# EFFECTS OF EXOGENOUS ENZYMES ON THE NUTRITIVE VALUE OF *MACADAMIA SPP*. NUT OIL CAKE AS A PROTEIN SOURCE FOR GROWING PIGS

**BY**

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## DECLARATION

I, Maemu Queen Rambau, hereby declare that this dissertation for a Master of Science in Agriculture (MSCANS) submitted to the Department of Animal Science, Faculty of Science Engineering & Agriculture, at the University of Venda has not been submitted previously for any degree at this or another university. It is original in design and in execution, and all reference material contained therein has been duly acknowledged.

**Signature**  **Date 30 January 2023**

RAMBAU MAEMU QUEEN

## DEDICATION

To my late father Thavha Petrus Rambau, mother Prescilla Rambau, my siblings Hulisani Rambau, Thifhelimbilu Rambau, Eve Rambau and lastly my handsome son Oritonda Edgar Ndou.

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## ABSTRACT

The current high cost and scarcity of the conventional stock feeds, and the predicted long-term impact of climate change on their production are major risks to sustainable animal production in the smallholder sector in South Africa. The present study investigated the nutritive value of Macadamia (Macadamia spp.) nut oil cake (MOC), and the efficacy of exogenous enzymes to enhance its value as an alternative protein source in growing pig diets. In an enzyme-screening *in vitro* study (experiment 1), 3-step (pepsin + pancreatin + Viscozyme) porcine digestion was employed to compare the digestive efficacy on MOC of a custom enzyme cocktail containing 3000 FTU $g^{-1}$ 6-phytase (IUB 3e1.3.26), 7270 U $g^{-1}$ endo-1,4-Beta-Xylanase (EC-3.2.1.8), 300 U $g^{-1}$ alpha amylase (EC-3.2.1.1), 6000 $g^{-1}$ subtilisin protease (EC-3.4.21.62), 532 U $g^{-1}$ endo-1,4-beta-glucanase (IUB 3.2.1.6), and Ronozyme® WX 2000 CT (UB 3.2.1.8 endo-1,4-β-xylanase, 200FXU/g) . The experiment was performed in a completely randomized design with six replicates per treatment. In experiment 2, the experimental diets were a balanced, standard commercial maize-soybean diet, and an iso-nutrient, 10% MOC-maize-growing diet, each with a duplicate supplemented with 500g/tonne of the custom enzyme cocktail. Eight F1 Large White X Landrace piglets (15.3 ± 1.91 kg live weight (LW) were assigned to diets in a 2 x 2 factorial arrangement, in a duplicated, balanced 4 × 4 Latin square design. An 8-day feeding period was used in the Latin squares, consisting of 3 days adaptation, and 5 days of feed intake measurement, and the total collection of faecal and urine excreta. *In vitro,* the enzymes did not affect DM digestibility($P>0.05$). Neither of the enzymes altered the partial gastric-small intestine (19.9-22-8), colon (21.3-22.8) and the total (41.2-44.4) IVDMD of MOC (0.05). *In vivo*, the 10% MOC diet had low ($p<0.05$) digestibility of crude protein, with no ($p>0.05$) effect on the digestibility of other chemical components. Scaled to the LW, 10% MOC dietary inclusion reduced ($p<0.05$) the NR. The NR was higher ($p<0.05$) on the 0%, compared to the 10% MOC diets only when the diets contained the exogenous enzymes. Scaled to the $LW^{0.75}$, the 0% MOC dietary inclusion reduced ($p<0.05$) the NR. Dietary inclusion of MOC at 10% marginally reduced the digestibility of CP, and the NR. In

conclusion, the IVDMD of MOC was low, and not improved by fortification with fibrolytic exogenous enzyme cocktails. *In vivo nutrient* digestibility, and N balance responses to the 10% MOC diet were similar to a standard diet, which supported the 10 % dietary inclusion of MOC as an alternative protein source for weaned, fast-growing Large White pigs. A more potent exogenous proteinase and or carbohydrase enzymes could be effective tools to improve dietary efficacy and protein efficiency.

**Key words**: *Macadamia integrifolia, Macadamia tetraphylla,* exogenous enzymes, *in vitro* digestibility, *in vivo* digestibility, nitrogen balance

## Contents

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ADF | Acid detergent fibre |
| ADL | Acid detergent lignin |
| ANOVA | Analysis of variance |
| Ca | Calcium |
| CP | Crude protein |
| DM | Dry matter |
| FNO | Faecal nitrogen output |
| IVDMD | *In vitro* dry matter digestibility |
| MOC | Macadamia oil cake |
| N | Nitrogen |
| ND | Apparent nitrogen digestibility |
| NDF | Neutral detergent fibre |
| NI | Nitrogen intake |
| NR | Nitrogen retention |
| NRC | National Research Council |
| NU | Nitrogen utilization |
| OM | Organic matter |
| P | Phosphorus |
| TDN | Total digestible nitrogen |
| TNE | Total nitrogen excretion |
| UNO | Urine nitrogen output |

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# CHAPTER 1 INTRODUCTION

## 1.1 Background

For some time, the global livestock industry has been subjected to escalating feed shortages and costs (Acheampong-Boateng *et al.*, 2017). Conventional protein sources such as soybean oil cake are not readily available, particularly to small scale-farmers (Nkosi *et al.*, 2011b). To sustain viable livestock production, farmers are increasingly under pressure to revert to agro-industrial by-products (Acheampong-Boateng *et al.*, 2017). However, local agro-industrial by-products are still largely excluded from mainstream stockfeed value chains. Their integration into both smallholder and advanced commercial feeding systems demands nutritional characterisation for efficient utilisation.

Macadamia (*Macadamia Integrifolia, Macadamia Tetraphylla, Macadamia spp hybrids)* nut oil cake (MOC) is a potential dietary protein substitute for conventional feeds (Wallace and Walton, 2011). However, the MOC is produced in a process that requires the inclusion of soybean hulls to facilitate efficient oil expeller function (Acheampong-Boateng *et al.*, 2008). Consequently, though typically high (19.5%) in crude protein, MOC also contains substantial (24.9%) crude fibre (Acheampong-Boateng *et al.*, 2008).

Compared to ruminant livestock, non-much is known on the nutritive value of MOC as a protein source for monogastric livestock such as pigs. In monogastric species, the high fibre content imposes a limit on its dietary inclusion, given the risk of fibre-induced restriction of the feed intake, and of nutrient digestion (Jha *et al.*, 2019). Exogenous enzymes might be effective tools to overcome this challenge. Cocktails which include phytase, protease and amylase activities can theoretically augment the action of endogenous pig enzymes, while fibrolytic enzymes additionally benefit nutrient digestion by degrading otherwise indigestible, nutrient-protecting complex non-starch polysaccharides to expose nutrients to digestion (O'Neill *et al.*,

2014). However, to be efficient, exogenous enzymes need to be customised to carefully examined for correct matching to the dietary chemical matrix (Apajalahti *et al.*, 2001).

The aim of the present study was to evaluate the effects of what we considered to be a high (10%) dietary inclusion of a locally available, highly fibrous MOC product as a protein source in a maize-based diet for growing pigs, and to evaluate the efficacy of a custom cocktail of amylolytic, fibrolytic and proteolytic exogenous enzymes in enhancing the dietary utilisation.

### 1.2 Statement of the research problem

Conventional plant protein sources which traditionally provide the bulk of protein in stock feeds are increasingly becoming expensive and scarce (Nkosi *et al.*, 2011a). There is a need to identify alternative feedstuffs such as MOC to replace the increasingly scarce and expensive conventional feed ingredients partially or completely in pig diets (Tiwari and Jha, 2017). Protein is usually the most limiting nutrient in smallholder growing pig diets (Khanyile *et al.*, 2014), largely due to the both cost and scarcity of protein feeds. Despite low-cost, alternative protein sources should still provide high quality protein to meet both quantitative and qualitative requirements of a pig. The key for efficient pig utilization of MOC based diet is content of essential amino acids, fibre and other antinutrients which may limit pig performance, for which there is limited information. Unlike ruminants, pigs cannot effectively digest fibre if excessive, and may need substrate-matching exogenous fibrolytic and proteinase enzyme complexes to help digest the MOC for efficient N retention. Therefore, there is a need to identify potent enzyme blends which match the unique complex polymer matrix of MOC diets. There is an additional need to establish the levels of dietary inclusion of the MOC for optimum nutritional and cost benefits.

### 1.3 Justification

It might be possible to reduce the high feed costs by switching from conventional protein sources to those that are more affordable, efficient, and easily accessible locally (Berrocoso *et al.*, 2017). It is important to develop a pig feeding programmes based on the readily available

alternative feedstuffs that are also economically viable (Tiwari and Jha, 2017). Macadamia nut oil cake could be such an alternative protein source, given it contains 19.5% crude protein. However, the high fibre content and undefined protein quality for pigs demand cautious inclusion in pig diets to avoid nutritional risk. There is potential to mitigate nutritional risk through the application of exogenous enzyme technology, to aid digestion of MOC. Information obtained from this study will particularly benefit the smallholder farmers who cannot afford or access the conventional protein sources and will contribute to the incorporation of MOC in the stockfeed value chain, with economic benefit to both the livestock and macadamia industry.

