (meat & milk). Therefore, there is a need to find new approaches to develop feeding strategies for ruminants based on inexpensive protein feedstuffs to use as components of concentrate diets (Yacout, 2016). The supplementation strategies based on cheaper protein sources will help enhance the utilization of low-quality roughages, primarily by providing nitrogen (N) to the rumen microorganisms (Malebana *et al*., 2018; Washaya *et al*., 2018). Owens *et al*., (2010) concluded that, supplementing pasture-based diets with protein sources promotes feed intake, digestibility, performance and the efficiency of the animal.

Therefore, scientific research for MOC as a protein supplement is critical; the information of MOC as an alternative cheaper protein source in ruminant feeding and the utilization attained from this study will be useful to nutritionists by expanding the use of MOC as a protein source. Small holder farmers and commercial ruminant farmers could improve their feeding approaches and have informed choices on protein sources which are within reach in terms of price and distance. In that regard, this study evaluated the utilization of MOC on apparent digestibility, nitrogen (N) retention and microbial protein yield on goats.

1.4 Objectives

1.4.1 Main objective

The main objective of the study was to determine the efficient utilization of Macadamia Oil Cake (MOC) based diets in ruminants.

1.4.2 Specific objectives

The specific objectives of the study were to:

i. Determine the apparent digestibility of Dry matter (DM) and Crude protein(CP) of MOC based diets in goats
ii. Evaluate the nitrogen (N) retention from MOC based diets in goats
iii. Estimate microbial protein yield in goats fed MOC based diets

1.5 Hypotheses

1.5.1 Null hypothesis

The null hypotheses were that:

i. The inclusion of MOC at different levels in goat diets does not significantly affect the apparent digestibility of DM and CP

ii. MOC inclusion in goat diets does not significantly affect nitrogen (N) retention

iii. MOC inclusion in goat diets does not significantly affect the microbial protein yield

1.6. References

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CHAPTER 2

LITERATURE REVIEW

2.1 General introduction

In the Southern part of Africa, ruminants (cattle, goats and sheep) are sources of income, food security and major protein sources for humans as plant protein are scarce and expensive (Ng'Ambi *et al*., 2018). These ruminants depend on natural pastures as their primary feedstuff. However, the natural pastures are low in protein and high in fibre content. This may limit the intake and degradability in the rumen, resulting in lower productivity (Beyene & Mlambo, 2012; Mokgakane *et al*., 2021; Msiza *et al*., 2021).

Generally, feed cost makes roughly 70 - 90% of the total ruminants' production (Gonzalez-Razo *et al*., 2010). An upsurge in feed cost results in reduced profit margins, which adversely affects the ruminant production and thus the production of ruminants' protein for consumers.The commercial diet of ruminants is mainly comprised of maize and Soyabean meal (SBM) as sources of energy and protein, respectively. These ingredients are the commonly used in ruminant feed due to their fundamental feeding value (Stein *et al*., 2009).

The demand for both maize and Soyabean as ingredients is constantly growing to provide for the ruminant production. Countries in the Sub-Saharan region rely on these ingredients as staple food for humans (Khojely *et al*., 2018). Therefore, there is competition for maize and soyabean between humans and livestock (Gidlund *et al*., 2015). Prices for these ingredients are increasing (R10 561/ton of Soyabean) (Mnisi & Mlambo, 2018), (R4 898/ton of yellow maize and R4 837/ton of white maize) (Famine Early Warning Systems Network (FEWS NET, 2022)). Agricultural-industrial by-products could be used to supplement or completely replace conventional feedstuffs for diet of animals.

There are several alternative protein sources for ruminant diets like cotton seed cake (CSC), groundnut meal (GNM) and other agro-products (Jelantik & Bellli, 2010; Nagalakshmi *et al*., 2011; Marghazini *et al.,* 2013). Macadamia Oil Cake (MOC) is also one feedstuff which can be assimilated into animal feed. Numerous research have been carried out to evaluate MOC as a supplementary protein feed for ruminants (Skenjana *et al*., 2006; Acheampong-Boateng *et al.,*

2008; Mikasi *et al.*, 2018) and the results suggest that MOC can replace SBM in ruminant diets without posing any adverse effects on the
general performance of the animal, digestibility, and nutrient retention.

2.2 The use of conventional oil cakes in ruminant nutrition

In South Africa, Soyabean meal (SBM), Cottonseed meal (CSM) and Sunflower meal (SFM) are used as principal protein supplements (Nkosi & Maseeke, 2010; Brand *et al*., 2012). However, these conventional oil cakes are becoming expensive due to their high demand (Malebana *et al*., 2018). On the other hand, they are non-available in the rural areas due to market and transportation problems. Generally, CSM is imported mainly from China and India. SBM is used by financially stable farmers; therefore, these oilcakes are not readily accessible to smallholder farmers (Beres *et al*., 2017).

2.2.1 Soyabean meal

The main protein source in ruminant feed is Soyabean (*Glycine max*) meal (SBM) (Zagorakis *et al*., 2018). The SBM provides protein, energy, fibre and fat of good quality to ruminant animals (Ruzic-Muslic *et al.,* 2014). South Africa produces insufficient Soyabean, therefore to meet demand, it depends on imports from China (Bureau for Food and Agricultural Policy, 2018). However, transporting Soyabean from regions of its production is costly.

The ever increasing population and the increasing demand for protein-rich foods stimulated the need of cheaper and available alternative feedstuffs to replace SBM in animal diets (Malebana *et al*., 2018). This results in the small-holder famers not being able to afford quality fodder for their livestock. The dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF) of SBM (%) range from 89.90 - 90, 45.4 - 47.7, 8.2 - 14.1, 5.3 - 6.59, respectively (Stein *et al*., 2015; Alves *et al*., 2016). SBM has a protein digestibility of about 88% (Wickramasuriya *et al*., 2015).

2.2.2 Cottonseed meal

Cottonseed (*Gossypium Spp*) meal (CSM) is the residue obtained during the process of pressing cotton seeds for their oil. CSM is a common protein source used in ruminants, as it is rich in protein (McDonald *et al*., 2011). Several researchers studied the chemical composition of CSM and reported ranges (%) 22.97 - 41.74 CP (Mawona, 2010; Habib *et al*., 2013; Tripathi *et al*., 2014; Thirumalaisamy *et al*., 2016). CSM may be included up to 15% in cattle diets (Weisbjerg *et al.,* 2007). When fed to ruminants, the CSM is a good resistor for degradation in the rumen.

The protein content in CSM is a good resistor of degradation in the rumen when fed to ruminants. It provides positive results for maintenance, milk production and fattening, of livestock. The main concern of incorporating CSM into ruminants' diets is the presence of gossypol. Gossypol in its free form is not allowed over 100 ppm due to its toxicity in complete diets for ruminants (Gadelha *et al.,* 2014). It is purified inside the rumen either by bonds formed with soluble proteins or by a dilution effect, which slows down the absorption of gossypol (Brand *et al*., 2012).

2.2.3 Sunflower meal

Sunflower (*Helianthus annuus*) meal (SFM) may be used as an alternative feedstuff for ruminants, due to its moderate levels of protein and energy (Mendoza *et al*., 2008). Nutritional studies conducted on the sunflower meal (SFM) discovered that it has high levels of fat (160 g/kg on DM basis) and protein (249 g/kg on a DM basis) (Neto *et al*., 2014). SFM has been recommended for supplementation or substitutions of soyabean meal (SBM) in feed for goats (Oliveira *et al*., 2014), lambs (Benaglia *et al*., 2016) and steers (Goes *et al*., 2012; Oliveira *et al*., 2019).

SFM could be a promising protein supplement to feedstuffs that are slowly degradable in the rumen (Ruzic-Muslic *et al.,* 2014) and its corporation enhances the use of nitrogen (N) and the efficacy of microbial metabolism (Agy *et al*., 2012; Mesacasa *et al*., 2012). However, when given in high quantities, SFM can increase the content of ether extract (EE) in the diet, which can result in reduced total neutral detergent fibre (NDF) digestibility associated to the high fat content which influences the metabolism of microorganisms (Lillis *et al*., 2011).

Incorporating SFM in ruminants' diets is mainly limited by its high fiber content (González-Vega and Stein, 2012). Dehulled SFM has a fibre content of approximately 30% neutral detergent fibre (NDF) , which is about four times higher than in Soybean meal (National Research Council (NRC), 2012). The presence of anti-nutritional factors in the SFM, markedly: tannins, phytic acid alkaloids, oxalate flavonoids and saponins are 0.39%, 79.1%, 1.23%,1.76%, 0.87% and 2.36% ,respectively. The anti-nutrients make SFM bitter, and as a result causes challenges in terms of acceptability for different species of animals (Fasuyi *et al*., 2010).

