Response in nitrogen balance, fibre digestibility and physicochemical characteristics of digesta in Windsnyer pigs fed on incremental levels of Amarula nut cake

By

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DECLARATION

I, Fortunate Yenziwe Hlongwana, declare that this dissertation entitled "**Response in nitrogen balance, fibre digestibility and physicochemical characteristics of digesta in Windsnyer pigs fed on incremental levels of Amarula nut cake**" is my original research, except where otherwise stated and was conducted under the supervision of Prof. M. Chimonyo and Dr. R.S. Thomas. This dissertation has not been submitted to any other higher education institution and research except the University of KwaZulu-Natal.

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GENERAL ABSTRACT

The broad objective of the study was to determine the response in nitrogen balance, fibre digestibility and physicochemical characteristics of digesta in slow-growing Windsnyer pigs fed on incremental levels of amarula nut cake (ANC) based diets. Thirty clinically healthy growing Windsnyer pigs (21.5 kg ± 4.97) (mean ± standard deviation) were individually assigned to separate pens in a completely randomized design. Five iso-energetic dietary treatments were formulated to contain 0, 50, 100, 150, and 200 g/kg dry matter (DM) of ANC. Six pigs were fed on each experimental diet *ad libitum*. Pigs were given ten days of the dietary adaptation period.

In Experiment 1, nitrogen (N) balance trial was conducted after thirty-one days of feeding pigs with an average body weight of 30.7 kg ± 6.57. The collection of faeces and urine took place every morning at 08h00 for five consecutive days. The hand-picking method was used to collect all faecal material from each pen and those captured by a 1 mm sieve suspended underneath the pen. Urine was collected using plastic trays and treated with 2 ml of sulphuric acid to reduce N volatilisation. The collected samples were frozen at -20 ºC pending analyses. Nitrogen intake (NI), total N excretion (TNE), urinary pH levels, N retention (NR), N absorption (NA), N digestibility (ND), N utilization (NU), net protein utilization (NPU), and biological value of feed protein (BVFP) were estimated. The average daily feed intake (ADFI), average daily gain (ADG), and gain: feed (G: F) ratio were also estimated weekly.

There was a quadratic increase in ADFI ($P < 0.05$), while ADG ($P < 0.05$) and G: F ratio ($P < 0.05$) increased linearly with incremental levels of ANC. Nitrogen (N) intake increased linearly with

ANC inclusion levels ($P < 0.01$). There was an increasing quadratic response in NA, apparent ND, and NR in pigs fed on increasing levels of ANC ($P < 0.05$). A positive linear response in NPU and BVFP to ANC inclusion was observed ($P < 0.01$). Nitrogen utilization increased at the rate of 0.63 g for each 1 g increase in ANC. There was a negative linear response in TNE through urine and faeces as ANC inclusion increased ($P < 0.01$). The relationship between urinary pH levels and ANC inclusion was described by the quadratic equation $Y = 0.0115x^2 - 0.3491x + 4.872$ ($P < 0.01$).

In Experiment 2, apparent total tract digestibility (ATTD) of fibre and physicochemical characteristics of colon digesta in growing Windsnyer pigs fed on ANC was determined. The experimental diets were blended with 3 g chromium oxide (Cr_2O_3) and pigs were acclimatized to this diet three days before the collection period. Representative feed samples for each diet were stored at room temperature pending Cr_2O_3 analysis. Faecal material was collected using the grab sampling method for five consecutive days between 08h00 and 13h00 and immediately chilled at -20 °C for further analysis. Digestibility of dry matter (DMD), acid detergent fibre (ADFD), acid detergent lignin (ADLD), hemicellulose (HemiD), and neutral detergent fibre (NDFD) were determined. After the digestibility trial, pigs weighing 34 kg ± 6.25 kg were fasted for 24 hours prior to slaughter, and routine abattoir procedures were followed. About 15 to 20 g digesta samples were obtained from the proximal colon and frozen immediately at -20 ºC within 1 hour of collection pending analysis. The DM content, pH level, water retention capacity (WRC), and swelling capacity (SWC) in the colonal digesta were estimated. The digesta pH was determined by inserting Crison 52 02 glass pH electrode immediately after collection. The WRC and SWC were measured using the centrifugation method and modified bed volume technique, respectively.

There was a quadratic increase in DMD as ANC inclusion increased ($P < 0.01$). A positive linear relationship between HemiD and increasing levels of ANC was observed ($P < 0.01$). There was also an increasing linear response in NDFD as ANC inclusion increased ($P < 0.01$). Apparent digestibility of ADF and ADL increased quadratically in response to ANC inclusion ($P < 0.01$). There was an increasing quadratic relationship between digesta DM content and ANC inclusion ($P < 0.01$). The digesta pH level decreased quadratically with ANC inclusion levels ($P < 0.01$). The quadratic equation $Y = -0.0017x^2 + 0.0867x + 3.0929$ and $Y = 0.017x^2 + 0.0389x + 2.9637$ described the response in swelling capacity (SWC) ($P < 0.01$) and water retention capacity (WRC) ($P < 0.05$) to ANC inclusion levels, respectively. It can be concluded that dietary ANC improves N utilisation and fibre digestibility while reducing N excretion into the environment. Further, fibrous ANC increases the physicochemical properties of colonal digesta, predicting increased fibre fermentation. Hence, ANC can be a potential dietary protein source.

Keywords: ammonia volatilization, by-products, digesta properties, dietary fibre, digestion, dry matter content, nitrogen absorption, nitrogen intake, nitrogen utilisation, swine, urinary pH level

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DEDICATIONS

To the entire Hlongwana family, exceptionally my grandmother (Ntombencane Sephronia Hlongwana), my mother (Ntombifikile Margaret Hlongwana), my older sister (Slindile Success Hlongwana), and my younger brother (Asanda Kwanele Hlongwana)

To my late father, Mafika Sydney Mkhonza, your presence will forever reside in my heart, and I will always love you. This dissertation is a tribute to you.