## 1.4 Research objectives

### 1.4.1 Overall objective of the study

The overall objective of the study was to evaluate the potential of Macadamia nut oil cake as a protein source for growing pigs, and the potential for nutritive enhancement through exogenous enzymes.

### 1.4.2 Specific objectives

To determine the following:

i. Comparative efficacy of a custom enzyme cocktail containing 3000 FTU $g^{-1}$ 6-phytase (IUB 3e1.3.26), 7270 U $g^{-1}$ endo-1,4-Beta-Xylanase (EC-3.2.1.8), 300 U $g^{-1}$ alpha amylase (EC-3.2.1.1), 6000 $g^{-1}$ subtilisin protease (EC-3.4.21.62), 532 U $g^{-1}$ endo-1,4-beta-glucanase (IUB 3.2.1.6 and Ronozyme® WX 2000 CT (UB 3.2.1.8 endo-1,4-β-xylanase, 200FXU/g) in enhancing dry matter digestibility of MOC measured in an *in vitro* porcine digestion model.
ii. Effects of 10% dietary inclusion of MOC on *in vivo* nutrient digestibility and N utilisation by growing pigs.
iii. Effects of supplementary exogenous enzymes on the utilisation of 10% MOC growing pig diet.

## 1.5 Research hypotheses

### 1.5.1 Null hypotheses

i. A custom enzyme cocktail containing 3000 FTU $g^{-1}$ 6-phytase (IUB 3e1.3.26), 7270 U $g^{-1}$ endo-1,4-Beta-Xylanase (EC-3.2.1.8), 300 U $g^{-1}$ alpha amylase (EC-3.2.1.1), 6000 $g^{-1}$ subtilisin protease (EC-3.4.21.62), 532 U $g^{-1}$ endo-1,4-beta-glucanase (IUB 3.2.1.6), and Ronozyme WX 2000 CT (UB 3.2.1.8 endo-1,4-β-xylanase, 200FXU/g) does not affect *in vitro* dry matter digestibility of MOC.

ii. A 10% MOC dietary inclusion level does not significantly affect the *in vivo* nutrient digestibility and N retention of growing pig diets.

iii. A supplementary custom enzyme cocktail containing 3000 FTU $g^{-1}$ 6-phytase (IUB 3e1.3.26), 7270 U $g^{-1}$ endo-1,4-Beta-Xylanase (EC-3.2.1.8), 300 U $g^{-1}$ alpha amylase (EC-3.2.1.1), 6000 $g^{-1}$ subtilisin protease (EC-3.4.21.62), 532 U $g^{-1}$ endo-1,4-beta-glucanase (IUB 3.2.1.6) does not affect *in vivo* nutrient digestibility and N retention of growing pig diets.

# CHAPTER 2 LITERATURE REVIEW

## 2.1. Introduction

In South Africa smallholder pig production is constrained by feed shortages and high cost (Acheampong-Boateng *et al.*, 2017). There is need to identify potential feed alternatives to sustain pig industry. The local and global Macadamia industries are rapidly expanding, currently valued at estimated R39.41/per kg (SAMAC, 2020). Protein-rich MOC is a candidate stockfeed grade by-product from the macadamia oil industry, for which limited information is available for potential utilization as pig feed. This review of literature discusses the chemical composition of MOC, optimum inclusion in growing pig diets, and the scope for the application of exogenous enzymes on such diets.

## 2.2. Origin, adaptability, and production of Macadamia

Macadamia trees are members of the Proteaceae family that originated in Australia (McNeil *et al.*, 2010). The smooth shelled (*Macadamia Integrifolia*) and rough shelled (*Macadamia Tetraphylla*) are cultivated for their edible nut (Peace *et al.*, 2008). In South Africa, macadamia nuts are becoming the fastest-growing tree crop business and are developing into a significant crop (DAFF, 2019). South Africa is one of the world's top producers of macadamia nuts (SAMAC, 2020). Three provinces in South Africa produce most the country's macadamia nuts: Mpumalanga (Hazyview to Barberton), which continues to be the principal production region, Limpopo (Tzaneen and Levubu districts), and the north and south coastal regions of KwaZulu Natal (SAMAC, 2020).

## 2.3 Effect the of complex constituent of fibre on DM digestibility in pigs

Macadamia nut oil cake has high fibre content of 25%, depending on the method of oil extraction (Phosa, 2009). Due to the pig digestive system's short transit rates caused by high fiber levels, nutrients are not effectively utilised (De Vries *et al.*, 2012). The high fibre content may render MOC unsuitable as an ingredient for pig diets, acting as a physical barrier to the animal's own enzymes, while encapsulating compressing useful nutrients (Bedford and Partridge, 2010), to inhibit nutrient digestibility and absorption(González-Alvarado *et al.*, 2007;

Kalmendal *et al.*, 2011). A study on corn by product showed a decrease in digestibility of ADF and NDF (Liu *et al.*, 2018).

**2.4 Chemical composition and nutritive value of Macadamia oil cake**

Macadamia nut oil cake of 93.8% dry matter contained 19.5% crude protein, crude protein 19.5%, 51.8% and 41.8% NDF and ADF (Acheampong-Boateng *et al.*, 2008). In 94.1% DM MOC, (Skenjana *et al.*, 2006) reported 20.93% crude protein, 49.84% NDF and 40.04% ADF in 94% dry matter MOC, reported 13.2% crude protein, 36.5% crude fibre, 22.8% Crude fat (Van Ryssen *et al.*, 2014). DM 94% with 13% crude protein was observed by (Phosa, 2009), which is comparable to the figure reported by (Van Ryssen *et al.*, 2014). The different chemical content might be due to the oil extraction method and the extent of soyabean hulls inclusion during oil extraction (Mikasi, 2018). Overall, the chemical composition makes it a suitable alternative protein source for pigs.

**2.5 *In vitro* dry matter digestibility of macadamia oil cake.**

*In vitro* methods are preferred for their simplicity, and do not require the use of animals which is ethically unacceptable (Cheli *et al.*, 2012). (Tiwari and Jha, 2017) reported porcine *in vitro* MOC dry matter digestibility of 76.7%. This shows that the Macadamia residues are highly digestible in porcine small intestines.

There are several *in vitro* methods, such as the 2-step (Boisen and Fernández, 1997) and step method (Noblet and Jaguelin-Peyraud, 2007), that can be used to determine digestibility. These methods simulate endogenous pig enzymatic digestion in the stomach and small intestine of pigs (2-step), and colon microbial hydrolysis of complex carbohydrates in the large intestine (3-step), to respectively estimate upper tract (gastric-ileal) and summative total tract digestibility of dry matter (DM) (Noblet and Jaguelin-Peyraud, 2007).

The *in vitro* gas production method (IVGPT) was created for ruminants in order to replicate microbial fermentation and to estimate the quality of forages in comparison to concentrates (Storm *et al.*, 2012). Similarly, the in vitro gas and short chain fatty profiles could be important

in determining the extent and rate of intestinal fermentation in the hindgut given potential benefits of microbial fermentation and its end products in pigs (Fushai *et al.*, 2019). Dietary fiber from various feedstuffs has been evaluated for its potential prebiotic effects in pigs using an in vitro approach (Jha *et al.*, 2011).

### 2.6 Nutritive value of MOC for monogastric livestock

Proteins are defined as biological compounds consisting of one or more polypeptide folder into a globular or fibrous form, associated with a specific biological function (Vermeulen, 2017). Protein digestion starts in the stomach and continues in the small intestines through the process of hydrolysis (Brodkorb *et al.*, 2019), where polypeptides are enzymatically incrementally broken down into simple subunits which can be absorbed through the gut epithelium. The amino acid content of feed protein, its digestibility, and the amino acids' availability in cells are of utmost importance to monogastric in terms of the protein value (Apajalahti and Vienola, 2016).

Digestibility and animal growth response are the two major approaches to assessing availability of amino acids in feeds for pigs with digestibility being an important determining factor of amino acid availability. According to Karzai *et al* (2000) amino acid digestibility can be accessed both the ileal and faecal points, with the ileal digestibility preferred indicator of protein value (Adeola *et al.*, 2016).

MOC successfully replaced 50% soybean in tilapia fish (Latif *et al.*, 2008). Both Phosa (2009) and Van Ryssen *et al* (2014) recommended 10% MOC optimum dietary inclusion in broiler diets. For Ross broilers (Acheampong-Boateng *et al.*, 2016) recommended 25% MOC substitution for soybean cake.

### 2.7 Role of exogenous enzymes in digestibility of nutrients by growing pigs

Exogenous enzymes are frequently used in swine diets to improve nutritional value by reducing the negative impact of no-starch polysaccharides (NSP) on nutrient absorption. A cocktail of multi-carbohydrase enzymes improves nutrient utilization of pigs (Woyengo *et al.*,

2010). Dietary enzyme supplementation improves feed conversion efficiency, thereby reducing feed cost (Adeola and Cowieson, 2011).

*In vitro,* exogenous enzymes increased pig ileal digestibility of dry matter (Kang *et al.*, 2017). Enzymes positively affected the bacterial populations in the large intestine, which enhanced the growth performance of weaned pigs (Yi *et al.*, 2013). Proteases are commonly included with commercial exogenous enzyme blends to break down storage protein stored/bound within feed ingredients (Geraldo *et al.*, 2008). An energy-deficient diet's apparent total tract digestibility of DM, GE, and nitrogen increased when xylanase and glucanase were blended. A blend of xylanase and *β*-glucanase increased the apparent total tract digestibility of DM, GE and nitrogen of an energy-deficient diet (Swiatkiewicz *et al.*, 2016).