2.3 Macadamia Oil Cake (MOC) as an alternative protein source

2.3.1 Production of Macadamia Oil Cake

Macadamia integrifolia and *Macadamia tetraphylla* trees originated in Australia and are members of the Proteaceae family. The trees have been grown in the United States, predominantly in Hawaii and California. The trees cannot endure frost and are suited best to areas where there are alternate wet and dry seasons. The Macadamia nuts are customarily produced in Mpumalanga, Limpopo and Kwazulu-Natal due to their subtropical conditions. Mpumalanga province is the largest Macadamia nuts production area followed by the Limpopo province (South African Macadamia Growers' Association (SAMAC),2020).

Macadamia nuts comprise (by weight) of 75% fat , proteins that ranges from 27% - 30% and sugars of 6% - 8% (Department of Agriculture, Forestry and Fisheries (DAFF), 2019). Annually about 6 000 tons of macadamia nuts are produced in South Africa, costing R34/kg (South African Macadamia Growers' Association (SAMAC), 2020).

The Macadamia Oil Cake (MOC) is obtained from Macadamia nuts that are either from cracked or insect-damaged or poorly developed nuts during the oil refinement process (Navarro *et al.,* 2016). A foundation of soybean hulls is used to avert the nuts from sticking inside the oil extracting machine (Navarro *et al.,* 2016). The oil rich MOC after the cold pressing extraction method, can be used as a protein supplement in animal feeding (Navarro *et al.,* 2016). The usage of MOC as a supplement in the diets of ruminants will directly provide animal feed security. The inclusion of MOC in animal forage-based systems could also benefit farmers by allowing them to engage in alternative feed production, which will decrease the demand for humans and livestock.

2.3.2 Nutritional value of Macadamia Oil Cake (MOC)

Numerous researchers have studied the nutritional value of Macadamia Oil Cake (MOC) (Skenjana *et al.,* 2002; Acheampong-Boateng *et al.,* 2008; Phosa, 2009; Van ryssen *et al.,* 2014; Tiwari & Jha, 2017; Mikasi *et al.*, 2018 and Marconato *et al*., 2021) and they reported, crude protein (CP) content of 24.5%, 19.5%, 14.0%, 13.2%,14.7% and 9.86%, respectively. The different protein contents may be due to the diverse methods used to extract oil from the cake and edaphic factors affected by the climate of the region where MOC is grown (Lee *et al*., 2018). The cake composes of a neutral detergent fibre (NDF) of 358 g/kg) which produces volatile fatty acid, improves fibre fermentation, microbiota and health gut (Tiwari & Jha, 2017).

Several scientific research has been conducted to determine the feeding potential of MOC in ruminants, replacing soyabean meal (SBM) as a typical protein source in animal feed (Sherrod & Ishikazi, 1996; Acheampong-Boateng *et al*., 2008/17; Phosa, 2009; Van Ryssen *et al*., 2014 ; Jha & Tiwari, 2017; Mikasi *et al*., 2018). Sherrod & Ishizaki (1996) fed sheep concentrate diets that contain macadamia nuts and concluded that ruminants can be fed low levels of feed grade Macadamia nut as feed grains. Acheampong-Boateng *et al.,* (2008) evaluated MOC in the diets of feedlot cattle and discovered that MOC may be used alone or mixed with SBM.

A study conducted by Mikasi *et al*., (2018) who supplemented lamb diets with 10 and 15% MOC, discovered that MOC does not affect dry matter (DM) intake (kg d^{-1}).The researchers suggested that MOC can be incorporated in ruminant diets up to 15%, without posing any negative effects. Additionally, the protein requirements were accomplished without compromising the performance of livestock fed MOC based diets (Acheampong-Boateng *et al.,* 2008). Although MOC is known as a good source of protein, it comprises high levels of fibre from the soyabean hulls added during processing which may interfere with digestion and normal rumen function.

2.3.2 The effect of integrating Macadamia Oil Cake (MOC) into ruminant diets

Understanding the nutritional value of Macadamia Oil Cake (MOC) in ruminants necessitates a research of nutrient digestibility and nitrogen balance to know the accepted threshold as a potential protein source in ruminant feeding strategies. Skenjana *et al.,* (2006) assessed the extent of dry matter (DM) digestibility of MOC based diets using an *in vitro* digestibility method and reported that MOC give rise to an overall DM digestibility of 79.2 %, higher than the 63.39%

recorded by Mikasi *et al*., (2018). However, the findings of Skenjana *et al*.,(2006) were comparable to the 75.5% reported by Tiwari & Jha (2017). Mikasi *et al*., (2018) reported that the low DM digestibility can be a result of the high fibre levels present in MOC, resulting from soyabean hulls included during processing compared to the Macadamia nut used by Tiwari & Jha (2017) which did not have any soyabean hulls. In general, oilcakes without hulls have high digestibility (Souza *et al*., 2009).

The apparent digestibility of organic matter (OM) by lambs in MOC-based diets showed non-significant differences in a study carried by Mikasi et al., (2018). This indicated that MOC did not have a negative impact in microbial population at lower levels. Mlambo et al., (2011) reported differences in OM digestibility of three different protein sources (Soyabean meal, Sunflower meal and Marula seed cake).

Sherrod & Ishizaki (1996) reported that supplementation with Macadamia nuts in lamb diets enhanced nitrogen retention, with a positive response suggesting efficiency in protein gain. This agrees with a recent research conducted by Mikasi *et al*., (2018), who discovered substantial differences in nitrogen retention amongst lambs fed MOC based diets at various levels. The positive values of nitrogen retention are evidence that the lambs acquired protein efficiently.

2.4 Estimation of microbial protein yield as an indicator of efficiency of protein utilization using the purine derivative (PD) (allantoin in urine) technique

Microbial protein yield refers to the amount of microbial protein assessed by purine derivative (PD) in the urine (Chen and Gomes, 1992). Microbial protein substantially makes up about 60 to 80% of the amino acids supplied to the duodenum (Pathak, 2008). Estimating microbial protein is critical for formulating diets for ruminants. The purine derivative (PD) allantoin is a product of purine metabolism excreted in urine. Its secretion in urine has been long considered as a pointer to recovery of microbial protein accessible at the duodenal level, which forms a key component of metabolizable protein in ruminants (Lamothe *et al*., 2002; Mota *et al*., 2008).

The advantage of using allantoin excretion in urine is that it is a non-invasive method, which can be used to a larger number of animals compared to the use of cannulated animals (Lamothe *et al.,* 2002). The analysis of the PD allantoin in urine is simple, inexpensive, and quick (Gonzalez-Ronquillo *et al*., 2003). The calculation of microbial protein yield can be added into digestibility and nitrogen (N) experiments because just urine is needed, which reduces the amount of additional labour input (Singh *et al*., 2007). However, compared to the direct procedures in the omasum or duodenum, allantoin excretion in urine results in reduced estimates of the duodenal microbial protein yield (Tas, 2007). The supplementation of MOC to lamb diets at 10 and 15% did not significantly influence microbial protein yield or urinary allantoin excretion. (Mikasi *et al*., 2018).

Several studies (e.g. Chen *et al*., 1992; Susmel *et al*., 1994, Singh *et al.,* 2007; Ramgaokar *et al.,* 2008) have been carried out to investigate the relationship of nutrient utilization parameters, including digestible dry matter (DDM) and digestible organic matter intake (DOMI) in ruminants. Balcells *et al*., (1991) reported urinary allantoin may be useful for assessing the amount of purines entering the duodenum when animals are fed close to or above their maintenance requirements. These findings are consistent with those of Chen *et al*., (1991) and Fujihara *et al*., (2005), who found that urinary allantoin can be utilized as a primary predictor of microbial protein yield. Mota *et al*., (2008) working with both goats and sheep reported a greater excretion of allantoin in goats compared to sheep.

2.5 Efficiency of nitrogen (N) utilization in ruminants

Improving nitrogen (N) efficiency in ruminants is a significant component in lowering feed costs. Generally, ruminants have a low efficiency of N utilization, with an average value of approximately 0.25 (g N in product/g N intake) (Kohn *et al*., 2005). The efficiency of N in protein rich feeds such as oilcake is often improved, as a result of moderate concentrations of energy (Moorby *et al*., 2016). However, Calsamiglia *et al*., (2010) reported efficiency factors ranging between 0.15 - 0.40 among different feeds and feeding practices. This indicated that diets and feeding practices have a significant impact on ruminant N utilization efficiency. Dietary N is used more efficiently in the body to synthesize milk protein than muscle tissues. Therefore, grazing beef animals have the lowest N efficiency in this range, while nursing dairy cows have the greatest (Metcalf *et al*., 2008).

2.6 Summary of literature review

Goats play an invaluable role in the South African economy, contributing to food, nutrition security and poverty alleviation (Ng'ambi *et al*., 2018). During the dry season in South Africa, goats graze on pastures with insufficient nutrients to support and optimize microbial development (Gulwa *et* *al*., 2017). During the dry season, natural pastures are expected to have low protein and high fibre content, which may limit intake and degradability in the rumen.