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To all those who died because of COVID-19

To my future husband and children

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LIST OF ABBREVIATIONS

ABBREVIATION	DESCRIPTION	Units
ADF	Acid detergent fibre	g/kg DM
ADFD	Acid detergent fibre digestibility	%
ADFI	Average daily feed intake	kg/day
ADG	Average daily gain	kg/day
ADL	Acid detergent lignin	g/kg DM
ADLD	Acid detergent lignin digestibility	%
ANC	Amarula nut cake	-
AOAC	Association of Official Analytical Chemists	-
ARC	Agricultural Research Council	-
ATTD	Apparent total tract digestibility	%
BVFP	Biological value of feed protein	%
CP	Crude protein	g/kg DM
Cr_2O_3	Chromium oxide	g
DE	Digestible energy	MJ/kg DM
DM	Dry matter	%
DMI	Dry matter intake	g/kg
EE	Ether extract	g/kg DM
FNO	Faecal nitrogen output	g/day
GE	Gross energy	MJ/kg DM
G: F ratio	Gain: Feed ratio	-
HemiD	Hemicellulose digestibility	%

NA	Nitrogen absorption	g/day
NDF	Neutral detergent fibre	g/kg DM
NDF	Neutral detergent fibre digestibility	%
NI	Nitrogen intake	g/day
ND	Nitrogen digestibility	%
NSPs	Non-starch polysaccharides	-
NPU	Net protein utilisation	%
NR	Nitrogen retention	g/day
NU	Nitrogen utilisation	%
REG	Regression	-
SWC	Swelling capacity	ml/g
TNE	Total nitrogen excretion	g/day
UNO	Urinary nitrogen output	g/day
UKZN	University of KwaZulu	-
WRC	Water retention capacity	g_{water}/g_{feed} DM

CHAPTER ONE: General Introduction

1.1 Background

The ever-growing human population causes proliferation of urbanization, loss of biodiversity, and degradation of natural vegetation compounded by persistent droughts and water shortages. Shortages in cereal grain production are likely to be experienced (Daryanto *et al*., 2016) due to a surge in protein demand for livestock production. Such impediments exacerbate feed ingredient competition amongst humans and livestock, which inflates the cost of livestock feed. Pig production plays an integral role in sustaining economic development. Pigs require a small piece of land (Halimani *et al*., 2010), have superiority in converting feed efficiently into high-quality protein (Adesehinwa, 2008), ability to utilize agricultural by-products (Ncobela *et al*., 2018) and high prolificacy. Therefore, the pig industry warrants the search for alternative feed ingredients to reduce competition on conventional feeds.

Commercial pig farmers engage more on imported pig breeds in harmony with attributes feasible for profitable and sustainable pig enterprises meeting the market demand. In Southern Africa, local pig breeds are of economic value due to their long-term merits, such as utilizing fibrous diets and adaptability to low-input production systems (Chimonyo *et al*., 2010; Halimani *et al*., 2010). One example is the South African Windsnyer indigenous pig (SAWIP), characterized by its small body-frame size, slow growth rate, and reliance on scavenging in most rural settlements depriving their contribution into formal market chains (Madzimure *et al*., 2017). The population of Windsnyer pigs is dwindling as threatened by the potential of being replaced by fast-growing pig genotypes (Halimani *et al*., 2010). The absence of comprehensible policies for conserving slow-growing pigs and uncontrolled mating leads to inbreeding and uncontrolled genetic admixture (Chimonyo & Dzama 2007; Halimani *et al*., 2010). These forces aggravate the danger of extinction of slow-growing pigs.

The main drawback in pig production is the narrow range of feed ingredients, chiefly conventional agro-industrial by-products as prime protein and energy sources. Soybean oilcake (SBOC) is the predominant protein-rich ingredient containing digestible nutrients and amino acid profile close to ideal, particularly lysine, arginine, and tryptophan but deficient in methionine, cysteine, threonine, and valine (Mthiyane & Mhlanga, 2017). The United States of America, Brazil, and Argentina lead the production of soybean globally, enabling exportation to other countries scarce of SBOC, including South Africa and sub-Saharan African countries (OECD/FAO, 2018). In developing countries, the exorbitant cost of SBOC is prohibitive and inaccessible for most pig farmers (Nurfeta, 2010). It is of paramount importance to introduce non-conventional oilseed by-products as an alternative replacement for SBOC, such as amarula nut cake (ANC).

Amarula (*Sclerocarya birrea* subsp. *caffra*) nut cake is the by-product of oil-extraction from amarula fruits processed by commercial and local manufacturers in Southern Africa (Molelekoa *et al*., 2018). The seed nuts are typically discarded into the environment and the implementation of oil-extraction techniques yields a protein-rich nut cake (Malebana *et al*., 2018). Amarula nut cake is available in large quantities with commercial and local initiatives processing about 2 000 tonnes and 80 to 100 tonnes, respectively (Wynberg *et al*., 2002). The cake is safe for feeding livestock, mainly dairy cattle (Mdziniso *et al*., 2016), fattening beef cattle and goats (Mlambo *et* *al*., 2011), with an exception for poultry requiring supplementation of dietary antioxidant and mycotoxin binders (Mthiyane & Mhlanga, 2017). It contains about 390 g crude protein/kg dry matter (DM), 411 g fats/kg DM, gross energy of 29 MJ/kg DM, 168 g acid detergent fibre/kg DM, and 338 g neutral detergent fibre/kg DM (Malebana *et al*., 2018; Nkosi *et al*., 2019). The protein content closely simulates that of SBOC and is comparable to other conventional ingredients. The ANC is, however, high in fibre and oil contents and contains polyphenolic compounds that may limit its utilization by pigs (Muhammad *et al*., 2011). High dietary fibre influences nutrient digestibility, nitrogen retention, and excretion (Len *et al*., 2007) and increases substrate availability for fibre fermentation, which promotes the synthesis of volatile fatty acids. The ANC, therefore, has potential to be efficiently utilized by SAWIP, which utilizes fibrous diets efficiently compared to their fast-growing counterparts.