Microbial phytases such as Ronozyme P improve amino acid digestibility when rice bran is included in the diet (Rutherfurd *et al.*, 2002). The *in vitro* DM digestibility of palm kernel cake was found to be 62.58% with a cocktail of phytase, xylanase and multipurpose enzyme (Jimoh, 2018). An increase in digestibility of dry matter for palm cake indicates the positive effect of enzymes and the cocktails. Even though it depends on the test ingredients, an *in vitro* experiment found that adding enzyme complexes (xylanase, protease, and phytase) increased the ileal digestibility of dry matter (Kong *et al.*, 2015).

Supplementation of cocktail enzymes (phytase, multipurpose and xylanase) was reported to improve in *vitro* dry matter (62.58), crude protein (75.31%), crude fibre (71.16%) and EE (68.55%) digestibility of palm kernel cake (Jimoh, 2018). The apparent ileal digestibility (AID) of amino acids in piglets increased when proteases were added to a diet based on soybean (Guggenbuhl *et al.*, 2012). Weaned pigs' meals included proteases, which appeared to increase the total tract's apparent ability to digest nitrogen (N), as well as a few essential amino acids and minerals (Pan *et al.*, 2016). Protease-supplemented diets given to weaned pigs resulted in enhanced nutritional digestibility (Zuo *et al.*, 2015; Tactacan *et al.*, 2016).

Pigs fed higher doses of xylanase showed improvements in nutritional digestibility (Tapingkae *et al.*, 2008). Increasing amounts of phytase to piglet meals resulted in linear increases in growth and P digestibility (Zeng *et al.*, 2015). When a blend of enzymes was added to the diet, dry matter digestibility increased (Skenjana, 2011). A study Yi *et al* (2013) reported that enzyme mixtures had a positive impact on nutrients digestibility.

### 2.8. Effect of Macadamia nut oil cake on nitrogen retention

Lowering the dietary CP of growing pigs from 16.9-15.6 to 14.6-13.5% resulted in a decrease in urine nitrogen excretion but not faecal nitrogen excretion, according to (Gatel and Grosjean, 1992). A low-protein diet caused nitrogen intake to be 53 g/d, faecal nitrogen to contain 6.56 g/d, urinary nitrogen to contain 10.12 g/d, total nitrogen to contain 16.69 g/d, and nitrogen retention to contain 68.48% g/d (Zhao *et al.*, 2019). N retention declines as protein inclusion rises; at CP 18%, N retention was 37.7 g/day. At CP 18.47%, it was 37.5, and at CP 18.97, it was 36.9 g/day (Antongiovanni *et al.*, 2007). High fibre low protein diet with 122g/kg CP observed N intake 45.6, N in faeces 9.80, urinary N 17.8, total N excreted 27.6 and N retained 18 (Galassi *et al.*, 2010). Pigs offered diets containing 160 g CP/kg with and without enzyme excreted 22.11 and 19.73 urinary N, 8.28 and 9.35 faecal, 31.40 and 29.09 total N excretion and 34.4 and 36.7 N retained.

### 2.9 Summary

Feed shortage and high cost are major constraints in smallholder pig production. Macadamia nut oil cake is rich in protein but contains high fibre which limits the dietary inclusion to avoid depressing nutrient digestibility and dietary intake. There is limited information regarding Macadamia nut oil cake as an alternative source of protein in pig diets, and on the benefit of

exogenous enzymes. Therefore, there is a need to evaluate the effects of dietary inclusion of MOC in growing pigs, and the potential to improve dietary utilisation through correctly matched enzymes.

# CHAPTER 3

## EFFECTS OF EXOGENOUS ENZYMES ON *IN VITRO* DRY MATTER DIGESTIBILITY OF MACADAMIA OIL CAKE

### Abstract

The objective of this study was to evaluate the chemical composition of MOC and the effects of Axtra® (custom enzyme cocktail containing 3000 FTU g $^{-1}$ 6-phytase (IUB 3e1.3.26), 7270 U g $^{-1}$ endo-1,4-Beta-Xylanase (EC-3.2.1.8), 300 U g $^{-1}$ alpha amylase (EC-3.2.1.1), 6000 g $^{-1}$ subtilisin protease (EC-3.4.21.62), 532 U g $^{-1}$ endo-1,4-beta-glucanase (IUB 3.2.1.6) and Ronozyme® WX (endo-1,4-beta-xylanase) enzyme blends on *in vitro* dry matter digestibility (IVDMD) of Macadamia nut oil cake (MOC). The standard, three step (pepsin, pancreatin, viscozyme). Porcine *in vitro* procedure was modified for micro (0.5 g) digestion of MOC within Ankom® 57 filter bags procedure was used to determine gravimetric exogenous enzymatic hydrolytic efficacy in a completely randomized design replicated seven times. Samples of MOC were fortified with either 500g/tonne of Axtra® (endo-1,4-ß-xylanase and endo1,3(4)-ß-glucanase) or 150g/tonne Ronozyme® (UB 3.2.1.8 endo-1,4-β-xylanase, 200FXU/g). The enzymes did not affect DM digestibility($p>0.05$) of MOC. Neither of the enzymes altered the partial gastric-small intestine (19.9-22-8) and colon (21.3-22.8) and the total (41.2-44.4) IVDMD of MOC ($p>0.05$). In conclusion, the IVDMD was low, and not improved by fortification with fibrolytic exogenous enzyme cocktails.

**Key words**: Enzymes, Chemical composition, *In vitro* digestibility

### 3.1 Introduction

Macadamia (*Macadamia Integrifolia, Macadamia Tetraphylla)* oil cake is a by-product of oil extraction from the nuts. Depending on the source of the macadamia nut and the oil extraction process, MOC contains variable protein content, ranging from as low as 13.2% (Van Ryssen *et al.*, 2014), to as high as 19.5% (Acheampong-Boateng *et al.*, 2008). The oil extraction process uses soybean hulls to prevent the cake from sticking to the sides of the oil-extracting machine, which increases the fibre content to as much as 24.9% crude fibre (Acheampong-Boateng *et al.*, 2008), thereby limiting its dietary inclusion to avoid depression of intake and digestibility on pigs. Therefore, subject to MOC batch protein content and quality, and to the fibre content, the by-product could at least partially, significantly replace more expensive protein sources in growing pig diets.

For such chemically complicated diets, exogenous enzymes could promote nutrient digestion and absorption (Apajalahti *et al.*, 2001). Subject to the enzyme match to the dietary chemical matrix, exogenous enzymes may directly augment endogenous enzyme digestion of nutrient polymers, or act indirectly by degrading nutrient encapsulating non-starch polysaccharides (O'Neill *et al.*, 2014), to enable greater dietary.

The *in vitro* porcine digestion method is a standard procedure to estimate pig feed digestibility, which can be adapted to evaluate the efficiency of different exogenous enzymes in improving swine nutrient digestibility. Experiment 1 evaluated the effects of two commercial exogenous cocktails on the *in vitro* DM digestibility (IVDMD) of MOC

## 3.2 Method and materials

### 3.2.1 Description of the study site

The study was conducted at the University of Venda, which is in Thohoyandou, Vhembe district, Limpopo province, in South Africa (22°58'32''S, 30°26'45''E). The annual minimum temperatures for the area vary, from 10 $^0$C during winter, to a summer maximum between 34 $^0$C to 38 $^0$C (Kom *et al.*, 2022). The sample analyses were done in the animal science nutrition laboratory.

### 3.2.2 Source of Macadamia nut oil cake

Bulk Macadamia nut oil cake was sourced from Royal Macadamia (Pty) Ltd in Levubu, Limpopo province. It could not be established whether a single or several species or varieties of *Macadamia spp* or their hybrids were included in the batch of cake that was used in the study, hence the indefinite species description. However, since the *Macadamia Integrifolia* is the commercially preferred, and dominant species in South Africa, it likely contributed most, if not all the nut used in the study.

### 3.2.3 Chemical analyses

Macadamia nut oil cake samples were dried at 60ºC in an oven for 48 hours to determine the dry matter (AOAC, 1990), method 930.15). Ash was analysed by combustion at 500 °C overnight (AOAC, 1990), method 942.05). Nitrogen was determined using the Kjeldahl procedure (AOAC, 1990), method 984.13 Fats and oils were extracted by the sohxlet procedure method (AOAC, 1990), 920, 39). Neutral detergent fibre and Acid detergent fibre was analysed using the technique of (Van Soest *et al.*, 1991).

The chemical composition of Macadamia nut oil cake is presented in Table 3.1

Table 3.1 Analyzed chemical composition (DM basis) of Macadamia nut oil cake.

| Nutrient | % |
|---|---|
| DM | 93.91 |
| Ash | 3.51 |
| CP | 15.17 |
| ADF | 37.88 |
| NDF | 52.63 |
| EE | 23.62 |
| Ca | 0.27 |
| P | 0.17 |
| Essential amino acids (g/100g DM) | |
| Arginine | 1.34 |
| Alanine | 0.42 |
| Asparagine | 0.52 |
| Glutamine | 0.37 |
| Glycine | 0.15 |
| Histidine | 0.97 |
| Isoleucine | 0.45 |
| Leucine | 0.64 |
| Lysine | 0.88 |
| Methionine | 2.09 |
| Phenylalanine | 0.54 |
| Proline | 0.89 |
| Serine | 0.67 |
| Threonine | 0.95 |
| Tyrosine | 0.42 |
| Valine | 0.54 |

DM: Dry matter, ADF: Acid detergent fibre, NDF: Neutral detergent fibre, CP: Crude protein, Ca: Calcium, P: Phosphorus.