Protein feedstuff are expensive, particularly Soyabean meal (SBM), which is the most commonly utilized protein source in ruminant diets (Silva *et al*., 2016). The increasing cost and inaccessibility of conventional feedstuffs has resulted in search for alternative, less expensive protein sources such as Macadamia Oil Cake (MOC). MOC is a by-product of oil processing obtained from Macadamia nuts as a result of oil produced from cracked, insect-damaged or poorly developed nuts (Navarro *et al.,* 2016) and its potential for use in ruminant diets is influenced by its nutritional qualities and accessibility. Consequently, the utilization of MOC by ruminants needs to be studied further.

Microbial protein output in the rumen accounts for 60-85% of the amino acid supply to the duodenum (Chen & Orskov, 2003). As a result, estimating microbial protein supply is a critical component in developing goat diets. Comparatively, the *in vivo* method necessitates the use of cannulated animals to assess microbial protein yield. Alternatively, the purine derivative (PD) urinary allantoin excretion method can be employed to estimate microbial protein yield to the duodenum in a non-invasive and repeatable manner (Henke *et al*., 2017).

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CHAPTER 3

APPARENT DIGESTIBILITY AND NITROGEN RETENTION OF MACADAMIA OIL CAKE BASED DIETS FED TO GOATS

ABSTRACT

The objective of the study was to investigate the apparent digestibility and nitrogen (N) retention in goats fed Macadamia Oil Cake (MOC) supplemented diets. Eight Boer and eight Pedi intact male goats with an average initial weight of 34 kg ± 8.39 aged 18 to 24 months were indiscriminately allocated to four *iso*-nitrogenous dietary treatments consisting of MOC inclusions as treatments: 0, 10, 15 and 20% inclusion. The goats were placed in metabolic cage for 21 days, consisting of 14 days of adaptation and seven days of recording feed intake and collecting feaces and urine. No noteworthy differences (P>0.05) were observed for dry matter intake (DMI), organic matter intake (OMI) and crude protein intake (CPI). However, ether extract intake (EEI), neutral detergent fibre intake (NDFI) and acid detergent fibre intake (ADFI) were significantly affected (P<0.05) by the inclusion of MOC in the diets of goats. NDFI decreased, while EEI increased with an increase in MOC inclusion level. The apparent digestibility of DM, CP, NDF and ADF did not differ significantly among the four treatments (P>0.05). However, OM and EE digestibility were affected (P<0.05) by MOC inclusions. N retention was not significantly affected (P>0.05) by MOC inclusion, all values were positive. Therefore, MOC can be incorporated in goat diets up to 20% without causing any negative effects on DM, CP digestibilities and N retention.

Key words: Apparent digestibility, goats, *iso*-nitrogenous, Macadamia Oil Cake (MOC), nitrogen retention.

3.1 Introduction

Goats (*Capra hircus*) play an vital role in the enhancement of livelihood in developing countries, by ensuring food security as they are hardy and drought tolerant (Ng'Ambi *et al*., 2018). The global population of goats has increased during the last decade and currently exceeds 1 billion goats with approximately 95% of those found in developing countries (Food and Agriculture Organization Statistics (FAOSTAT), 2019). Goats are cheap to acquire and reproduce quickly, thus have a faster population growth (Peacock, 2005). They are also efficient in the utilization of land and very easy to market given their small carcass (Abdul-Aziz, 2010). Their high productivity and growth rate make them suitable for small-scale farmers (Rumosa Gwaza *et al*., 2009). Goat meat has high nutritional attributes owing to low quantities of saturated fat and cholesterol (Tsvuura, 2020). Goat nutrition is primarily based on natural pastures and that poses the most severe problem since the composition of natural pasture species and levels of nutrients are poor especially in dry seasons (Shinde & Mahanta, 2020). Supplementation during the dry season embodies a valuable economic alternative to improve the quality of diet and lessen nutritional problems for smallholder farmers (Arguello, 2011).

Under circumstances of poor-quality forages, conventional protein sources like Soyabean (*Glycine max)* meal have been used as supplements to enhance nutritional quality of low-quality feeds (Silva *et al*., 2016). Alternatively, Sunflower meal (*Helianthus annuus*) which is cultivated nationwide for oil extraction has a crude protein (CP) content of 29 - 45 % (Habib *et al.,* 2013). Cottonseed meal (*Gossypium spp*) is also used as feed for ruminants due to high levels of proteins ranging between 23 - 45 % and energy (Thirumalaisamy *et al.,* 2016). However, the prices of these conventional protein sources are high in South Africa. To counter this, Macadamia Oil Cake (MOC) has been identified as a potential alternative protein source for animal production (Mikasi *et al*., 2018). The objective of this study was to evaluate the effect of feeding MOC as a supplementary feed on apparent digestibility and nitrogen (N) retention in goats on complete MOC pellet-based diet.

3.2 Materials and methods

3.2.1 Ethical considerations

Before embarking on this research project, permission was granted by the University of Venda Ethics Committee (SARDF/19/ANS/12/2211) and the researcher adhered to the ethical standards outlined by the University for Research involving animals.

3.2.2 Description of the study area

The study was carried out at the Faculty of Science, Engineering and Agriculture Experimental Farm, University of Venda, Thohoyandou, Limpopo province (22°58’32’’ S, 30°26’45’’E; Altitude of 596 m above sea level). The study site experiences summer temperatures ranging from 25°C to 40°C and winter with average minimum and maximum temperatures of 11°C to 27°C, respectively (Tadross *et al*., 2006).

3.2.3 Macadamia Oil Cake (MOC) sample preparation

Macadamia Oil Cake (MOC) was obtained from Green Farms (Pty) Ltd Company in Levubu, Limpopo, South Africa. To produce the MOC, oil was extracted from Macadamia nuts which were either cracked or insect damaged. The cake samples were dried at 105°C until constant weight (method 930.15; AOAC, 2010) and ground using a grinder (Retsch® 2M 200 mode Retsch laboratories Ltd, Germany) to pass through a 1mm sieve. A sample of the MOC was subjected to chemical analysis and preceded diet formulation.

3.2.4 Animal management and experimental design

Eight Boer and eight Indigenous male intact goats with an average initial body weight of 34± 8.39 kg aged 18 to 24 months were carefully chosen from the existing herd in the University of Venda Experimental Farm and purchased from Banabakgomo farm just outside Bronkospruit, Gauteng province, respectively.

The goats were weighed with an electronic scale at the commencement of the trial, ranked based on the breed and assigned to four treatment diets containing different MOC inclusions except the control treatment which was commercial goat pellets containing gluten feed as a protein source (Table: 3.1). The diets were formulated to be *iso*-nitrogenous. The experiment utilized a completely randomized designs (CRD) due to the random assignment of goats to formulated treatments using Microsoft excel, and one factor which has the potential to affect the parameters under investigation. The goats were housed in individual metabolic cages (1.18 m × 0.58 m × 1.33 m) with removable feeders and water troughs as shown in Figure: 3.1.

3.2.5 Experimental diets and composition of diets

Four treatment diets were formulated to contain different levels of MOC: 0 (control), 10, 15 and 20% inclusion and coded MOC0, MOC10, MOC15, and MOC20 respectively. The four dietary treatments were formulated and prepared by the Brennco Feed Company (Pty) Ltd in Louis Trichardt, Limpopo province, South Africa .The diets were prepared according to Brennco Feed Company (Pty) Ltd growing goats physio-chemical properties and provided approximately 180 g/kg DM which met the requirements of growing goats (NRC, 2007). The formulation and chemical composition of the diets used in the study is provided in Table 3.1 and mineral composition in Table 3.2, no chemical analysis of the individual ingredients was conducted prior to experimental diets formulation, except for MOC. In general, the MOC20 diet had marginally higher at content than the MOC0, MOC10 and MOC15 diets.