Understanding nitrogen balance can determine the efficiency of nitrogen retention and utilization as a function of body protein accretion, maintenance and metabolism. Consequently, inefficient protein utilization is costly to the pig industry and results in increased nitrogen excretion to the surroundings. Feeding the fibrous ANC can reduce the profound effects of nitrogen excretion on ammonia emissions and nitrogenous pollutants, inflaming offensive odour in pig barns (Jha & Berrocoso, 2016; Mpendulo *et al*., 2018). Fibrous by-products characterized by large quantities of soluble fibre have high viscosity and water-binding capacity, which delays gastric emptying (Serena *et al*., 2008). Exploiting dietary fibre has beneficial effects on nutrient digestion, intestinal function, gut microbiota, and fermentation is associated with changes in digesta physicochemical properties (Anguita *et al*., 2007; Jha & Berrocoso, 2016). These properties influence transit time, fermentability, water retention capacity and swelling capacity of digesta. Thus, it is worthwhile to assess nitrogen balance, fibre digestibility and physicochemical characteristics of colon digesta in growing pigs fed on ANC to validate its protein potential without compromising pig growth, health and welfare.

Utilizing ANC can expand the pool of protein sources for pig diets. Although ANC has been successfully reusable to feed livestock, its utilization in pigs as a protein source has not been investigated. It is unclear whether increasing inclusion levels of ANC in pig diet exerts a response on nitrogen utilization and excretion, fibre digestibility and physicochemical characteristics of colon digesta in Windsnyer pigs. Ascertaining the acceptability of the cake can reveal whether ANC can replace SBOC as an alternative protein source in SAWIP without compromising growth and production performance.

1.2 Justification

By-products from amarula production are generally discarded (Molelekoa *et al*., 2018). Defatting ANC can reduce its excess fat. Full exploitation of defatted ANC requires generating knowledge on the nutritional value, nitrogen balance, digestibility of fibre and changes in physicochemical characteristics of digesta. The fibre richness of ANC can improve gut health and the welfare of pigs. Availing information on physicochemical properties of digesta can reveal the nutritional, physiological and functional attributes of the fibrous components of ANC. These factors influence voluntary feed intake and help feed compounders predict the optimum inclusion level of ANC without depressing nutrient digestion and growth performance. Extrapolating response in physicochemical characteristics of colon digesta can relate gut capacity, restrictions and fermentability strength in growing pigs fed on ANC. The availability of such information can be recommended for pig nutritionists by expanding the protein pool and options for diet formulation. Smallholder and commercial pig farmers could further intensify their production systems.

The government and municipalities could benefit from the exploitation of ANC in pig feeding through inventing an eco-friendly environment, elicit protection of natural resources, and reduce dependence on imports. Use of ANC can also encourage local production of amarula, even in resource-limited communities. Researchers can further investigate appropriate methods of defatting the oil cake and exploring strategies to increase nutritive value of ANC, with the intention of increasing pig productivity and reducing environmental pollution. Furthermore, rural settlements could strengthen their economies and livelihoods through exploiting ANC. Lastly, feed formulators can nurture emerging pig farmers through innovating low-cost pig diets and promoting sustainable development of local pork markets.

1.3 Objectives

The broad objective of the study was to determine the response in nitrogen balance, fibre digestibility and physicochemical characteristics of digesta in slow-growing Windsnyer pigs fed on incremental levels of amarula nut cake (ANC) based diets. The specific objectives were to:

1. Determine the response in nitrogen balance of Windsnyer growing pigs fed on ANC; and
2. Assess the relationship between ANC inclusion and fibre digestibility, and digesta physicochemical characteristics in Windsnyer pigs.

1.4 Hypotheses

The hypothesis tested was that

1. Amarula nut cake inclusion reduces nitrogen excretion from slow-growing pigs
2. Dietary ANC increases fibre digestibility and physicochemical properties of bulkiness in colon digesta of slow-growing pigs.

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CHAPTER TWO: Literature Review

2.1 Introduction

Pig enterprises face a dual challenge of promoting efficient pork production for the ever-growing human population and developing sustainable production systems with a compact contribution to environmental pollution (Phillipe *et al*., 2011). The narrow range of feed ingredients compounded by frequent climate fluctuations aggravates competition for ingredients between humans and livestock. Inaccessibility and prohibitive prices of conventional protein sources such as soybean become a prodigious threat to pig farming. Pigs are reared for financial security, source of protein (bacon and pork), and insurance against changing climate, of which high expenditure prejudice sustainability of production systems (Ncobela *et al*., 2017). A conceptual framework from biodiesel and environmental policies incentivizes by-products to mitigate the hazardous waste impact on the environment (Muzenda, 2014). There is a need to exploit the disposal of oil cakes in pig production owed to the dearth of information on utilizing indigenous fruit-bearing trees.

Sub-Saharan Africa is diverse with about 1 200 species of indigenous fruit-bearing trees and approximately 477 of them produce nuts (Chivandi *et al*., 2015). Amongst indigenous fruit trees that produce seed nuts, amarula (*Sclerocarya birrea* subsp. *Caffra*), which is well known as the elephant tree, is one of the most treasured fruits for its macro-and micro-nutrients (DAFF, 2010). The popularity of amarula widely distributed in Southern Africa expands its domestication internationally due to cooperatives processing large quantities of fruits manufacturing value-added commodities. Approximately 2 000 tonnes of amarula fruits are processed (Wynberg *et al*., 2002), vast amounts of valuable by-products are generated and typically discarded into the environment, as such under-utilized (Molelekoa *et al*., 2018). Implications embedded in disposing of waste to landfills are associated with low availability and costs stipulated to techniques converting by-products into value-added products. The challenge is adopting affordable methods of processing amarula nuts in extracting high oil content and maintain an environmentally friendly establishment. Utilising amarula nut cake could extend the pool of pig ingredients to reduce the disposal of by-products from environmental health concern perspectives.