### 3.3 *In vitro* digestion

#### 3.3.1 Macadamia nut oil cake preparation

Macadamia nut oil cake was milled to pass through a 1mm screen. Samples were dried in an oven to a constant weight in a Labotec® drought oven and cooled in a desiccator. A 0.5 g

sample was weighed into an Ankom® F57 filter bag which had been pre-rinsed in acetone (Acetone for HPLC, ≥99.8%, Sigma-Aldrich 34850) and air-dried.

### 3.3.2 Exogenous enzymes

Samples of MOC were fortified with either 500g/tonne of a custom enzyme cocktail containing 3000 FTU $g^{-1}$ 6-phytase (IUB 3e1.3.26), 7270 U $g^{-1}$ endo-1,4-Beta-Xylanase (EC-3.2.1.8), 300 U $g^{-1}$ alpha amylase (EC-3.2.1.1), 6000 $g^{-1}$ subtilisin protease (EC-3.4.21.62), 532 U $g^{-1}$ endo-1,4-beta-glucanase (IUB 3.2.1.6) or 150g/tonne Ronozyme® (UB 3.2.1.8 endo-1,4-β-xylanase, 200FXU/g). The samples which were to serve as controls were not treated with any enzyme.

## 3.4 Treatments, experimental design and digestion procedures

### 3.4.1 Experimental design and digestion procedures

A randomized design was used to compare the efficacy of two enzyme blends in digesting MOC in a one-way experiment with seven replicates per treatment. Treatments included MOC (control) and MOC fortified with either Axtra® XB or Ronozyme®. Samples were subjected to a modified three-step (pepsin-pancreatin-viscozyme) *in vitro* porcine digestion model (Boisen and Fernández, 1997). The digestion setup used 250 ml tight- sealing jars immersed in a 39° C shaking water bath, each holding 7 X sample replicates + 1 X no sample (blank) Ankom® F57 filter bag for a treatment. In a run of each digestion step, each treatment had three such digestion jars. The samples were incubated for digestion as follows:

*Step 1 (Gastric) digestion:* 2-hour incubation in buffered digestion medium [600 mL 0.1 M, pH 6.0 phosphate buffer + 240 mL 0.2 M HCl (adjusted to 2.0 pH using 1 M solutions of HCl or NaOH)] + 0.6 g fresh pepsin solution (porcine, 2000 FIP-U/g, Merck no. 7190) + 12 mL of chloramphenicol (Sigma no. C-0378, 0.5 g/100 mL ethanol).

*Step 2 (Small intestine digestion):* 5-hour incubation after topping up the step 1 digestion medium with high pH buffer media [240 mL of phosphate buffer (0.2 M, pH 6.8) + 120 mL 0.6 M NaOH) (pH adjusted to 6.8 using 1 M HCl or NaOH)] + 2.4 g pancreatin (porcine, grade IV, Sigma no. P-1750).

*Step 3 (Colon digestion)*: 24-hour incubation in 750 mL fresh phosphate buffer (0.1 M, pH 4.8) + 12 mL Viscozyme (Viscozyme L® V2010 120 L, mixture including β-glucanase, xylanase, arabinase, cellulase (120 FBG/g)), Novo Nordisk.

Dry matter, which disappeared after the second (ileal partial digestibility) and third (colon partial digestibility) were estimated gravimetrically by washing off the soluble residue through sequential gentle rinsing in warm tap water, 95% ethanol, and 99% acetone, before forced air oven-drying at 85 °C for 18 hours.

### 3.4.3 Mathematical analyses

The compartmental and the total IVDMD were defined and calculated as:

$$\textbf{Three} - \textbf{step compartmental digestion model}$$

$$= [\,step\ 1\ (Pepsin, Gastric) + \ \ Step\ 2\ (Pancreatin, Small\ intestine)] + \ Step\ 3\ (Viscozyme\ Large\ intestin$$

$$\textbf{IVDMD}$$

$$= \frac{Initial\ \ (bag + sample)DM\ \ \ (g) - Final(bag + residual\ sample)\ DM\ (g\ ) - [Final\ DM\ Blank\ (g) - Initial\ DM\ Blank\ (g)}{Sample\ DM\ (g)}$$

### 3.5 Statistical analyses

A one way ANOVA of dry matter digestibility coefficients was performed using the General Linear Models (GLM) procedure of Minitab software vision 19. Means were separated using Tukey's procedure, at the $p<0.05$ level of significance.

Statistical model that was used is as follows:

$Y_i = \mu + \alpha_i + \varepsilon_i$

Where:

$Y_i$ = $I^{th}$ observation

$\mu$ = Overall mean

$\alpha_i$ = Enzyme effect

$\varepsilon_i$ = random error

### 3.6 Results

Table 3.2 shows the partial IVDMD coefficient of MOC with and without different exogenous enzymes. Neither of the enzymes altered the partial gastric-small intestine (18.93-22.65) and colon (21.29-22.25) and the total (41.18-44.40) IVDMD of MOC (P>0.05).

Table 3.2 Partial compartmental of *in vitro* DM digestibility of Macadamia nut oil cake in a porcine digestion model

| Enzyme | | In vitro dry matter digestibility (%) | | |
|---|---|---|---|---|
| Treatments | n | [1]Step1 +[2]Step2 | [2]Step3 | Total |
| No Enzyme | 7 | 22.65 | 21.75 | 44.40 |
| [4]Custom mix | 7 | 22.20 | 21.29 | 43.48 |
| [5]Ronozymes® | 7 | 18.93 | 22.25 | 41.18 |
| SEM | | 2.090 | 1.300 | 2.450 |
| P-value | | 0.41 | 0.87 | 0.64 |

[1]Gastric digestion- 2-hour incubation in buffered digestion medium [600 mL 0.1 M, pH 6.0 phosphate buffer + 240 mL 0.2 M HCl (adjusted to 2.0 pH using 1 M solutions of HCl or NaOH)] + 0.6 g fresh pepsin solution (porcine, 2000 FIP-U/g, Merck Product 7190) + 12 mL of chloramphenicol (Sigma Product C-0378, 0.5 g/100 mL ethanol); [2]Small intestine digestion- 5-hour incubation after topping up the step 1 digestion medium with high PH buffer media [240 mL of phosphate buffer (0.2 M, pH 6.8) + 120 mL 0.6 M NaOH) (pH adjusted to 6.8 using 1 M HCl or NaOH)] + 2.4 g pancreatin (porcine, grade IV, Sigma no. P-1750). [3]Colon digestion- 24-hour incubation in 750 mL fresh phosphate buffer (0.1 M, pH 4.8) + 12 mL Viscozyme (Viscozyme L® V2010 120 L, mixture including β-glucanase, xylanase, arabinase, cellulase (120 FBG/g)), Novo Nordisk. [4]Axtra® (endo-1,4-ß-xylanase and

endo1,3(4)-ß-glucanase), at 0.089/3.5g, [5]Ronozyme® WX (endo-1,4-beta-xylanase), at 0.390/3.5g. SEM-Standard error of the mean.

### 3.7 Discussion

In the present study, exogenous enzymes did not improve the partial gastric-small intestinal (19.9-22-8) and colon (21.3-22.8) IVDMD, which resulted in a low total (41.2-44.4). The low digestibility was expected, given the high ADF (378.8 g/kg) and NDF 526.3 g/kg).

The ileal digestibility, which is pepsin+pancreatin (step 1+2), was similar (P>0.05), although the custom mix was higher than Ronozyme, despite there being no statistically significant difference (P>0.05). The findings of this study contradict those of Tiwari and Jha (2017), who found that MOC had a high DM digestibility *in vitro*. The addition of enzyme complexes tended to increase the ileal digestibility of dry matter *in vitro* tests as reported (Kong *et al.*, 2015), even though it relied on test ingredients, which fits with the current study's findings of an increase in ileal (step1+2). Low enzyme dosage in this study may be the cause of low dry matter digestibility. Enzymes had a beneficial impact on nutrient digestibility in the large intestine of weaned pigs (Yi *et al.*, 2013), which is contradictory to the findings of the current study.

### 3.8 Conclusion

Enzyme inclusion did not have any effect on IVDMD, with low digestibility attributed to the high ADF and NDF content. This suggests that either the enzyme dosage was suboptimal, or the activities did not match the MOC chemical matrix.