Figure 3.1: Metabolism cage/crate used for housing the goats

Table 3.1: Feed formulation and chemical analysis of experimental diets with inclusion of Macadamia oil cake (g/kg DM) fed to goats

	MOC inclusion level (g/kg DM)			
Ingredients(g/kg)	MOC0	MOC10	MOC15	MOC20
Yellow maize	235.0	235.0	235.0	233.0
Wheat bran	150.0	150.0	150.0	150.0
Gluten feed	200.0	100.0	50.0	0.0
Urea	4.0	4.0	4.0	8.0
Salt	5.0	5.0	5.0	5.0
Molasses	80.0	80.0	80.0	80.0
Goat Premix	5.0	5.0	5.0	5.0
Limestone	16.0	16.0	16.0	14.0
Ammonium Sulfate	2.5	2.5	2.5	2.5
Ammonium Chloride	2.5	2.5	2.5	2.5
MOC	0.0	100.0	150.0	200.0
Lucerne	300.0	300.0	300.0	300.0
Total	**1000**	**1000**	**1000**	**1000**

MOC0=0% Macadamia oil cake; MOC10=10% Macadamia oil cake; MOC15=15% Macadamia oil cake; MOC20=20% macadamia oilcake (Macadamia oil cake inclusions); MOC=Macadamia oil cake

Table 3.2: Chemical and mineral composition of experimental diets

	MOC inclusion level (g/kg DM)			
Chemical composition (g/kg DM	MOC0	MOC10	MOC15	MOC20
DM (g/kg)	917.1	918.2	919.0	920.8
Ash	91.2	81.7	81.8	85.1
EE	33.5	42.4	50.4	54.6
CP	175.3	165.2	177.8	186.7
NDF	342.9	321.7	309.5	280.9
ADF	148.2	159.0	174.2	151.3
ADL	24.7	29.3	29.7	30.5
Minerals				
Macro minerals (g/kg DM)				
Ca	8.8	8.0	7.3	6.9
Mg	3.5	3.1	3.0	2.9
K	14.8	13.5	13.4	13.1
Na	3.3	2.9	2.6	2.5
P	4.1	3.5	2.9	2.8
Micro minerals (mg/kg DM)				
Zn	0.1	0.1	0.1	0.1
Cu	0.0	0.0	0.0	0.0
Mn	0.1	0.1	0.1	0.1
Fe	0.8	1.0	0.8	0.7

MOC=Macadamia oil cake; MOC0=0% Macadamia oil cake; MOC10=10% Macadamia oil cake; MOC15=15% Macadamia oil cake; MOC20=20% macadamia oilcake (Macadamia oil cake inclusions); DM=Dry matter; CP=Crude protein; NDF=Neutral detergent fibre; ADF=Acid detergent fibre; ADL=Acid detergent fibre; Ca=Calcium; Mg=Magnesium; K=Potassium; Na= Sodium; P=Phosphorus; Zn=Zinc; Cu=Copper; Mn=Manganese; Fe=Iron

3.2.6 Determination of apparent digestibility

The digestibility trial lasted for 21 days. The goats were adapted to the dietary treatments for 14 days and daily feed intake, faecal and urine output were measured for seven consecutive days. Each morning prior to feeding, feed refusals were measured, and feed intake was determined from; feed offered - feed refused. The quantity of feed offered was estimated at 5% body weight of the goats, adjusted when necessary (when the goats have finished the feed on the next feeding day).

Collection of feaces for digestibility was done daily using feacal bags after adapting the goats to the feacal bags for three days. The feacal bags were attached to the harness by clips, which were unfastened when the feaces were collected (Figure: 3.2) just before the morning feeding into sealable plastic bags, and the daily feacal output was weighed and recorded for each animal. Afterwards, 10% of the feacal output was sampled and stored in the refrigerator at -20°C pending chemical analysis.

Figure 3.2: Collection of feaces into feacal bags

3.2.7 Determination of nitrogen (N) retention

In addition to feed intake and feacal output data, daily urine output was collected in plastic buckets containing approximately 100 ml of 10% sulphuric acid to prevent the volatilization of ammonia in the urine and the volumes were recorded. A total of approximately 10% of the excreted urine was sampled for each goat and stored at -20 °C in the refrigerator pending N analysis.

3.2.8 Chemical analysis of feeds, refusals, feaces and urine

Feed, feed refusals, feaces and urine samples were analysed in duplicates. Dietary ingredients and chemical composition of formulated diets are shown in Tables 3.1 and 3.2, respectively. Dry matter (DM), ash, crude protein (CP), ether extract (EE) were analysed according to the Association of Official Analytical Chemists (AOAC, 2010) methods. DM content was determined by oven-drying at 105 ^{0}C until all free water evaporated as indicated by constant weight of the sample (method 930.15; AOAC, 2010). Organic matter (OM) was determined as the loss in weight upon combustion in the muffle furnace at 550 ^{0}C for 6 h (method 942.05; AOAC, 2010). CP content was determined following the micro-Kjeldahl method using the Kjeltec system through digestion; distillation and titration steps (method 2001.11; AOAC, 2010) and CP content was determined by multiplying N by 6.25 (N×6.25), where N represents nitrogen. The EE content was evaluated using the ANKOMXT15 fat extractor by refluxing samples with petroleum ether, an organic solvent. The residues after extraction were weighed and EE was determined by the difference from the initial sample weight (method 2003.05; AOAC, 2010). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined using the technique of Van Soest *et al*., (1991). The mineral contents (Calcium (Ca), Magnesium (Mg), Potassium (K), Sodium (Na), Phosphorus (P), Zinc (Zn), Copper (Cu), Manganese (Mn), and Iron (Fe)) of the samples were determined using Inductively Coupled Plasm Optical Omission Spectrometry (ICP-OES) following Standard Operating Procedure (SOP) (2005).

3.2.9 Calculations and statistical analysis

(i) The digestibility coefficient of each parameter was calculated by using the following equation on a DM basis (McDonald *et al*., 2011):

Apparent digestibility coefficient = $\frac{Nutrient\ in\ feed - Nutrient\ in\ feaces}{Nutrient\ in\ feed}$ (1)

(ii) Nitrogen retention was calculation from the following formula:

Nitrogen retention (g d^{-1}) = NI – (FN + UN) (2)

Where;

NI=Nitrogen intake; FN=Faecal nitrogen; UN=Urinary nitrogen

(iii) The digestible organic matter intake (DOMI) was calculated from the organic matter intake – faecal organic matter:

DOMI (g d^{-1}) = organic matter intake-feacal organic matter (3)

(iv) Metabolisable energy (ME) intake and digestible organic matter in the rumen (DOMR) were calculated from the DOMI content according to AFRC (1993)

Metabolisable energy intake (ME) (MJ d^{-1}) = 0.0157 × DOMI (4)

Digestible organic matter in the rumen (DOMR)(g d^{-1}) = 0.65×DOMI (5)

All data on voluntary nutrient intake, apparent digestibility and nitrogen (N) retention were analysed using the General Linear Model (GLM) procedures, one-way of variance for completely randomized design (CRD) procedures of SPSS (2016) statistical software. The treatment means were separated using Turkey's test. The initial weight of the animals was used as a covariate in the analysis. The level of significance was set at P<0.05. The model used is as follows:

$Y_{ijk}=\mu + Ti + \mathcal{E}_{ijk}$ (6)

Where;

Y_{ijk}= Observation/response variable (DM intake, Apparent digestibility and N retention)
μ = Overall mean
Ti=Effect of diets for i=1-4 (0, 10, 15 and 20%)
$\mathcal{E}_{ijk}$ =Experimental error

3.3 Results

3.3.1 Chemical composition of Macadamia Oil Cake (MOC)

Table 3.3 shows the results of the chemical and mineral composition of a representative sample of Macadamia Oil Cake (MOC). The information was used in the formulation of the rations.

3.3.2 Voluntary intake of nutrients by goats fed Macadamia Oil Cake (MOC) based diets

Table 3.4 shows data of the voluntary nutrient intake of Macadamia Oil Cake (MOC) based diets (CPI) by goats among all the treatments. However, the inclusion of MOC significantly affected (P<0.05) neutral detergent fibre intake (NDFI), acid detergent fibre intake (ADFI) and ether extract intake (EEI) by goats. Digestible organic matter intake (DOMI) and metabolisable energy intake (ME) were significantly decreased (P<0.05) with an increase in MOC supplementation. MOC0 resulted in the highest DOMI and ME compared to MOC20, however similar to MOC10 and MOC15.

3.3.3 Apparent digestibility of Macadamia Oil Cake (MOC) based diets in goats

Table: 3.5 shows data on apparent digestibility coefficient of nutrients in goats fed MOC based diets. No significant (P>0.05) differences were observed for dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) digestibility coefficients among all the treatments in this study. However, the results of organic matter (OM) and ether extract (EE) digestibility coefficients of MOC based diets fed to goats were significantly different (P<0.05). Goats fed MOC0, MOC10 and MOC15 had significantly high (P<0.05) apparent digestibility coefficient of OM compared to goats on MOC20. MOC10 had the highest EE digestibility than MOC20 and the rest were the same.

3.3.4 Nitrogen (N) intake and retention in goats fed Macadamia Oil Cake (MOC) based diets

Table 3.6 shows nitrogen (N) intake and retention data of Macadamia Oil Cake (MOC) based diets in goats. The results show that MOC supplementation did not significantly affect (P>0.05) N-intake by goats. However, MOC inclusion considerably affected (P<0.05) feacal-N, urinary-N and total nitrogen excretion (TNE). Observed urinary-N and TNE were significantly higher (P<0.05) for MOC0 compared to MOC15, which recorded a lower mean value (12.78 and 19.94 g d-1), respectively. N-retention was not considerably affected by the inclusion of MOC (P>0.05). All goats had positive N-retention values.