Amarula nut cake contains crude protein ranging from 360 to 470 g/kg DM, ether extract from 289 to 632 g/kg DM, the neutral detergent fibre of 338 g/kg DM and acid detergent fibre of 168 g/kg (Mdziniso *et al*., 2016; Nkosi *et al*., 2019). The potential of pigs in utilizing products that would otherwise be of no use for human consumption (Halimani *et al*., 2012a) could exploit amarula nut cake as a useful feed ingredient and improve the sustainability of pig production systems (Halimani *et al*., 2012a; b). Exploiting non-conventional feeds can assist in conserving genetic resources and promote efficiency of pig production systems.

It is economically viable to use applicable methods for converting discarded amarula seed nuts into valuable pig feed ingredients. Consequently, it urges evaluating the nutritional aspects of amarula nut cake as a potential protein source in pig feeding systems. The review captures constraints in slow-growing pig production systems, nutritional attributes of amarula nut cake and discusses oil-extraction processing techniques. It also outlines the nutritional aspects of amarula nut cake in reducing ammonia volatilization from pig production.

2.2 Production systems and constraints to slow-growing pig production

Outdoor production systems, lack of marketing opportunities, biasness in carcass grading schemes for lean-pork and policy disincentives are some of the constraints to the production of indigenous genotypes (Chimonyo & Dzama, 2007; Halimani *et al*., 2012b). They are characterized by slow growth and compact body frame with a maximum mature body weight of 100 kg resulting in small carcasses, constraining their use in commercial sectors. The small body frame is, however, advantageous to smallholder pig farmers who are more prone to feed shortages (Ncobela *et al*., 2018) and water scarcity. They also possess exceptional skin anatomical traits to withstand heat load in marginal areas exposed to persistent droughts (Moyo *et al*., 2018). Slow-growing pigs exhibit multiple roles as a protein source, uplifting livelihoods, and alleviate poverty in economically vulnerable communities (Halimani *et al*., 2010; Kanengoni *et al*., 2015). There are also additional non-monetary benefits of manure production for improving crop cultivation in mixed production systems. Profound inadequacy in the quality and quantity of feed restrain the introduction of imported pigs into the smallholder sector (Halimani *et al*., 2012a).

Characterization of commercial pig production systems is by adopting improved pig genotypes where breeding and rearing occur under confined infrastructure (Kariga *et al*., 2010). Intensive production systems are economically viable, strengthened by accessibility to high-quality commercial feedstock and market chain superiority. In developing countries, predominant factors constraining intensification in pig smallholder production systems include inaccessibility to high-quality feedstuffs, feasible market channels, and inadequate infrastructure (Antwi & Seahlodi, 2011). Other constraints to the production of indigenous pigs include high parasite and disease prevalences, poor management skills, and lack of institutional contributions (Chiduwa *et al*., 2008;

Halimani *et al*., 2012b). These limitations have led about 70 % of farmers in marginal regions of Southern Africa to adopt indigenous pigs to match the available resources (Halimani *et al*., 2012b). The prolificacy and capability of pigs to convert agro-industrial waste into high-quality protein mark an increasing number of emerging smallholder pig farmers (Martens *et al*., 2012). Indigenous pigs can, however, endure low-input production systems and recurring changing climate (Madzimure *et al*., 2012).

Slow-growing pigs are reared under extensive production systems, particularly backyard and free or semi scavenging production systems. In the backyard system, pigs are secured within the designated farm area and provided with kitchen scraps, rotten maize, coarse maize meal, maize cobs, maize husks, vegetables, pumpkins, watermelons, groundnut shells, fruits, grasses and brewers waste (Ncobela *et al*., 2017). The free scavenging production system is categorized as the cheapest practice with little start-up capital requirement. In this system, pigs can roam and scavenge freely all year round in search of feed and extensively feeding on forages (Phengsavanh *et al*., 2011). It is, however, characterized by the spread of diseases, resulting in reduced reproductive efficiency and high mortality rates (Kagira *et al*., 2010). The main threats to extensive production systems are endemic diseases and parasites. The adoption of these production systems in communal areas best suits indigenous-type pigs compared to their improved counterparts.

The feeds available to most smallholder farmers are usually nutritionally unbalanced and fibrous. Slow-growing pigs digest and utilize high-fibre diets efficiently, however, inadequate protein and energy supply results in reduced productivity and reproductive efficiency. Their population is dwindling, requiring policy interventions to conserve their genetic resources of economic value. The need to preserve the genetic erosion of indigenous pig breeds has mandated institutions such as Agricultural Research Council to keep a phenomenal population for research purposes (Mkhwanazi *et al*., 2018). To fully appreciate their meritorious attributes and increase meat choices amongst health-conscious consumers is by availing good quality protein sources. Further, exploiting inedible sources for humans, such as amarula nut cake into pig feeding systems, can better reduce competition for feed ingredients and drive pig growth. It is crucial to outline the protein requirements of pigs to closely match the potential of amarula nut cake in diet formulation.

2.3 Protein requirements of pigs

Farmers should provide high-quality feedstuffs containing essential nutrients to maximize production (Adesehinwa, 2008). The adequacy of the dietary protein level is determined by the capability of the protein source to provide sufficient essential amino acids in correct amounts and proportions (NRC, 2012). As pigs grow, their amino acid requirements decrease because requirements are greater during rapid growth and muscle development before weaning. In growing pigs, the amino acids are mostly used for maintenance and tissue protein accretion. The recommendations and allowances of dietary essential amino acid levels as pigs grow are outlined by the NRC (2012). Kanengoni *et al*. (2015) reported that indigenous pigs require less protein concentration than their imported counterparts. In slow-growing pigs weighing 35 kg, optimum nitrogen utilization and biological value of feed protein were attained at 135 g/kg protein level (Hlatini *et al*., 2020). Barea *et al.* (2007) reported that the transition to the finishing phase for maintenance in indigenous pigs weighing between 50 to 100 kg requires an optimum of 95 g/kg protein level.