# CHAPTER 4

## NUTRIENT DIGESTIBILITY AND NITROGEN BALANCE IN GROWING PIGS FED A 10% MACADAMIA (*INTEGRIFOLIA, TETRAPHYLLA*) OIL CAKE SUPPLEMENTED WITH EXOGENOUS ENZYMES

### Abstract

The study evaluated the effects of 10% dietary inclusion of Macadamia (*Integrifolia, Tetraphylla)* Oil Cake (MOC) with supplementary exogenous enzymes on nutrient digestibility and N balance in growing pigs. Experimental diets were a balanced, standard commercial maize-soybean diet, and an iso-nutrient, 10% MOC-maize-growing diet, each with a duplicate supplemented with 500g/tonne of a custom enzyme cocktail containing 3000 FTU $g^{-1}$ 6-phytase (IUB 3e1.3.26), 7270 U $g^{-1}$ endo-1,4-Beta-Xylanase (EC-3.2.1.8), 300 U $g^{-1}$ alpha amylase (EC-3.2.1.1), 6000 $g^{-1}$ subtilisin protease (EC-3.4.21.62), 532 U $g^{-1}$ endo-1,4-beta-glucanase (IUB 3.2.1.6). Eight F1 Large White X Landrace piglets (15.3 ± 1.91 kg live weight (LW)) were assigned to diets in a 2 x 2 factorial arrangement, in a duplicated, balanced 4 × 4 Latin square design. An 8-day feeding period was used in the Latin squares, consisting of 3 days of adaptation, and 5 days of feed intake measurement, and the total collection of faecal and urine excreta. The 10% MOC diet had a lower ($p<0.05$) digestibility of crude protein, with no ($p>0.05$) effect on the digestibility of other chemical components. Scaled to the LW, 10% MOC dietary inclusion reduced ($p<0.05$) NR. Nitrogen retention was higher ($p<0.05$) on the 0%, compared to the 10% MOC diets only when the diets contained the exogenous enzymes. Scaled to the $LW^{0.75}$, the 0% MOC dietary inclusion reduced ($p<0.05$) NR. Dietary inclusion of MOC at 10% marginally reduced the digestibility of CP, and NR. In conclusion, nutrient digestibility, and N balance responses to the 10% MOC diet were similar to a standard diet, which supported the 10 % dietary inclusion of MOC as an alternative protein source for weaned, fast growing Large White pigs. A more potent exogenous proteinase and or carbohydrase enzymes could be effective tools to improve dietary efficacy and protein efficiency.

**Key words**: Macadamia oil cake, enzyme, *in vivo* digestibility

## 4.1. Introduction

The global livestock industry has been subjected to escalating feed shortages and high costs (Acheampong-Boateng *et al.*, 2017). Conventional protein sources such as soybean oil cake are no longer readily available, particularly to small scale farmers (Nkosi *et al.*, 2011b). To sustain viable livestock production, farmers are increasingly under pressure to revert to agro-industrial by-products (Acheampong-Boateng *et al.*, 2017). However, local agro-industrial by-products are still largely excluded from mainstream stockfeed value chains. Their integration into both smallholder and advanced commercial feeding systems demand nutritional characterisation for efficient utilisation.

Macadamia oil cake (MOC) is a potential dietary protein substitute for the conventional feeds (Acheampong-Boateng *et al.*, 2008). However, the MOC is produced in a process which requires the inclusion of soybean hulls to facilitate efficient oil expeller function (Acheampong-Boateng *et al.*, 2008). Consequently, though typically high (19.5%) crude protein, MOC also contains substantial (24.9%) crude fibre (Acheampong-Boateng *et al.*, 2008).

Compared to ruminant livestock, little is known about the nutritive value of MOC as a protein source for monogastric livestock such as pigs. In monogastric species, the high fibre content imposes a limit on its dietary inclusion, given the risk of fibre-induced restriction of feed intake, and of nutrient, particularly protein digestion (Jha *et al.*, 2019). Exogenous enzymes might be effective tools to overcome this challenge. Cocktails which include phytase, protease and amylase activities can theoretically augment the action of endogenous pig enzymes, while fibrolytic enzymes additionally benefit nutrient digestion by degrading otherwise indigestible, nutrient-protecting complex non-starch polysaccharides to expose nutrients to digestion (O'Neill *et al.*, 2014). However, to be efficient, exogenous enzymes need to be carefully screened for correct matching to the dietary chemical matrix (Apajalahti *et al.*, 2001).

In experiment 1, the digestibility of MOC was predicted in vitro, with evaluation of exogenous enzyme match to its chemical matrix. Experiment 2 was conducted to further evaluate the

effects of 10% MOC inclusion into a complete diet, and of enzyme fortification, on the *in vivo* dry matter and specific nutrient digestibility, and on the N balance of growing pigs.

## 4.2. Methods and materials

### 4.2.1. Description of the study site

The study was conducted at the University of Venda, which is in Vhembe district, Limpopo province, in South Africa at geo- location 22°58'32''S, 30°26'45''E. The annual minimum temperatures for the area vary from 10 $^0$C during winter, whereas in summer the maximum varies between 34 $^0$C to 38 $^0$C (Kom *et al.,* 2022). The sample analyses were done in the animal science nutrition laboratory.

**Macadamia nut oil cake**

Bulk Macadamia nut oil cake was sourced from the Royal Macadamia (Pty) Ltd in Levubu, Limpopo province. Though it could not be established whether a single or several varieties of *Macadamia spp*, or their hybrids, were included in the cake that was used in the study, the *Macadamia Integrifolia* is the commercially cultivated species in South Africa. The chemical composition of a representative sample of the MOC is presented in Table 4.1.

Table 4.1 Analyzed chemical composition (DM basis) of Macadamia nut oil cake.

| Nutrient | % |
|---|---|

| | |
|---|---|
| DM | 93.91 |
| Ash | 3.51 |
| CP | 15.17 |
| ADF | 37.88 |
| NDF | 52.63 |
| EE | 23.62 |
| Ca | 0.27 |
| P | 0.17 |
| Essential amino acids (g/100g) | |
| Arginine | 1.34 |
| Alanine | 0.42 |
| Asparagine | 0.52 |
| Glutamine | 0.37 |
| Glycine | 0.15 |
| Histidine | 0.97 |
| Isoleucine | 0.45 |
| Leucine | 0.64 |
| Lysine | 0.88 |
| Methionine | 2.09 |
| Phenylalanine | 0.54 |
| Proline | 0.89 |
| Serine | 0.67 |
| Threonine | 0.95 |
| Tyrosine | 0.42 |
| Valine | 0.54 |

DM: Dry matter, Ether Extract: EE, ADF: Acid detergent fibre, NDF: Neutral detergent fibre, CP: Crude protein, Ca: Calcium, P: Phosphorus.

### 4.3 Enzymes

The enzyme cocktail was a custom mix designed to contain 3000 FTU g $^{-1}$ 6-phytase (IUB 3e1.3.26), 7270 U g $^{-1}$ endo-1,4-Beta-Xylanase (EC-3.2.1.8), 300 U g $^{-1}$ alpha amylase (EC-3.2.1.1), 6000 g $^{-1}$ subtilisin protease (EC-3.4.21.62), 532 U g $^{-1}$ endo-1,4-beta-glucanase (IUB 3.2.1.6).

### 4.4 Diets

Experimental diets (Table 4.2) were a standard maize-soybean diet, and a Macadamia nut oil cake (MOC) diet was included at 10%, each with (+) and without (-) 500g per tonne of the custom enzyme cocktail. 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 pig grower physico-chemical properties, and met the minimum nutrient standards recommended for growing pigs (National Research Council (NRC, 1998)).

Table 4.2 shows the feed ingredients and nutrient compositions of the test pig grower diets.

Table 4.2 Ingredients and analysed nutrient compositions of pig grower diets.

| Composition | Diets | |
|---|---|---|
| | Macadamia nut oil cake | |
| | 0% | 10% |
| Ingredients (% as is) | | |
| Maize | 58.4 | 33.9 |
| Corn gluten feed | 20.0 | 18.5 |
| Corn-hominy feed | 7.5 | 25.0 |
| Macadamia nut oil cake | 0.0 | 10.0 |
| Soybean Meal (>46.5 % CP) | 9.5 | 8.0 |
| Corn gluten meal (60% CP) | 3.0 | 3.0 |
| Limestone Flour | 0.4 | 0.4 |
| Mineral & Vitamin mix | 1.2 | 1.2 |
| Total | 100.0 | 100.0 |
| *Chemical components (g $kg^{-1}$ DM)* | | |
| Dry matter | 895.0 | 834.0 |
| Ash | 52.2 | 46.9 |
| Crude protein | 201.9 | 193.9 |
| Starch | 380.3 | 188.2 |
| Ether extract | 45.8 | 28.1 |
| Crude Fibre | 80.0 | 80.0 |
| Acid detergent fibre | 53.7 | 107.5 |
| Neutral detergent fibre | 233.4 | 324.8 |
| Acid detergent lignin | 9.6 | 17.8 |
| Ca | 4.2 | 5.1 |
| P | 4.2 | 3.9 |
| Essential amino acids (g/100g DM) | | |
| Arginine | 1.18 | 1.13 |
| Alanine | 1.23 | 1.10 |
| Asparagine | 1.50 | 1.43 |
| Glutamine | 3.52 | 3.30 |
| Glycine | 0.93 | 0.95 |
| Histidine | 0.73 | 0.72 |
| Isoleucine | 0.74 | 0.71 |
| Leucine | 2.28 | 2.12 |
| Lysine | 0.87 | 0.88 |
| Methionine | 0.57 | 0.43 |
| Phenylalanine | 1.24 | 1.13 |
| Proline | 1.60 | 1.46 |
| Serine | 1.00 | 1.00 |
| Threonine | 0.88 | 0.82 |
| Tyrosine | 0.97 | 0.88 |
| Valine | 0.93 | 0.94 |

Crude fibre, CP: Crude protein, Ca: Calcium, P: Phosphorus.