Table 3.3: Chemical and mineral composition of Macadamia Oil Cake (MOC)

Nutrients (g/kg DM)	MOC
DM (g/kg)	926.1
Ash	42.8
CP	137.0
EE	179.3
NDF	500.0
ADF	364.5
Mineral composition	
Macro minerals (g/kg DM)	
Ca	3.0
Mg	1.9
K	10.0
Na	0.7
P	1.6
Micro minerals (mg/kg DM)	
Zn	0.0
Cu	0.1
Mn	0.1
Fe	0.4

MOC=Macadamia oil cake; MOC0=0% Macadamia oil cake; MOC10=10% Macadamia oil cake; MOC15=15% Macadamia oil cake; MOC20=20% macadamia oil cake (Macadamia oil cake inclusions); DM=Dry matter; CP=Crude protein; NDF=Neutral detergent fibre; ADF=Acid detergent fibre; ADL=Acid detergent lignin; Ca=Calcium; Mg=Magnesium; K=Potassium; Na=Sodium; P=Phosphorus; Zn=Zinc; Cu=Copper; Mn=Manganese; Fe=Iron

Table 3.4: Voluntary intake (g d^{-1}) of nutrients by goats fed Macadamia Oil Cake (MOC) based diets

		Treatment					
Parameter Intake (g d^{-1})	N	MOC0	MOC10	MOC15	MOC20	SEM	P-value
DM	28	1186.24	974.49	1108.76	1120.34	57.00	0.07
OM	28	1078.55	887.79	987.25	1024.81	51.20	0.07
CP	28	207.96	173.19	183.07	189.02	9.88	0.09
EE	28	39.68^{b}	41.26^{b}	56.00^{a}	61.11^{a}	2.41	0.00
NDF	28	409.91^{a}	313.65^{ab}	342.94^{b}	314.89^{b}	18.3	0.00
ADF	28	175.73^{ab}	154.87^{b}	193.36^{a}	169.52^{ab}	8.78	0.02
DOMI	28	1023.68^{a}	912.22^{ab}	909.34^{ab}	823.15^{b}	49.90	0.05
ME intake (MJ d^{-1})	28	16.07^{a}	14.32^{ab}	14.28^{ab}	12.92^{b}	0.78	0.05

^{a,b} Means in the same row with different superscripts are significantly different at $P<0.05$; MOC0=0% Macadamia oil cake; MOC10=10% Macadamia oil cake; MOC15=15% Macadamia oil cake; MOC20=20% Macadamia oil cake (Macadamia oil cake inclusions); DM=Dry matter; OM=Organic matter; CP=Crude protein; NDF=Neutral detergent fibre; ADF=Acid detergent fibre; DOMI= Digestible organic matter intake ; ME= (Metabolisable energy intake); N= Number of observations; SEM=Standard error of mean

Table 3.5: Apparent digestibility co-efficient of nutrients in goats fed diets supplemented with different Macadamia Oil Cake (MOC) inclusions

		Treatment					
Apparent digestibility co-efficient (g d^{-1})	N	MOC0	MOC10	MOC15	MOC20	SEM	P-value
DM	28	0.76	0.74	0.75	0.66	0.04	0.27
OM	28	0.94^{a}	0.92^{a}	0.92^{a}	0.87^{b}	0.01	0.00
CP	28	0.77	0.75	0.75	0.65	0.43	0.20
EE	28	0.88^{ab}	0.91^{a}	0.89^{ab}	0.79^{b}	0.03	0.01
NDF	28	0.53	0.45	0.45	0.23	0.08	0.07
ADF	28	0.40	0.37	0.40	0.11	0.09	0.06

^{a,b} Means in the same row with different superscripts are significantly different at P<0.05; MOC0=0% Macadamia oil cake; MOC10=10% Macadamia oil cake; MOC15=15% Macadamia oil cake; MOC20=20% macadamia oil cake (Macadamia oil cake inclusions); DM=Dry matter; OM=Organic matter; CP=Crude protein; NDF=Neutral detergent fibre; ADF=Acid detergent fibre; N=Number of observation; SEM=Standard error of mean

Table 3.6: Nitrogen (N) intake and retention in goats fed Macadamia Oil Cake (MOC) based diets

		Treatments					
Parameter (g d^{-1})	N	MOC0	MOC10	MOC15	MOC20	SEM	P-value
N-intake	28	33.50	27.76	29.36	30.30	1.58	0.09
Faecal-N	28	7.26^{ab}	6.30^{b}	7.16^{ab}	8.61^{a}	0.49	0.01
Urinary-N	28	17.04^{a}	15.49^{ab}	12.78^{b}	16.87^{a}	0.97	0.01
TNE	28	24.49^{a}	21.79^{ab}	19.94^{b}	25.49^{a}	1.13	0.00
N-retention	28	9.02	5.97	9.40	4.08	1.70	0.16

^{a,b} Means in the same row with different superscripts are significantly different at P<0.05; MOC0=0% Macadamia oil cake; MOC10=10% Macadamia oil cake; MOC15=15% Macadamia oil cake; MOC20=20% Macadamia oil cake (Macadamia oil cake inclusions); N=Nitrogen; TNE=Total nitrogen excretion (Feacal-N + Urinary-N); N-retention= N-intake - TNE; N=Number of observations; SEM=Standard error mean

3.4 Discussion

3.4.1 Chemical composition of Macadamia Oil Cake (MOC)

The crude protein (CP) content of Macadamia Oil Cake (MOC) in the present study was 137 g/kg DM which is lower than the 260 g/kg DM of MOC reported by Tiwari & Jha (2017). However, Skenjana *et al*., (2006) and Mikasi *et al*., (2018) recorded 130 and 147 g/kg DM in MOC respectively, which is comparable to the CP of MOC in the present study. The lower CP content of MOC in the present study in comparison to that of Tiwari & Jha (2017) may be attributed to the different processing methods or varieties of MOC such as the hull percentage present in the cake. Extraction methods and edaphic factors affected by the climate of the region where MOC is grown influence the chemical composition of the resulting by-product and determine their nutritional values including digestibility (Lee *et al*., 2018; Dhillon *et al*., 2016).

The ether extract (EE) in the present study is 179.3 g/kg DM higher than the 119 g/kg DM reported by Tiwari & Jha (2017) and the 85 g/kg DM (Mikasi *et al*, 2018). The differences in the EE content between the cakes might be attributed to the different methods used to produce the cakes (Navarro & Rodrigues, 2016). Nevertheless, the high fat content (179.3 g/kg DM) in MOC of the present study means that it has high energy content, which makes it a good supplement for energy.

Fibre fractions have been reported to adversely affect the intake of DM in ruminants, especially if it exceeds 15% DM of the diet (Pulina *et al*., 2013). This means that the high neutral detergent fibre (NDF) and acid detergent fibre (ADF) present in the diet will reduce feed intake. The MOC in the present study contains higher fibre fractions (500 g/kg DM NDF and 364.5 g/kg DM ADF), which is higher than 358 g/kg DM NDF and 280 g/kg DM ADF reported by Tiwari & Jha (2017). However, the NDF fraction of MOC in the present study is comparable to the 554 g/kg DM NDF reported by Mikasi *et al*., (2018) in MOC. Len *et al*., (2007) reported that, high dietary fibre influences nutrient digestibility, nitrogen retention and excretion.

The present study has demonstrated that MOC has moderate CP content (13%), however, this is not good enough to supply enough protein to meet the animals requirements. MOC can be best suited when combined with other feedstuff (i.e. gluten feed, urea or Soyabean meal). Therefore making it a potentially good source of protein supplement for ruminants.

3.4.2 Voluntary nutrient intake

All the experimental goats voluntarily consumed MOC pellet based diets during the experiment. Significant differences (P<0.05) were observed on the ether extract intake (EEI), neutral detergent fibre (NDFI) and acid detergent fibre intake (ADFI). Significantly higher EEI was observed for treatment MOC15 which was similar to MOC20, albeit different from treatments MOC0 which was similar to MOC10. The linear increase (P<0.05) of EEI (39.68-61.11 g d^{-1}) can be attributed to the considerably higher fat content in MOC (179.3 g/kg DM), which resulted in an increase of this component in the diets. Similar results were reported on EEI (Marconato *et al*., 2021) on lambs fed macadamia nut cake based diets and found differences (P<0.05) in EEI. The treatment with the highest MOC% (MOC20) presented higher EEI mean, however it did not affect DMI.

Macadamia Oil Cake (MOC) is characterized by its fibrous composition (500 g/kg DM NDF; 360 g/kg DM ADF). It is known that some factors involved in the regulation of intake in ruminants, among them is the diet NDF content that is considered restrictive due to their slow degradation and low pass rate in the rumen (Banerjee, 2010). Nagalakshmi & Dhonakshmi (2015) fed lambs diets supplemented with castor seed cake (CSC) and reported no adverse effects (P<0.05). This is in disagreement with the results of NDFI in the current study (P>0.05). MOC0 had significantly higher (P<0.05) NDFI (409.91 g d-1) which was similar to MOC10 and lower than MOC15 and MOC20. The NDF intakes in the present study are therefore suggestive that MOC supplementation had an effect on the concentrate intake.