There is a direct linkage between protein quality and the balance of indispensable amino acid profile. Low protein quality results in inefficient nutrient utilization, retarded growth, increased carcass fatness, and reduced reproductive performance (Adesehinwa, 2008). Deprivation in amino acid supply depresses pig growth and production performance, while oversupply leads to deamination resulting in excessive nitrogen losses into the environment (Van Milgen & Dourmad, 2015). A sound pig diet formulation maintains an ideal protein profile for the growth, maintenance and lean pork. Low protein-based diets with supplementation of synthetic amino acids have been recommended for growing pigs to reduce excessive ammonia emissions (Lynch *et al*., 2008). Introduction of by-products in pig diets such as macadamia nut cake containing almost half crude protein of the conventional soybean exhibited good protein attributes with a high digestibility (Tiwari & Jha, 2017). It is crucial to feed protein sources with little impact on the environment through minimum nitrogenous-containing compounds. The potential of amarula nut cake as a protein ingredient in pigs is largely unknown, and its protein content is higher than other non-conventional oilseed cakes (Nkosi *et al*., 2019). Conducting dose-response trials in pigs fed amarula cake can reveal its dietary protein qualities.

2.4 Potential of oilseed by-products as protein sources in pig feeding systems

Progressive and sustainable development of pig production depends on the availability and accessibility to quality dietary protein and energy sources. The attention brought on dietary protein ingredients is invigorated by the necessity for efficient digestibility and utilization to acquire maximum growth performance and production. A good quality protein source provides a balanced essential amino acid profile crucial for the normal functioning of metabolic and physiological processes (Adesehinwa, 2008). In pigs, the protein sources that are widely utilized are plant-based. Animal-derived feedstuffs have been prohibited due to the detrimental effects and implications on pig growth and health (Stein *et al*., 2016). The banned dietary animal derivatives protein sources in animal feeds include meat meal, blood meal, bone meal, and fish meal (Malebana *et al*., 2018). The reason behind this lies in downgrading the quality of pork and bacon products interfering with the consumer's preference.

Various feed ingredients used in pig diet formulation are by-products from human food industries and other sectors, principally the biofuel production (Stein *et al*., 2016). Greater exploitation of agro-industrial waste from oilseeds has been common in the biodiesel industry (Woyengo *et al.*, 2017). Oilseed meals and cakes are the second most crucial feed ingredients after cereal grains in pig diets as protein-rich dietary sources (Messad *et al*., 2016). The dominant conventional protein sources are soybean, sunflower, cottonseed, rapeseed and groundnut cakes. Global production of soybean and other oilseeds projects 361.9 million tons and 152.9 million tons in 2019, with an estimated increase in production reaching 406.8 million tons and 173 million tons by 2027, respectively (OECD/FAO, 2018). Climate change, high market prices for conventional soybean oilcake and the competition for ingredients between humans and livestock have necessitated exploring indigenous oilseed by-products, such as amarula nut cake from amarula production industries.

2.5 Processing and production of amarula nut cake

A rapidly growing demand and enduring oil production in biodiesel industries increase perceptions of amarula nut cake availability. Approximately 5 x 10^3 to 9 x 10^4 seeds are produced by mature amarula trees per season (Mokgolodi *et al*., 2011). These seeds enclose two to three soft white kernels (nuts) favoured by people and animals, mainly elephants as seed-dispersers (Mojeremane & Tshwenyane, 2004). The commercial sectors and the local initiatives in Southern Africa process about 2 000 tonnes and 80 – 100 tonnes of amarula fruits, respectively (Wynberg *et al*., 2002), which prompts large quantities of seed nuts. The nuts are sold during the offseason, and oil extraction from these kernels produces amarula nut cake (Mdziniso *et al*., 2016). The resulting residue is protein-rich and abundant in oil and fibre. A profusion of fat and fibre in the nut cake may limit their full utilization capacity in pigs. Excessive residual oil is undesirable for feeding pigs instigating to provide useful processing tools and exploit its nutritional attributes. Various oil extraction processing techniques are hereinafter availed to mitigate detrimental effects from excessive oil accumulation and further improve the extent of amarula nut cake utilization in pigs.

2.5.1 Traditional methods

The traditional technique is referring to the manual press, which involves preliminary processing and hand pressing. Smallholder farmers mostly appreciate manual press due to accessibility and affordability. Amarula kernels are sun-dried, then subjected to decortication to remove shells enclosing the nuts protective layer, typically applying sizable stone manually (Attiogbe & Abdul-Razak, 2016). After decortication, the kernels are roasted between 43 and 45 °C before subjected to a manual hand to reinforce pressure (Mlambo *et al*., 2011). Critical setbacks for smallholder farmers to access modern methods include the degree of complexity of modern types of equipment, requirement for maintenance, spare parts, and availability of power sources (Ibrahim & Onwualu, 2005).

2.5.2 Modern methods

Industrial-scale oil extraction uses modern techniques consisting of mechanical and chemical extraction. These methods require a pre-extraction process as the preliminary processes involving removing amarula nutshells and pre-treatment conditioning, such as reducing moisture content, heat treatment, and pressure application (Ibrahim & Onwualu, 2005). The drying of seed kernels typically occurs in the oven at the range of 65 to 70 °C for about three to four days (Bhuiya *et al*., 2015).

2.5.2.1 Mechanical method

Mechanical extraction is one of the oldest methods and is widely used for extracting oil from seed kernels. It requires the use of devices such as an electric-powered screw or cold-screw press, hydraulic press, and oil-expellers to apply pressure on pre-treated amarula seed kernels. Amongst the power sources used, mechanical extraction using the oil expeller could produce oil from amarula kernels at a low cost (Bhuiya *et al*., 2015). Ibrahim & Onwualo (2005) reported that oil extracted could be high as 90 % through pressing using an oil expeller. Cold-pressing is the most preferred because it retains beneficial nutritious components such as antioxidants increasing the nutritive value of the resulting oil and extracted kernel (Rombaut *et al*., 2015). The seed kernels are roasted in the oven at a temperature approximating 43 °C for two hours before the oil is extracted mechanically by cold-pressing (Mlambo *et al*., 2011) to produce a cold-pressed nut cake that contains about 470 g/kg protein. Malebana *et al*. (2018) concurred with these findings with cold-press and hydraulic-press yielding 391 and 325 g/kg protein content in amarula nut cake, respectively. The cold press can effectively extract about 68 to 80 % oil content from the seed kernels (Bhuiya *et al*., 2020).