### 4.5 Pigs, housing, experimental design, and management

The trial facility was an open structure which housed movable individual (117.5 length x 57.8 width and x 83.9 cm height) steel-framed metabolic pig cages. The cage design allowed lateral and longitudinal adjustments to continuously accommodate pig growth, for both pig welfare and the efficient separation of pig faeces from the urine. Each cage had a nipple drinker to provide free access to water, which was strategically positioned to exclude water splashing into a frontal feeding trough designed for free access to feed, and for minimal feed spillage. The base of the metabolic cage was fabricated with steel gauze plates padded with serviceable plastic sheeting which was positioned to collect and funnel urine into a collection bucket, allowing free, tail-end fall of faeces to a clean floor. Eight F1 Large White X Landrace weaned piglets were dewormed with Virbamec® LA prior to transfer to the metabolic cages in the trial house and were acclimatised to the experimental setup for two weeks before assignment to treatments in a 2 (diet) x 2 (enzyme) factorial arrangement within two balanced 4 (period) X 4 (diet) Latin squares. Diets were evaluated using 8-day feeding periods, each split into 3 days adaptation plus 5 days for intake measurement, and total faecal and urine collection. Feed and water were provided *ad libitum.*

### 4.6 Measurements

Piglets were weighed at each period interchange. The initial weight of the piglets was 15.3 ±1.91 kg. The pigs had grown to 28 ± 0.92 kg by the end of feeding period 4. To estimate the period dry matter intake, the feed input, along with all leftovers and spillage, were fully accounted for on each day of each feeding period. Total faeces were collected daily between 08:00 – 09:00. The cage setup largely ensured that most faeces dropped directly to the floor, and any faeces pasted onto the cage base frames were scrapped by hand and mixed thoroughly with the floor faeces and weighed. Representative 10% faeces from the daily collections were retained and frozen at -20 ºC. At the end of collection periods, the frozen

daily faecal samples from each pig were thawed and pooled, mixed by gloved-hand, and oven-dried at 60 ºC prior to dry storage pending chemical analyses.

Daily total urine was collected into floor plastic buckets containing 100ml of 1M $H_2SO_4$ to reduce nitrogen volatilisation and prevent microbial growth. The total urine was filtered through a fine mutton cloth, to remove solid contaminants, which was weighed, and the volume measured. Aliquots (10% v/v) of the daily urine collection from each pig were frozen-stored at -20 ºC pending N analyses of 5-day pooled samples.

### 4.7 Chemical analyses

The dry matter content of feed and faeces was estimated oven-drying 1g samples to constant weight at 60 ºC (AOAC, 1990), method 930.15). Ash was analysed by overnight combustion of 0.5g at 500 °C (AOAC, 1990), method 942.05). Feed, faecal and urine N were determined using the Kjeldahl procedure (AOAC, 1990), method 984.13). Crude fat was extracted by the sohxlet procedure (AOAC, 1990), method 920, 39). Neutral detergent fibre and Acid detergent fibre were analysed according to (Van Soest *et al.*, 1991).

### 4.8 Mathematical and statistical analysis

Nitrogen balance parameters were computed using the equations listed in Table 4.3. The weight-dependent parameters (NI, FNO, UNO, TNE, NR, AN) were further scaled to the pig live (g $kg^{-1}$ of W) or metabolic (g $kg^{-1}$ of $W^{0.75}$) weight.

Table 4.3: Equations for estimating nitrogen balance components.

| Component | Formula |
|---|---|
| Nitrogen intake (NI) | (N feed/100) ×daily feed intake |
| Faecal nitrogen output (FNO) | (N faeces/100) ×daily faecal output |
| Urinary nitrogen output (UNO) | (N urine/100) ×daily urine output |
| Total nitrogen excretion (TNE) | FNO+UNO |
| Nitrogen retention (NR) | NI – TNE |
| Absorbed nitrogen (AN) | NI – FNO |
| Apparent nitrogen digestibility (ND) | (AN/ NI) |
| Nitrogen utilization (NU) | NR/ NI×100 |
| The biological value of feed protein (BVFP) | NR/ND×100 |

The GLM of MINITAB software (Minitab, 2020) was used to analyse the dry matter and nutrient digestibility coefficients, and the scaled and unscaled N balance parameters in a 2 (diet) X 2 (enzyme) X 4 (feeding period) factorial experiment, in a replicated 4 × 4 Latin square design using the model:

$Y_{ijklmn} = \mu + D_i + E_j + S_k + P_l + A_m + (\alpha\beta)_{ji} + \varepsilon_{ijklmn}$

Where,

$Y_{ijklmn}$ = is the observed parameter value,

$\mu$ = the overall mean,

$D_i$ =the fixed effect of the $i^{th}$ diet,

$E_j$ = the fixed effect of the $j^{th}$ enzyme dosage,

$S_k$ = the random effect of the $k^{th}$ Latin Square,

$P_l$ = the random effect of the $k^{th}$ period within a Latin square,

$A_m$ = the random effect of the $m^{th}$ animal within a Latin Square,

$(\alpha\beta)$ = the diet x enzyme interaction,

$\varepsilon_{ijklmn}$ = the residual error.

Means were separated using Tukey's procedure, at the $p<0.05$ level of significance.

### 4.9 Ethical considerations

The experimental procedures used in the study were approved by the Animal Care and Use Committee of the University of Venda (PROJECT NO: SARDF/19/ANS/16/0612)

### 4.10. Results

Table 4.4 shows the effects of 10% MOC and supplementary exogenous enzymes on the dry matter intake and coefficients of *in vivo* digestibility of DM, OM, CP FAT, NDF and ADF. Dietary inclusion of MOC at 10% significantly reduced (0.73-0.72) ($P<0.05$) the digestibility of crude protein, with no significant effect ($P>0.05$) on the digestibility of dry matter (0.86 versus 0.84), organic matter (0.87 versus 0.86) and fat (0.69 – 0.61), Neutral detergent fibre (0.71)

and Acid detergent fibre (0.66-0.59). The exogenous enzymes did not alter dry matter and specific nutrient digestibility (P>0.05).

Table 4.4 Effects of 10% dietary inclusion of macadamia oil cake, and of supplementary enzymes on dry matter intake and nutrient digestibility in growing Large White x Landrace pigs.

| Treatments | | Intake (g DM day $^{-1}$) | Digestibility coefficients | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | DM | OM | CP | FAT | NDF | ADF |
| Diet | [1]Enzyme | | | | | | | |
| 0% | + | 670.95 | 0.86 | 0.84 | 0.72 | 0.54 | 0.66 | 0.52 |
| | - | 703.36 | 0.86 | 0.88 | 0.72 | 0.55 | 0.66 | 0.52 |
| 10% | + | 649.03 | 0.83 | 0.88 | 0.73 | 0.66 | 0.70 | 0.62 |
| | - | 586.20 | 0.84 | 0.86 | 0.73 | 0.71 | 0.72 | 0.66 |
| SEM | | 98.100 | 0.024 | 0.018 | 0.006 | 0.059 | 0.042 | 0.060 |
| Diet | | - | | | | | | |
| 0% | | 687.16 | 0.86 | 0.86 | 0.72[a] | 0.55 | 0.66 | 0.52 |
| 10% | | 617.61 | 0.84 | 0.87 | 0.73[b] | 0.69 | 0.71 | 0.64 |
| SEM | | 69.400 | 0.013 | 0.013 | 0.004 | 0.042 | 0.029 | 0.043 |
| [1]Enzymes | | | | | | | | |
| - | | 644.78 | 0.85 | 0.87 | 0.72 | 0.63 | 0.69 | 0.59 |
| + | | 659.99 | 0.85 | 0.86 | 0.73 | 0.60 | 0.68 | 0.57 |
| SEM | | 69.400 | 0.013 | 0.013 | 0.004 | 0.042 | 0.029 | 0.043 |
| P-Values | | | | | | | | |
| Diet | | 0.50 | 0.30 | 0.59 | 0.04 | 0.05 | 0.29 | 0.08 |
| Enzyme | | 0.88 | 0.82 | 0.52 | 0.94 | 0.61 | 0.81 | 0.79 |
| Diet*Enzyme | | 0.64 | 0.75 | 0.20 | 0.92 | 0.74 | 0.88 | 0.69 |

[ab] Within each factor or factor interaction, for each parameter, means with different superscripts differ significantly at (P <0.05). DM: Dry matter; OM: Organic matter digestibility; CP: Crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; [1]Cocktail containing 3000 FTU g $^{-1}$ 6-phytase (IUB 3e1.3.26), 7270 U g $^{-1}$ endo-1,4-Beta-Xylanase (EC-3.2.1.8), 300 U g $^{-1}$ alpha amylase (EC-3.2.1.1), 6000 g $^{-1}$ subtilisin protease (EC-3.4.21.62), 532 U g $^{-1}$ endo-1,4-beta-glucanase (IUB 3.2.1.6). (-): diet without enzyme; (+): diet plus 500g tonne $^{-1}$ enzyme; SEM: Standard error of mean

Tables 4.5-4.7 show the effects of 10% dietary inclusion of Macadamia nut oil cake (MOC), and of supplementary enzymes on N balance parameters evaluated as measured, or scaled to the LW or $W^{0.75}$, respectively. Without scaling, diet x period interactions were significant ($p<0.05$) for FNO and TNE. Diet x period interaction for FNO and TNE arose from the quantitatively ($p>0.05$) parameter order 10% MOC > 0% for feeding periods 1, 2 and 4, which reversed for period 3, effects which were similar for both parameters when scaled to the LW, and when the TNE was scaled to $LW^{0.75}$. Scaled to the LW, 10% MOC dietary inclusion reduced ($p<0.05$) the NR, with significant period x diet and period x enzyme interactions($p<0.05$). The NR was higher ($p<0.05$) on the 0%, compared to the 10% MOC diets only when the diets contained the exogenous enzymes. The NR was significantly high ($p<0.05$) for pigs on the 0% MOC, no enzyme diet, compared to pigs on all dietary treatments during periods 3-4. Nitrogen retention decreased ($p<0.05$) with the feeding period. Scaled to the $LW^{0.75}$, the 0% MOC dietary inclusion reduced ($p<0.05$) the NR. The NR was the highest in period 2 ($p<0.05$), and higher in period 3 compared to period 4.