The absence of significant differences (P>0.05) of dry matter intake (DMI), organic matter intake (OMI) and crude protein intake (CPI) among treatments in the current study, could be an indication that MOC inclusion had no adverse effective on palatability and consumption by goats. The results in the current study are similar to those of Acheampong-Boateng *et al*., (2008) who reported similar DMI in cattle fed MOC based diets at different levels. Mikasi *et al*., (2018) reported non-significant differences (P>0.05) in OMI in sheep, which is in line with the results of the current study. In contrast, Ishizaki & Sherrod (1996) reported significant differences (P<0.05) indicating that DMI decreased with an increase in MOC in lamb rations. These differences may be attributed to the dissimilar species of animals used in the two experiments.

The non-significant differences in crude protein intake (CPI) can be attributed to the similar CP content present in the diets. The CPI's in the current study are consistent with the findings of Mikasi *et al*., (2018) who reported that supplementing lamb diets with MOC at 10 and 15% did not affect the CP intakes (14.6 ,14.5 g d^{1}), respectively. This study shows that you can still increase MOC inclusion up to 20% without any effect on CP intake. Therefore, there is still necessity to investigate the threshold levels of inclusion of MOC in ruminant diets.

Ruminants feedstuff are made up of several components, such as carbohydrates and fats which offer metabolisable energy. The metabolisable energy intake (ME) in the current study ranged from 12.92 – 16.07 MJ d^{-1}, with the lowest being for MOC20 and the highest for MOC0 , which was similar to MOC10 and MOC15. The findings of this study suggest that MOC might even maintain high producing ruminants such as lactating cows (NRC, 2007).

3.4.3 Apparent digestibility of nutrients

The non-significant differences (P>0.05) observed for dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) apparent digestibility coefficient could be attributed to the high fibre content of Macadamia Oil Cake (MOC), which is positively linked to a lower digestibility. Therefore, it was expected that the increase in MOC would result in lower digestibility (Menezes, 2008). The non-significant differences in the digestibility coefficient of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) imply that there was no adverse effect of MOC on DM, CP, NDF and ADF digestibility. Similar to the results of the current study, Mikasi *et al*., (2018) reported no significant differences (P>0.05) on NDF and ADF apparent digestibility of lambs fed MOC based diets. It is therefore acceptable that the level of feeding should not influence overall DM apparent digestibility of the diets (McDonald *et al*., 2011).

However, the current findings on the apparent digestibility of NDF and ADF in MOC contrasted that of Mlambo *et al*., (2011) who reported significant differences (P<0.05) on NDF and ADF digestibility of Amarula Oil Cake (AOC) and Sunflower Oil Cake (SOC). These differences may be attributed to the differences in the fibre content of the cakes used in the studies.

Results of OM and EE digestibility in the present study showed significant differences (P<0.05) among the treatments. The results of OM digestibility are at odds with OM digestibility reported by Mikasi *et al*., (2018) who found no differences (P>0.05) in sheep fed MOC at 10 and 15% inclusion levels. These differences may be attributed to the differences in the nutrient composition of the diets used in the two studies. The decreased OM digestibility coefficient at MOC20 in the current study, may be attributed to the increased fibre fractions present in the MOC20 diet.

3.4.4 Nitrogen (N) intake and retention

The inclusion of Macadamia Oil Cake (MOC) in the diets in the present study did not affect (P>0.05) nitrogen (N) intake by goats. However, differences were observed for urinary-N, feacal-N, total nitrogen excretion (TNE) and N-retention among the treatments (P<0.05). The similar N intake by goats could be due to the similar crude protein intake (CPI) among the treatments.

The feacal-N ranged from 6.17-8.58 g/day, the higher feacal excretion of N among treatments (8.58 g/day) was recorded in feaces of goats fed diet containing 20% MOC. In contrary Mikasi (2018) reported non-significant differences (P>0.05) of feacal-N by lambs fed MOC based diets, ranges of feacal-N (0.30-0.40 g/day). Mlambo *et al*., (2011) reported that the inclusion of Amarula Oil Cake (AOC) in diets resulted in the lowest feacal-N excretion, which contrasted the results in the present study. The findings of this study are comparable to the findings of Nazir *et al*., (2012) and Simbarashe *et al*., (2015) who reported feacal-N ranges of 5.11-7.08 g/day in sheep and goats fed cottonseed cake and forage legumes, respectively.

Higher urinary-N is associated with lower N efficiency in animals (Kohn *et al.,* 2005). The urinary-N results in the present study contradict the findings of Mikasi *et al*., (2018), who reported that the inclusion of MOC at 10 and 15% did not affect (P>0.05) urinary-N in lambs. These differences could be attributed to the differences in protein and energy of the diets used in the two studies.

The N retention in the present study showed no significant differences (P>0.05) among goats fed the four different diets, all values were positive. The results are consistent with the findings of

Sherrod & Ishizaki, 1996; Mikasi *et al*., 2018) who reported positive N retention in lambs fed MOC based diets. This suggests that the goats retained the digester long enough to allow for nitrogen absorption and not excrete it in the feaces and urine (Mlambo *et al*., 2011). In contrast, Simbarashe *et al* ., (2015), who fed forage legumes reported negative N retention by goat. The positive N retention in the present study is an indication that goats fed MOC based diets acquired protein efficiently to support the protein needs of the goats (Mlambo *et al*., 2011). Furthermore, this is also suggestive that there was good balance between protein and energy of the diets.

3.5. Conclusion

The present study indicated that Macadamia Oil Cake (MOC) at up to 20% of total DM does not have any effect on nutrient intake, dry matter (DM), crude protein (CP) digestibilities and nitrogen (N) retention. Therefore, it can be concluded that MOC as a protein supplement supported the N requirements for maintenance of growing goats. This is supported by the positive N retention values attained in the study. Therefore, farmers can incorporate MOC in the diets of ruminants. However, there is still a necessity to investigate the threshold inclusion level of this cake as well as investigation using animals at different physiological stages such as gestation and lactation.

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CHAPTER 4

ESTIMATION OF MICROBIAL PROTEIN YIELD USING URINARY ALLANTOIN METHOD IN GOATS FED MACADAMIA OIL CAKE BASED DIETS

ABSTRACT

Supplementation of protein to goats can sustain optimal microbial growth so that utilization of fibrous feed can be optimized. The objective of this study was to evaluate the rumen microbial protein yield based on urinary excretion of the purine derivative (PD) allantoin in goats fed Macadamia Oil Cake (MOC) based diets. Eight Boer and eight Pedi male intact goats with average initial weight of 34 ± 8.3 kg aged 18 to 24 months were housed individually in metabolic cages. The goats were randomly assigned to one of the four treatments according to completely randomized design (CRD) consisting of four replicates. The treatments were MOC0: complete feed containing 0% MOC, MOC10: containing 10% MOC, MOC15: containing 15% MOC and MOC20: containing 20% MOC. This study began with an adaptation period of 14 days; urine collection was carried out for seven days. Approximately 10% of urine samples were taken as spot sample from each goat, samples were frozen, and aliquots were analysed for the PD allantoin. The data were subjected to analysis of variance. The findings of the current study indicated that, the inclusion of MOC on goat diets results in increased ($P<0.05$) allantoin excretion. In general, the excretion of PD allantoin affected microbial protein yield by goats fed MOC based diets.

Keywords: allantoin, by-product, Macadamia Oil Cake (MOC), microbial protein yield, purine derivative, urine.

4.1 Introduction

Microbial protein is the major protein source, contributing 70 - 100 % of the total protein for ruminants (AFCR, 1993). Ruminants must consume the required amount of protein to meet their production potential (Chanjula *et al*., 2004). Measurement of microbial protein yield of the gastrointestinal tract is extremely vital in the estimation of the protein needs of ruminants (Chen & Gomes, 1992). The knowledge of microbial protein yield contributes to the development of supplementation strategies for enhancing ruminant production. Methods used to predict the microbial protein yield is the measurement of either total purine derivatives (PD) or allantoin only, which are present in urine.

Purine derivatives are primarily produced by microbes (Chen & Gomes, 1992). The excretion of PD can be used as an indicator of rumen microbial protein supply for ruminants. The cost of conventional protein sources for ruminants has resulted in farmers using oilcakes as feed like Macadamia Oil Cake (MOC). This oilcake has modest crude protein (CP), and its utilization in goats highly depends on the rumen microbial activity to produce microbial protein. Like other ruminants, goats have the ability to utilize lignocellulose and convert it to animal products of high nutritional value, such as meat, milk, hide and manure. This is due to the dense and diverse rumen microbial population belongings to a different group of flora and fauna (Agrawal *et al*., 2014). Therefore, the present study was conducted to estimate the microbial protein yield using the PD allantoin excretion method in goats fed MOC based diets.