2.5.2.2 Chemical extraction

Chemical extraction technique uses organic solvents as the mediator for recovering oil from the kernels (Ibrahim & Onwualu, 2005). Solvent extraction is the most effective oil extracting modern method due to its consistency in performance and high oil yield. Its widely used in commercial biodiesel industries and relatively expensive due to the exorbitant costs of solvents. The prime solvent is n-hexane, the reduction of residual oil in the seed kernel is mediated by hexane-binding. Attiogbe & Abdul-Razak (2016) used n-hexane for oil extraction of *Sclerocarya birrea* seed oil with a roasted kernel containing lower moisture content and residual oil compared to raw kernel. Raw and roasted seed kernel oils had a saponification value of 12.5 and 11.4 mg KOH/g, respectively (Attiogbe & Abdul-Razak, 2016). These values are not within the standard range of about 200 mg KOH/g as an index of a high proportion of fatty acids of low molecular weight. It shows that the saponified amarula oil does not exhibit potential for use in the soap making industry. Solvent extraction recovers almost all the oil and leaves approximately less than 0.7 % residual oil, resulting in nut cake (Topare *et al*., 2011). Despite the proficiency of solvent extraction, there is a risk of solvent contamination with the nut cake and its impact on the environment from a health and safety perspective (Bhuiya *et al*., 2020)

Figure 2.1 shows the steps that are followed when preparing seed kernels for oil extraction (Ibrahim & Onwualo, 2005). Discrepancies in oil extraction techniques cause variation in the physical and chemical composition of feed ingredients. Several factors influence the varying nutritive value and quality of amarula nut cake includes heating time, temperature and pressure application of the oil-extraction adopted. Efficient pre-treatment of amarula kernels to emanates moisture content prior to oil-extraction could reduce substantial residual oil that negatively affects nutrient digestion and absorption. An oil-extraction technique for processing amarula seed kernels should sustain the nut cake's nutritive value and cost-effectiveness. Therefore, it is worth assessing the nutritional characteristics of defatted amarula nut cake for pig feeding systems.

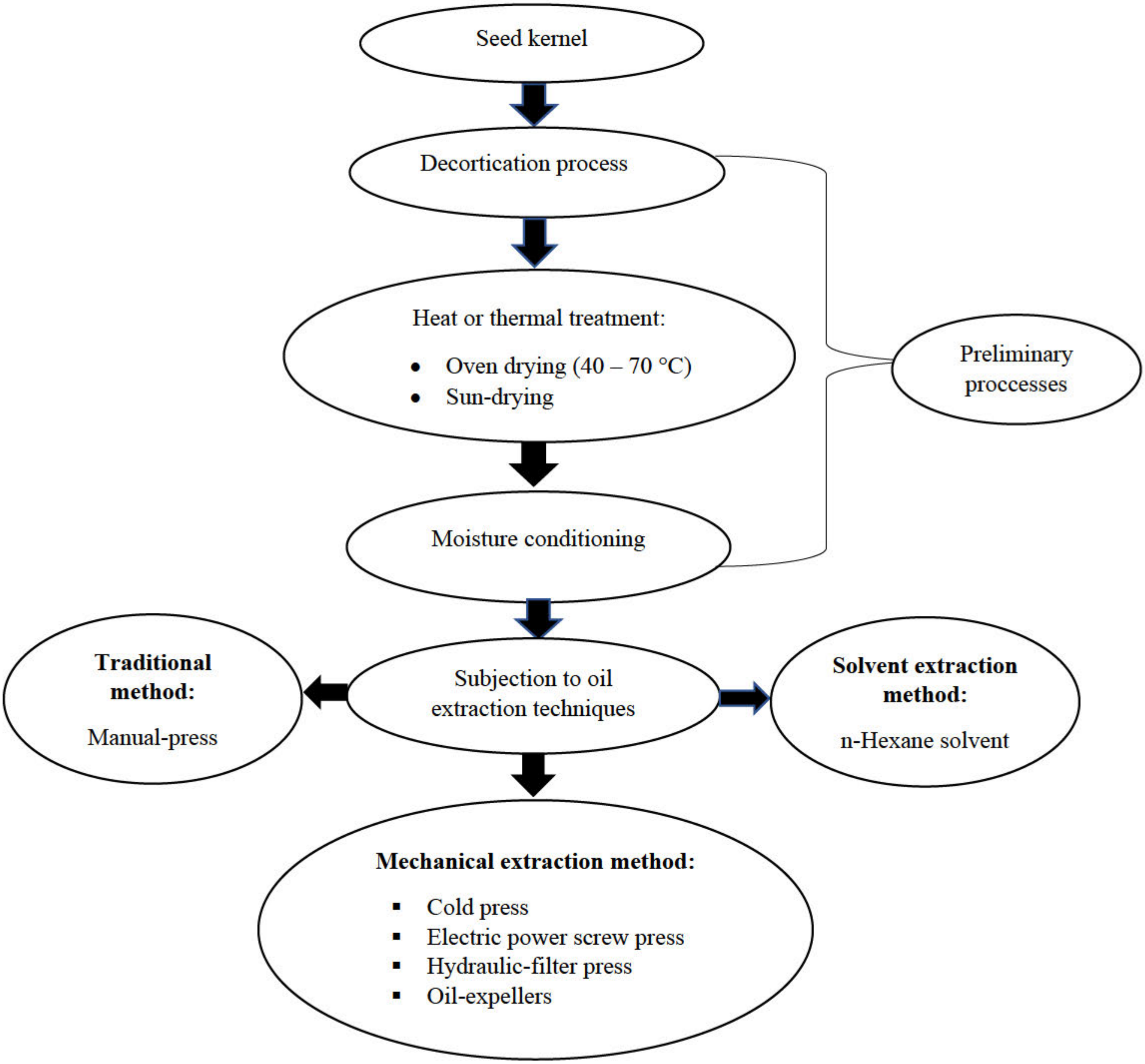

Figure 2. 1 Flowchart of oil extraction primary steps for oil-bearing seed kernels (Ibrahim & Onwualo, 2005)