Table 4.5 Effects of 10% dietary inclusion of Macadamia nut oil cake (MOC) and of supplementary enzymes on the intake, balance, and efficiency of nitrogen utilization in growing Large White x Landrace pigs.

| Treatments | | NI | N Utilisation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | UNO | FNO | TNE | AN | NR | NU | ND | BVFP |
| Diet | [1]Enzyme | g day $^{-1}$ | g day $^{-1}$ | g day $^{-1}$ | g day $^{-1}$ | g day $^{-1}$ | g day $^{-1}$ | % | | % |
| 0% | + | 227.29 | 2.79 | 27.67 | 30.46 | 196.83 | 86.22 | 39.68 | 0.88 | 0.45 |
| | - | 245.88 | 2.96 | 27.14 | 30.09 | 215.78 | 87.77 | 37.59 | 0.89 | 0.42 |
| 10% | + | 202.38 | 2.63 | 31.44 | 39.19 | 169.22 | 83.62 | 46.52 | 0.85 | 0.55 |
| | - | 240.42 | 1.73 | 36.57 | 33.17 | 201.22 | 83.1 | 35.85 | 0.84 | 0.43 |
| SEM | | 20.600 | 0.688 | 4.630 | 4.740 | 19.300 | 2.170 | 4.820 | 0.021 | 0.055 |
| Diet | 0% | 236.58 | 2.87 | 27.41 | 30.28 | 206.30 | 86.99 | 38.63 | 0.88 | 0.44 |
| | 10% | 221.40 | 2.18 | 34.00 | 36.19 | 185.22 | 83.36 | 41.19 | 0.84 | 0.49 |
| SEM | | 14.500 | 0.486 | 3.270 | 3.350 | 13.700 | 1.540 | 3.410 | 0.015 | 0.039 |
| [1]Enzymes | - | 243.15 | 2.79 | 31.85 | 34.65 | 208.49 | 85.43 | 36.72 | 0.87 | 0.43 |
| | + | 214.84 | 2.26 | 29.56 | 31.82 | 185.52 | 84.92 | 43.09 | 0.86 | 0.50 |
| SEM | | 14.500 | 0.486 | 3.270 | 3.350 | 13.700 | 1.540 | 3.410 | 0.015 | 0.039 |
| P Values | | | | | | | | | | |
| Diet | | 0.48 | 0.31 | 0.11 | 0.14 | 0.19 | 0.19 | 0.60 | 0.16 | 0.40 |
| Enzymes | | 0.21 | 0.42 | 0.55 | 0.46 | 0.85 | 0.85 | 0.21 | 0.83 | 0.21 |
| Period | | 0.63 | 0.43 | 0.36 | 0.29 | 0.70 | 0.70 | 0.52 | 0.77 | 0.63 |
| Diet*Enzyme | | 0.65 | 0.58 | 0.46 | 0.41 | 0.69 | 0.70 | 0.38 | 0.72 | 0.46 |

[ab] Within each factor or factor interaction, for each parameter, means with different superscripts differ significantly at (P <0.05). **: P<0.01; (NS) not significant: (P > 0.05); NI: Nitrogen intake; UNO: Urine Nitrogen Output; FNO: Faecal Nitrogen Output; TNE: Total Nitrogen Excretion; AN: Absorbed Nitrogen; NR: nitrogen retention; NU: Nitrogen utilisation; BVFP: Biological Value Feed Protein; ND: Apparent N digestibility; [1]Cocktail containing 3000 FTU g $^{-1}$ 6-phytase (IUB 3e1.3.26), 7270 U g $^{-1}$ endo-1,4-Beta-Xylanase (EC-3.2.1.8), 300 U g $^{-1}$ alpha amylase (EC-3.2.1.1), 6000 g $^{-1}$ subtilisin protease (EC-3.4.21.62), 532 U g $^{-1}$ endo-1,4-beta-glucanase (IUB 3.2.1.6). diet without enzyme; (+): diet plus 500g tonne $^{-1}$ enzyme (-): SEM: Standard error of mean

Table 4.6 Effects of 10% dietary inclusion of Macadamia nut oil cake (MOC) and of supplementary enzymes on scaled (g/day/kg live weight (LW)) N balance in growing Large White pigs.

| Treatments | | N Utilisation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | NI | UNO | FNO | TNE | AN | NR | NU | ND | BVFP |
| Diet | [1]Enzyme | g kg$^{-1}$ LW | g kg$^{-1}$ LW | g kg$^{-1}$ LW | g kg$^{-1}$ LW | g kg$^{-1}$ LW | g kg$^{-1}$ LW | % | | % |
| 0% | + | 8.36 | 0.09 | 1.05 | 1.15 | 8.26 | 3.18$^{a}$ | 39.68 | 0.88 | 0.45 |
| | - | 7.99 | 0.09 | 0.87 | 0.96 | 7.77 | 2.94$^{ab}$ | 37.59 | 0.89 | 0.42 |
| 10% | + | 6.60 | 0.05 | 0.99 | 1.39 | 6.55 | 2.69$^{b}$ | 46.52 | 0.85 | 0.55 |
| | - | 8.25 | 0.09 | 1.3 | 1.05 | 8.16 | 2.9$^{ab}$ | 35.85 | 0.84 | 0.43 |
| SEM | | 0.577 | 0.024 | 0.171 | 0.178 | 0.578 | 0.195 | 4.820 | 0.021 | 0.055 |
| Diet | 0% | 8.18 | 0.09 | 0.96 | 1.06 | 8.01 | 3.06$^{a}$ | 38.63 | 0.88 | 0.44 |
| | 10% | 7.43 | 0.07 | 1.15 | 1.22 | 7.35 | 2.79$^{b}$ | 41.19 | 0.84 | 0.49 |
| SEM | | 0.408 | 0.017 | 0.121 | 0.126 | 0.408 | 0.138 | 3.410 | 0.015 | 0.039 |
| [1]Enzymes | - | 8.12 | 0.09 | 1.08 | 1.18 | 7.96 | 2.29 | 36.72 | 0.87 | 0.43 |
| | + | 7.48 | 0.08 | 1.02 | 0.10 | 7.40 | 2.93 | 43.09 | 0.86 | 0.50 |
| SEM | | 0.408 | 0.017 | 0.121 | 0.126 | 0.408 | 0.138 | 3.410 | 0.015 | 0.039 |
| P Values | | | | | | | | | | |
| Diet | | 0.24 | 0.23 | 0.20 | 0.25 | 0.32 | 0.02 | 0.60 | 0.16 | 0.40 |
| Enzymes | | 0.30 | 0.39 | 0.67 | 0.57 | 0.39 | 0.93 | 0.21 | 0.83 | 0.21 |
| Period | | 0.17 | 0.56 | 0.52 | 0.48 | 0.21 | 0.01 | 0.52 | 0.77 | 0.63 |
| Diet*Enzyme | | 0.12 | 0.36 | 0.12 | 0.09 | 0.13 | 0.04 | 0.38 | 0.72 | 0.46 |

[ab] Within each factor or factor interaction, for each parameter, means with different superscripts differ significantly at (P <0.05). **: P<0.01; (NS) not significant: (P > 0.05); NI: Nitrogen intake; UNO: Urine Nitrogen Output; FNO: Faecal Nitrogen Output; TNE: Total Nitrogen Excretion; AN: Absorbed Nitrogen; NR: nitrogen retention; NU: Nitrogen utilisation; BVFP: Biological Value Feed Protein; ND: Apparent N digestibility; [1]Cocktail containing 3000 FTU g $^{-1}$ 6-phytase (IUB 3e1.3.26), 7270 U g $^{-1}$ endo-1,4-Beta-Xylanase (EC-3.2.1.8), 300 U g $^{-1}$ alpha amylase (EC-3.2.1.1), 6000 g $^{-1}$ subtilisin protease (EC-3.4.21.62), 532 U g $^{-1}$ endo-1,4-beta-glucanase (IUB 3.2.1.6). diet without enzyme; (+): diet plus 500g tonne $^{-1}$ enzyme (-): SEM: Standard error of mean

Table 4.7 Effects of 10% dietary inclusion of Macadamia nut oil cake (MOC) and of supplementary enzymes on scaled (g/day/kg metabolic weight ($LW^{0.75}$) N balance in growing Large White pigs.