4.2 Materials and methods

4.2.1 Ethical considerations

The study was approved by the Ethics Committee of the University of Venda as described in Chapter 3.2.1.

4.2.2 Description of study area

The study was conducted at the University of Venda, Faculty of Science, Engineering and Agriculture Experimental farm as described in Chapter 3.2.2

4.2.3 Macadamia Oil Cake (MOC) preparation

Macadamia Oil Cake (MOC) was collected from Green farms (Pty) Ltd and samples were prepared as described in Chapter 3.2.3.

4.2.4 Experimental animals and management

Experimental animals were managed and fed as described in Chapter 3.2.4. The feed chemical and mineral composition of each treatment is presented in Table 3.1 and Table 3.2, respectively.

4.2.5 Analysis of purine derivative (PD) allantoin

The observed variable in this study was excretion of the purine derivative (PD) allantoin in urine. Urine sample for each goat was collected daily in plastic buckets containing 100 ml of 10% sulphuric acid for seven days. Approximately 10% of urine was taken as spot sample from each goat. Immediately after collection, the urine was filtered through gauze and frozen later to determine the concentrations of allantoin. Distilled water was added to dilute urine in such a way that the concentrations of allantoin in the final sample fall within the range of standards (10-50 mg/L) (appendix 1). The urine allantoin contents were estimated by the colorimetric method at an absorbance rate of 522 nm, according to the specifications of Chen & Gomez (1992). The readings were taken on a digital spectrophotometer.

4.2.6 Calculations

Microbial protein yield

The microbial protein yield was calculated from equations as described by Chen & Gomes (1992):

$$Y= 0.84X + (0.15W^{0.75}e^{-0.25X}) \quad (1)$$

Y= purine derivative excreted in the urine (mMol/d),
X=concentration of microbial purines absorbed after duodenal and intestinal digestion (mMol/d), The calculation of X will be performed by means of Newton-Raphson iterative process until it approaches a constant value (Chen & Gomez, 1992), $W^{0.75}$= the metabolic body weight of animal (kg).

Microbial Nitrogen yield

$$MNY (g/d) = X*70/ (0.83*0.116*1000) =0.727X \quad (2)$$

Where;

70 is the N content of purines (mg N/mMol), 0.83 is the average digestibility of mixed microbial purines based on observations of Chen & Gomez (1992), 0.116 is the proportion of purine N in the total microbial nitrogen of mixed rumen microbes, 1/1000 is used to convert the estimate from mg to g

Microbial true protein (MTP), digestible microbial true protein (DMTP) and efficiency of microbial nitrogen supply (E_{mns}) were calculated according to the AFRC (1993)

$$MTP (g/d) = 0.80×MNY×6.25 \quad (3)$$
$$DMTP (g/d) = 0.85×MTP \quad (4)$$
$$E_{mns} (g/kg DOMR) = MNY/DOMR \quad (5)$$

4.2.7 Statistical analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) . The treatment means were separated using Turkey's test the General Linear Model (GLM) using the SPSS (2016) statistical software. The level of significance was set at $p<0.05$. The model used is as follows:

$$Y_{ijk}=\mu + Ti + \varepsilon_{ijk} \qquad (6)$$

Where;

Y_{ijk}= Observation/response variable (allantoin, microbial protein yield)

μ = Overall mean

Ti=Effect of diets for i=1-4 (0, 10, 15 and 20%)

ε_{ijk} =Experimental error

4.3 Results

4.3.1 Purine derivative (PD) allantoin excretion in urine of goats fed Macadamia Oil Cake (MOC) based diets

Table 4.1 shows the excretion of the purine derivative (PD) allantoin in the urine of goats fed MOC based diets. The excretions of allantoin in the urine were significantly different ($P<0.05$) among the treatments. Allantoin excretion increased with increasing MOC supplementation. Observed allantoin excretion in the urine of goats was high for MOC20 compared to MOC0. However, it was similar to MOC10 and MOC15.

4.3.2 Microbial protein yield

Table 4.1 shows data on estimated microbial nitrogen (N) yield (MNY), calculated microbial true protein (MTP) (AFRC, 1993), digestible organic matter in the rumen (DOMR) and the efficiency of microbial nitrogen synthesis ($^{1}E_{mns}$) of goats fed MOC supplemented diets. MNY and MTP were significantly ($p<0.05$) higher in goats fed MOC20 than goats fed the MOC0 diet. However, it was similar to goats fed MOC10 and MOC15 diets. The inclusion of MOC at 0, 10, 15 and 20% did not significantly affect ($P>0.05$) DOMR and the $^{1}E_{mns}$ by goats.

Table 4.1: Microbial protein yield based on urinary allantoin method in goats fed Macadamia Oil Cake (MOC) based diets

	Treatments					
Parameter	MOC0	MOC10	MOC15	MOC20	SEM	P-value
Allantoin (mMol/d)	14.2^{b}	15.7^{ab}	15.5^{ab}	16.3^{a}	0.40	0.00
Microbial N yield(g d^{-1})	12.3^{b}	13.5^{a}	13.4^{ab}	14.0^{a}	0.34	0.00
Microbial true protein (g d^{-1})	61.3^{b}	67.9^{a}	67.9^{ab}	70.0^{a}	1.72	0.00
Digestible organic matter in the rumen (DOMR)	660.6	534.0	589.8	591.8	32.3	0.06
[1]E_{mns} (g/kg DOMR)	0.02	0.03	0.02	0.03	0.01	0.31

[a,b] Means in the same row with different superscripts are significantly different MOC0=0% Macadamia oil cake; MOC10=10% Macadamia Oil Cake; MOC15=15% Macadamia Oil Cake; MOC20=20% Macadamia Oil Cake (Macadamia oil cake inclusions); N=Nitrogen; [1]E_{mns}=Efficiency of microbial nitrogen synthesis (AFRC,1993); SEM=Standard error of mean

4.4 Discussion

4.4.1 Allantoin excretion in the urine of goats fed Macadamia Oil Cake (MOC) based diets

The differences (P<0.05) in the urinary excretion of the purine derivative (PD) allantoin in mMol/d observed in response to the increase of Macadamia Oil Cake (MOC) in the goat diets, suggests that there is greater flow of microbial protein from the rumen (Moorby *et al*., 2006). The results in the current study are in line with the findings of Fonseca *et al*., (2006), who reported substantial differences (P<0.05) in allantoin excretion in urine of goats fed cottonseed meal (CSM) ranging from 12.9 - 16 mMol/d which are comparable to the 14.25 -1 6.30 mMol/day reported in the current study. These results suggest that MOC supplementation has an effect on the metabolism of nutrients especially protein, resulting in greater ruminal microbial activity. In contrast, Mikasi *et al*., (2018) reported no substantial differences (P>0.05) in allantoin concentrations in the urine of lambs fed MOC based diets at 10 and 15% MOC inclusion levels. The differences in the two studies may be attributed to the different animal species used and the different diet compositions.

4.4.2 Estimation of microbial protein yield in goats fed Macadamia Oil Cake (MOC) based diets

Considering the crude protein (CP) levels that maximize microbial yield, Detmann *et al*., (2014) reported that 8% CP is required for ruminal microbes not to utilize endogenous sources of nitrogen (N) sources. According to Lazzarini *et al*., (2009), forages that contain CP content lower than 7% may not adequately support microbial activity in the rumen. Therefore, the CP content of the diets (approximately 18%) in the present study suggests that it will support microbial activities in the rumen.

Differences in rumen microbial nitrogen (N) yield are related to dietary N concentration of the diet. Microbial N yield showed substantial differences (P<0.05), goats in diets MOC10, MOC15 and MOC20 had relatively high microbial N yield compared to MOC0. The results in the present study are similar to the results reported by Santos *et al*., (2014) who fed goats protein sources (Soyabean meal, Cottonseed meal and Leucaena hay) and found significant differences (P<0.05) in microbial N yield. Khandaker *et al*., (2012) reported substantial differences in digestible organic matter in the rumen (DOMR), and MN yield in cattle fed Mustard Oil Cake.

In contrast, Mikasi *et al*., (2018) reported no significant differences (P>0.05) in allantoin concentrations in the urine, DOMR and microbial N yield of lambs fed MOC based diets at 10 and 15% MOC inclusion levels. These differences could be attributed to the differences in the availability of energy and nitrogen (N), as they are the main restrictive factors in the development of ruminal microorganisms (Borja *et al*., 2014).