2.6 Nutritional value of amarula nut cake

Disposal of amarula seed nuts in large quantities into the environment expands the availability of amarula by-products as livestock feed ingredients. Amarula nut cake has not been adopted in pig production feeding systems. Their palatability and effect on pig growth performance, nutrient digestibility and utilization in pigs need to be ascertained.

Nutritional characterization of amarula nut cake has been assessed by numerous researchers (Mdziniso *et al*., 2016; Malebana *et al.*, 2018; Nkosi *et al*., 2019). Defatting oilseed cakes exploits richness in protein concentration ranging between 350 and 600 g/kg, and varies with oilseed source (Moure *et al.*, 2006). Defatted amarula nut cake has been reported to contain a crude protein content between 360 and 470 g/kg DM, higher than the cottonseed cake, groundnut meal, and sunflower cake (Mdziniso *et al*., 2016; Nkosi *et al*., 2019). The varying protein concentration is influenced mainly by different oil extraction methods and edaphic factors affected by climatic conditions of regions growing amarula. A recent improvement in mechanical oil-extraction yielded 3.65 % oil lower in the study of Mthiyane & Mhlanga (2018) compared to residual oil of 380 g/kg (Mlambo *et al*., 2011). Malebana *et al*. (2018) also outlined variation in the physicochemical composition of amarula nut cake processed differently with low crude protein than solvent-extracted soybean, comparable to other conventional and non-conventional oilcakes. Table 2.1 shows the chemical composition of *Sclerocarya birrea* kernel compared to other oilseeds containing considerably monogastric animals' protein content. The cake is fibrous with a neutral detergent fibre of 338 g/kg DM, enhancing gut health and microbiota, improving fibre fermentation, hence volatile fatty acid production.

Table 2.1 Chemical composition of *Sclerocarya birrea Caffra* seed kernels compared to other oilseed meals

Oilseed cakes	Components (g/kg dry matter basis)									References
	DM	OM	CP	EE	NDF	ADF	ADL	Ash	GE#	
Conventional oilseeds										
Soybean	892	942	484	8.3	67	29	-	58	17.6	Baker & Stein (2009); Rodriguez *et al.* (2013)
Canola	909	925	368	37	334	200	80	75	-	Almeida *et al.* (2014)
Cottonseed	896	919	423	-	246	171	-	81	18.2	Rodriguez *et al.* (2013)
Sunflower	958	969	221	-	81	76	-	31	29.8	Rodriguez *et al.* (2013)
Non-conventional oilseeds										
Amarula	947	944	360-470	345-394	208-338	168-180	94-101	54-56	28.5	Mlambo *et al*., (2011); Mthiyane & Mhlanga (2017); Nkosi *et al.* (2019)
Avocado	949	-	156	-	518	393	258	-	-	Skenjana *et al*. (2006); Van Ryssen *et al.* (2013)
Baobab	907	-	228	54.1	-	-	-	-	-	Magonka *et al*. (2018)
Macadamia	912	963	255	119	358-404	280-327	118	37	23.4	Skenjana *et al.* (2006); Tiwari & Jha (2017)

= MJ/kg DM; CP = crude protein; DM = dry matter; EE = ether extract; GE = gross energy; ADF = acid detergent fibre; ADL = acid detergent lignin; NDF = neutral detergent fibre; OM = organic matter.

Amarula oil contains high amounts of monounsaturated fatty acids (MUFAs) dominated by oleic acid (18:1 n-9) (Glew *et al.*, 2004; Malebana *et al*., 2018; Mthiyane & Mhlanga, 2017). Substantial MUFAs have a beneficial effect on sustaining oxidative stability and promote general health. However, polyunsaturated fatty acids (PUFAs) acquire the least proportion in the fatty acid composition of amarula. Malebana *et al*. (2018) reported that amarula seed nuts contain 23 times more oil content with a dilution effect on crude protein concentration than soybean meal. Consequently, the copious residual oil quantities could prompt lipid peroxidation, which causes rancidity and shortens the shelf life of feed (Malebana *et al*., 2018). Attiogbe & Abdul-Razak (2016) reported that raw and roasted amarula seed kernels had peroxide values lower than the maximum acceptable value of 10 meq KOH/g. This index shows that fat content in amarula nut cake-based diets is within the safety margin from going rancid. Efficient oil-extraction of amarula nut cake may better meet maintenance energy requirements and tissue protein accretion, possibly not merely as a protein source but may also provide additional energy.

Table 2.2 depicts a good amino acid profile containing predominantly glutamic acid and arginine (Mariod & Aldelwahab, 2012) and limiting in lysine (Mariod *et al*., 2005). The cold-pressed cake had the highest concentration of arginine compared to hydraulic-filter pressed cake (Table 2.2). Supplementing lysine on pig diets containing amarula nut cake can improve its nutritional value for maximum protein deposition. The mineral profile in Table 2.3 indicates that amarula nut cake contains adequate amounts of phosphorus, calcium, magnesium and potassium (Mariod & Aldelwahab, 2012). It also shows a slight variation in nutritional composition caused by differences in processing methods. The amino acid and mineral composition profiles largely influence nutrient digestibility and absorption for growth performance and production. Despite the evidence that amarula nut cake has appreciable protein quantities comparable to other conventional protein-bearing sources (Mdziniso *et al*., 2016), the effect of its nutritional characteristics on nutrient digestibility, nitrogen utilisation, and absorption and its influence on physicochemical properties of digesta and volatile fatty acid production as a protein source in pigs remain mostly unrevealed. The successful use of amarula nut cake in other livestock feeding systems gives a better potential for feeding pigs.