| | | N Utilisation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Diet | [1]Enzyme | NI g/day/kg $LW^{0.75}$ | UNO g/day/kg $LW^{0.75}$ | FNO g/day/kg $LW^{0.75}$ | TNE g/day/kg $LW^{0.75}$ | AN g/day/kg $LW^{0.75}$ | NR g/day/kg $LW^{0.75}$ | NU % | ND | BVFP % |
| 0% | + | 19.01 | 0.238 | 2.37 | 2.59 | 16.41 | 7.23 | 39.68 | 0.88 | 0.45 |
| | - | 18.79 | 0.23 | 2.04 | 2.27 | 16.51 | 6.86 | 37.59 | 0.89 | 0.42 |
| 10% | + | 19.11 | 0.13 | 2.36 | 2.49 | 13.02 | 6.33 | 46.52 | 0.85 | 0.55 |
| | - | 15.50 | 0.21 | 2.99 | 3.20 | 15.9 | 6.69 | 35.85 | 0.84 | 0.43 |
| SEM | | 1.440 | 0.049 | 0.313 | 0.327 | 1.350 | 0.176 | 4.520 | 0.020 | 0.052 |
| Diet | 0% | 18.89 | 0.23 | 2.21 | 2.44 | 16.46 | 7.05[a] | 38.63 | 0.88 | 0.44 |
| | 10% | 17.30 | 0.17 | 2.67 | 2.84 | 14.46 | 6.51[b] | 41.19 | 0.84 | 0.49 |
| SEM | | 1.010 | 0.035 | 0.222 | 0.231 | 0.958 | 0.125 | 3.190 | 0.014 | 0.038 |
| [1]Enzymes | - | 18.95 | 0.22 | 2.52 | 2.74 | 16.21 | 6.78 | 36.72 | 0.87 | 0.43 |
| | + | 17.25 | 0.18 | 2.36 | 2.54 | 14.71 | 6.78 | 43.09 | 0.86 | 0.50 |
| SEM | | 1.010 | 0.035 | 0.222 | 0.231 | 0.958 | 0.125 | 3.190 | 0.014 | 0.038 |
| P Values | | | | | | | | | | |
| Diet | | 0.29 | 0.25 | 1.17 | 0.21 | 0.20 | 0.03 | 0.60 | 0.16 | 0.40 |
| Enzymes | | 0.27 | 0.40 | 0.63 | 0.53 | 0.33 | 0.10 | 0.21 | 0.83 | 0.21 |
| Period | | 0.29 | 0.52 | 0.49 | 0.43 | 0.36 | 0.02 | 0.52 | 0.77 | 0.63 |
| Diet*Enzyme | | 0.21 | 0.41 | 0.16 | 0.12 | 0.36 | 0.11 | 0.38 | 0.72 | 0.46 |

[ab] Within each factor or factor interaction, for each parameter, means with different superscripts differ significantly at ($P < 0.05$). **: $P<0.01$; (NS) not significant: ($P > 0.05$); NI: Nitrogen intake; UNO: Urine Nitrogen Output; FNO: Feacal Nitrogen Output; TNE: Total Nitrogen Excretion; AN: Absorbed Nitrogen; NR: nitrogen retention; NU: Nitrogen utilisation; BVFP: Biological Value Feed Protein; ND: Apparent N digestibility; [1]Cocktail containing 3000 FTU $g^{-1}$ 6-phytase (IUB 3e1.3.26), 7270 U $g^{-1}$ endo-1,4-Beta-Xylanase (EC-3.2.1.8), 300 U $g^{-1}$ alpha amylase (EC-3.2.1.1), 6000 $g^{-1}$ subtilisin protease (EC-3.4.21.62), 532 U $g^{-1}$ endo-1,4-beta-glucanase (IUB 3.2.1.6). diet without enzyme; (+): diet plus 500g tonne $^{-1}$ enzyme (-): SEM: Standard error of mean

### 4.11. Discussion

The South African Macadamia nut industry has seen rapid expansion to current production of 44775 hectares/annum (SAMAC, 2020a). The nut oil expeller process produces a moderately high protein, but high fibre by-product, which is yet to be fully utilised in the local stockfeed value chain. To test the influence of MOC inclusion on the dietary protein utilisation by growing commercial pigs, in the present study, a 10% MOC dietary inclusion level was evaluated, which was the approximate dietetic upper limit imposed by a modest CP (152 g $kg^{-1}$), high crude fat (23.62 g $kg^{-1}$ ) and high fibre (ADF -379 g $kg^{-1}$ , NDF 526 3 g $kg^{-1}$) content of the test MOC. Dietary inclusion of MOC at 10% only marginally (0.73-0.72) depressed the CP digestibility, without effect on the digestibility of dry matter, DM, OM, CP FAT, NDF and ADF.

A higher level of MOC dietary inclusion would likely incrementally affect dietary efficacy, since high dietary fibre has a negative effect on feed intake and nutrient digestion (Nortey *et al.*, 2008). However, such deleterious effects could be mitigated by more potent, matched exogenous enzymes (Zijlstra *et al.*, 2010). The exogenous proteinase and carbohydrase enzymes used in the present study were not effective in terms of the DM, specific nutrient, and fibre digestibility. Depressed CP digestibility with 10% MOC dietary inclusion was associated with increased faecal N wastage, and with increased total N excretion. Relationships between the excretion parameters FNO, UNO, and the efficiency indicators NR, NU and BVFP can be complex.

The correlation is subject to both the pig, and its gut microbial N metabolism. Imbalance in dietary amino acids leads to catabolism of excess amino acids, which increases urea excretion through urine (Ball *et al.*, 2013). Dietary crude protein which evades digestion in the upper tract offloads nitrogenous substrates which increase colon microbial fermentation (Bindelle *et al.*, 2009). If the diet contains the requisite colon fermentable energy, the energy is used to assimilate both the endogenous blood urea N, and the indigestible dietary protein N, causing a shift in blood urea N excretion from urinary, to faecal excretion as microbial protein (Bindelle

*et al.*, 2009). Such effect was confirmed by (Hlongwana *et al.*, 2021), who observed high N excretion through faeces as a consequence of colon microbial assimilation of escape dietary and the endogenous blood urea N. If the diet is deficient in fermentable energy which escapes to the colon, increased colon protein fermentation is undesirable since it may yield toxic nitrogenous metabolites (Tuśnio *et al.*, 2017).

In the present study, the magnitude of increase in faecal N wastage suggested it was the major contributor to the relatively low NR on the 10 % MOC diet. There was more marked difference in NR when both diets contained exogenous enzymes also implied relatively more beneficial exogenous enzyme action on protein digestion or its metabolism from the 0%, compared to the 10% MOC diet. The non-phased feeding regime across feeding periods which spanned over 32 days of pig feeding supplied constant profile of dietary amino acids which did not track the changing pig amino acid requirement. When expressed on a LW or $W^{075}$ basis, a decrease in NR over time was therefore expected, consistent with declining efficiency of amino acid utilisation due to the mismatch in supply versus demand. Though less so for a lean pig genotype, with a decreasing metabolic requirement for muscle growth with pig age, pigs should incrementally metabolise surplus dietary amino acids to accrue body fat, with increased excretion of the waste N in urine (Remesar and Alemany, 2020). However, perhaps also due to the short feeding period, declining N or protein efficiency was not confirmed through the NU and BVFP. In multiphase-feeding systems, commercial growing pigs attain high N utilisation efficiency, as high as 40% (Rotz, 2004). In this study, comparatively high dietary protein efficiency was obtained for all diets, as indicated BVFP of 42-55% and NU of 35-46%. For the fast growing, lean-type Large White breed used in the present study, superior protein efficiency was expected given the experimental pig weight or age range.

## 4.12 Conclusion

Dietary inclusion of MOC at 10% marginally reduced the digestibility of CP, and NR. Overall, nutrient digestibility and N balance responses to the 10% MOC diet were similar to a standard diet, which supported the 10 % dietary inclusion of MOC as an alternative protein source for weaned, fast growing Large White pigs. Despite the lack of enzyme effect on nutrient and fibre digestibility, the observed relative reduction in NR in pigs fed the 10% MOC which contained exogenous enzymes suggested more potent exogenous proteinase and or carbohydrase enzymes could be effective tools to improve dietary efficacy and protein efficiency.

# CHAPTER 5

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

Enzymes did not improve the IVDMD of MOC, with low digestibility attributed to the high ADF and NDF content. Dietary inclusion of MOC at 10% marginally reduced the digestibility of CP, and NR. Overall, nutrient digestibility and N balance responses to the 10% MOC diet were similar to a standard diet, which supported the 10 % dietary inclusion of MOC as an alternative protein source for weaned, fast growing Large White pigs. Despite the lack of enzyme effect on nutrient and fibre digestibility, the observed relative reduction in NR in pigs fed the 10% MOC which contained exogenous enzymes suggested that more potent exogenous proteinase and or carbohydrase enzymes could be effective tools to improve dietary efficacy and protein efficiency.

It is recommended that, depending on the cost benefit analyses, 10% MOC can be included in growing pig diet to reduce the feed cost without marked adverse effects on pig productivity and growth efficacy. It is also recommended that more studies be done to test different enzyme dosages or identify enzyme activities which match the MOC chemical matrix.

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