According to Pathak (2008), the efficiency of microbial protein synthesis ($^{1}E_{mns}$) greatly differs in animals fed similar diets. The non-significant differences (P>0.05) in $^{1}E_{mns}$ in the current study could be attributed to the high neutral detergent fibre (NDF) 500 g/kg DM in the Macadamia Oil Cake (MOC), which limits the supply of metabolizable protein of microbial origin in the small intestines (Argolo *et al.,* 2010). Calsamiglia *et al.,* (2010) reported ranges of $^{1}E_{mns}$ within the range 10 - 40% in ruminants, which is comparable to the 20 – 30% ranges reported in the current study. In contrast, Baloyi *et al*., (2014) reported higher range values of $^{1}E_{mns}$ (7.67 – 13.3 g/kg DOMR) for forage legume hays based diets fed to lambs. These differences could be attributed to the different OM digestibility co-efficient in the two studies as $^{1}E_{mns}$ is influenced by OM intake and digestibility.

4.5. Conclusion

The inclusion of Macadamia Oil Cake (MOC) in diets for goats improved the excretion of urinary allantoin and improved microbial protein yield. Digestible Organic Matter in the rumen (DOMR) and the efficiency of microbial protein synthesis (^{1}Emns) were not affected by the MOC inclusions. This suggests that MOC supplementation in the diets of goats up to 20% may help improve the utilization of low-quality roughages, by supplying nitrogen (N) to rumen microbes.

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CHAPTER 5

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. General discussion

Ruminants (goats) in South Africa are mostly raised on pasture grazing systems. The quality and quantity of the pastures limit production especially in the winter season. Therefore, there is necessity for supplementation in order to maintain, and enhance production at a low cost. Soyabean meal (SBM) is an expensive protein source for most small-scale farmers. Moreover, SBM is not readily available or easily accessible in the rural areas where most goats are reared. Macadamia Oil Cake (MOC) is a promising ruminant feed ingredient because it is less expensive (R3450.00 per ton) compared to Soyabean, a tonne of Soyabean costs approximately (R117.00 per kg). The chemical composition in this study (Table: 3.3), shows that MOC contains 137 g/kg crude protein (CP), which is adequate for small growing ruminants' protein requirements as per NRC (2007).

The non-existence of differences in the apparent digestibility coefficients of nutrients, suggests that MOC has no negative effect on the digestibility of nutrients by goats. Exceptions were observed for the intake of EE, NDF and ADF and apparent digestibility of OM and EE which showed significant differences among treatments . Similar N-retention was observed for goats among all the treatments, the positive N-retention values (4.08 – 9.40 g d^{-1}) from the MOC based diets emphasises that MOC supplementation did meet the maintenance requirements for goats as shown by the positive N-retention values. Inclusion of MOC in goat diets affected the excretion of purine derivative (PD) allantoin, indicating that there was a greater flow of microbial protein from the rumen. Generally, microbial protein yield was improved by MOC supplementation, making the urinary allantoin method a promising tool to estimate microbial protein yield in ruminants.

5.2. General conclusion

The study's specific objectives were to determine the apparent digestibility of Dry matter (DM) and Crude protein (CP), nitrogen (N) retention and microbial protein yield from Macadamia Oil Cake (MOC) based diets in goats. It was hypothesized that feeding MOC based diets to goats would not have any significant effect (P>0.05) on DM and CP apparent digestibility, Nitrogen (N) retention and microbial protein yield. The study confirmed the null hypotheses.

Based on the results of the current study, it can be concluded that the CP levels of the pellet-MOC based diets meet the supplementation requirements of growing goats. Therefore, to improve the quality of pastures during dry seasons MOC can be used as a protein supplement in the diets of ruminants. The results of this study will open prospect of formulating affordable and efficient MOC-based diets for ruminants.

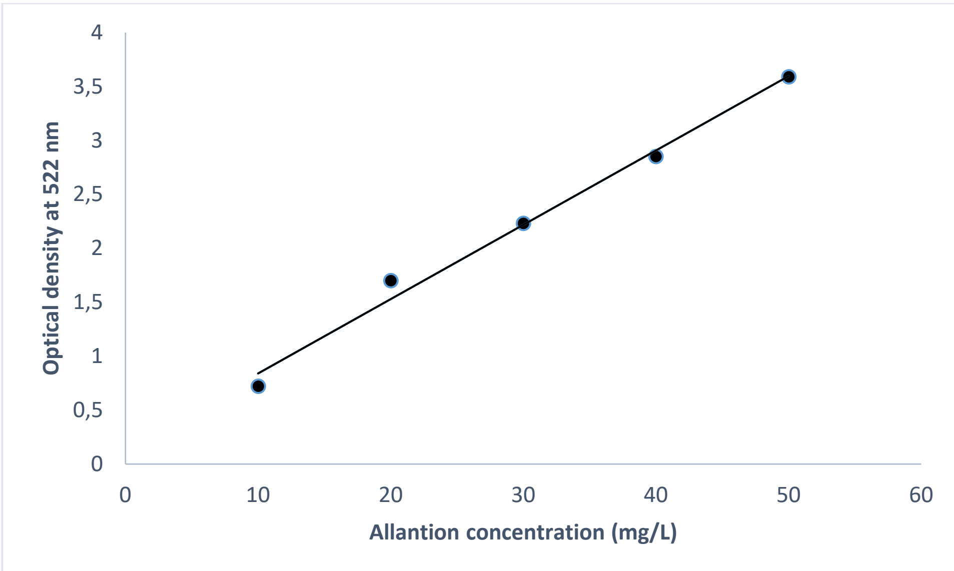
The efficient utilization of nitrogen (N) is increasingly becoming important in ruminant production. This is as a result of the increasing prices of protein sources. The results obtained in this study showed that the urinary excretion allantoin method is a promising tool that can be used to estimate microbial protein yield, which is a fraction of N indicating nitrogen utilization efficiency.

5.3. Recommendations

The current study did not carry out *in vivo* digestibility and rumen degradability trials. Consequently, it is recommended that more research be carried out on ruminants especially goats to study the effects of MOC on goats and on all other parameters of efficient utilization evaluation. Moreover, research on allantoin as an estimate of microbial protein yield in goats fed MOC based diets is currently limited. Only a study carried out by Mikasi *et al*., (2018) on lambs is available, therefore further research on goats is necessary. This will provide information on the efficient utilization of MOC in goats and test the hypotheses. Finally, the inclusion of MOC should be increased or replace Soyabean meal (SBM) totally in order to determine MOC full potential in the ruminant industry.

APPENDICIES

Appendix 1: Allantoin standard curve used to calculate concentrations of the unknown based on this equation: Y= 0.06X + 0.15 (R^2= 0.96)



Appendix 2: Analysis of variance for voluntary intake (g d^{-1}) and apparent digestibility coefficient

Source	Df	DMI	OMI	CPI	EEI	NDFI	ADFI	DOMI	ME intake
Diet	3	226806	180566	5981	3188**	53262	7081*	177140**	43.66*
Error	107	90193	73229	2724	162	9344	2158	68872	16.98

**:P<0.01; *:P<0.05; df=Degree of freedom; DMI= Dry matter intake; CPI= Crude protein intake; EEI= Ether extract intake; NDFI=Neutral detergent fibre intake; ADFI= Acid detergent fibre intake; DOMI= Digestible Organic Matter Intake; ME intake= Metabolisable energy (MJ d^{-1})

Appendix 3: Analysis of variance for apparent nutrients digestibility co-efficient (g d^{-1})

Source	Df	DMD	OMD	CPD	EED	NDFD	ADFD
Diet	3	0.058069	2298**	0.08063	0.07749**	0.449720	0.574129
Error	107	0.044108	0.003588	0.0511	0.02065	0.184909	0.230196

**:P<0.01; *:P<0.05; df=Degree of freedom; DMD= Dry matter digestibility; OMD=Organic matter digestibility; CPD= Crude protein digestibility; EED= Ether extract digestibility; NDFD= Neutral detergent fibre digestibility; ADFD= Acid detergent fibre digestibility

Appendix 4: Analysis of variance for nitrogen retention (g d^{-1}) of goats fed Macadamia Oil Cake (MOC) based diets

Source	Df	N intake	N-feaces	Urinary-N	TNE	N-retention
Diet	3	153.11	25.730**	110.32**	174.06**	110.32**
Error	107	69.74	6.58	26.37	35.88	26.37

**:P<0.01; *:P<0.05; df= Degree of freedom; N= Nitrogen; TNE= Total nitrogen excretion

Appendix 5: Analysis of variance for microbial protein yield based on urinary allantoin method in goats fed Macadamia Oil Cake (MOC) based diets

Source	Df	Allantoin	MNY	MTP	DOMR	[1]Emns
Diet	3	20.6544**	15.4751**	386.68**	74842*	0.001280
Error	107	4.4404	3.3055	82.64	29099	0.001062

**:P<0.01; *:P<0.05; df= Degree of freedom; MNP= microbial nitrogen yield; MTP= microbial true protein; DOMR= digestible organic matter in the rumen ; [1]Emns= Efficiency of microbial nitrogen synthesis (AFRC, 1993).