Table 2.2 Amino acid composition of *Sclerocarya birrea Caffa* nut cake mechanically processed using a hydraulic press and cold press extraction

Components (g/kg DM)	Mean (g/kg DM)	
	Hydraulic filter press	Cold press
Indispensable amino acids		
Arginine	50.9	76.3
Histidine	5.7	6.8
Isoleucine	12.3	12.9
Leucine	16.9	16.7
Lysine	7.7	7.7
Methionine	6.3	7.5
Phenylalanine	12.4	11.9
Threonine	6.9	6.0
Tryptophan	3.4	6.3
Valine	13.0	13.0
Dispensable amino acids		
Alanine	8.9	8.1
Aspartic acid	19.8	19.4
Cysteine	2.8	9.7
Glutamic acid	66.1	61.5
Glycine	13.6	11.5
Hydroxyl-proline	0.6	0.3
Proline	13.0	9.1
Serine	12.2	10.4
Tyrosine	9.7	12.6

Source: Malebana *et al*. (2018)

Table 2.3 Mineral composition of *Sclerocarya birrea Caffra* nut cake processed using different techniques (g/kg dry matter)

	Mean (g/kg DM)	
Component	Hydraulic filter press	Cold press
Macro mineral elements		
Calcium	1.43	1.10
Phosphorus	10.00	10.90
Magnesium	4.87	5.50
Potassium	6.33	9.30
Sodium	0.03	0.10
Micro mineral elements		
Copper	0.02	0.03
Iron	0.69	0.04
Zinc	0.05	0.06
Sulphur	4.23	5.92

Source: Malebana *et al*. (2018)

Anti-nutritional factors in oilseed by-products and their influence on feed intake and availability of nutrients have been reported (Woyengo *et al.*, 2017). Antinutrients present in amarula nut cake include hydrocyanic acid, nitrates, oxalate, phytates, and tannins/proanthocyanidins (Muhammad *et al*., 2011). Most of these compounds partially contribute to the exhibition of medicinal properties (Mariod & Aldelwahab, 2012) but reduce nutritive value. Phytate possesses the capability to chelate divalent mineral components like calcium. Simultaneously, oxalate stimulates the removal of calcium in calcium oxalates from the blood as insoluble salt, which inhibits mineral absorption (Muhammad *et al.*, 2011). Adverse malabsorption of essential nutrients elicits poor performance perpetuating adverse effects on animal productivity.

Adverse effects arise from proanthocyanidins, a polyphenolic compound either hydrolyzable or condensed. These compounds precipitate dietary proteins and digestive enzymes, forming indigestible complexes (Jansman *et al*., 1993). A higher tannin concentration of 3136.39 mg/100 g dry weight (DW) in the amarula seed kernel could be due to the astringent flavour of nuts. Muhammad *et al*. (2011) reported high phytate content of 423 mg/100g DW, however, mineral bioavailability predictions indicated that this value is below the critical level that causes a deficiency in divalent elements. Mthiyane & Mhlanga (2018) supplemented phytase and DL-methionine for inhibiting deleterious effects of phytate and hydrocyanic acid in diets containing amarula nut cake but did not improve production performance. Consequently, depressed growth performance suggested toxin-bound infestation, which interfered with feed intake. It is, therefore, important to assess response in nutrient digestibility, protein utilization, and nitrogen excretion in growing pigs fed on incremental levels of amarula nut cake for its acceptability in pig feeding systems.

2.7 Nutrient digestibility and nitrogen balance in pigs fed on amarula nut cake-based diets

Understanding the nutritional characteristics of amarula nut cake in pigs requires a dose-response trial of nutrient digestibility and nitrogen balance to ascertain their acceptability as a potential protein source in pig feeding systems. Mdziniso *et al*. (2016) used an *in vitro* digestibility approach to assess the extent of organic matter digestibility and nitrogen degradability of amarula nut cake-based diets for dairy cattle. Mdziniso *et al*. (2016) reported that amarula nut cake resulted in a nitrogen digestibility coefficient of 0.81, close to 0.85 standardized ileal digestibility of nitrogen in soybean meal (Brestenský *et al*., 2013). High nitrogen digestibility comparable to conventional protein ingredients indicates the finite capacity of pigs to digest and absorb ingested nitrogen in amarula nut cake efficiently. The remaining undigested dietary protein fraction escapes digestion and absorption during transit through the stomach to small intestines and becomes available for fermentation (Varley *et al*., 2011). The precision in pig diet formulation relies mostly on standardized ileal digestibility of amino acids and crude protein, accounting for the correction of basal endogenous losses (Van Milgen & Dourmad, 2015). There is a scarcity of information on standardized ileal digestibility of amino acids and crude protein in growing pigs fed on amarula nut cake, hence it requires practical assessment.

Mlambo *et al*. (2011) conducted a nutrient metabolism trial to determine the apparent digestibility of acid detergent fibre and neutral detergent fibre in goats fed on amarula nut cake. About 0.52 fibre digestibility coefficient was observed compared to 0.64 in soybean meal-based diets (Mlambo *et al*., 2011). Urriola & Stein (2010) reported that dietary fibre in soybean meal is more digestible, soluble, and contains highly fermentable oligosaccharides, which stimulates microbial activity. The extent of suppressed digestibility depends mainly on the degree of fibre solubility state (Zhang *et al*., 2013), which influences water retention capacity, nutrient absorption rate, gut-fill, and gastric emptying. High fibre content in amarula nut cake could interfere with feed intake as the inclusion level increases total dietary fibre concentration. Mthiyane & Mhlanga (2018) reported that feeding broilers on amarula nut cake inclusion level exceeding 200 g/kg up to 300 g/kg resulted in depressed growth and production performance. Amarula nut cake incorporation up to 315 g/kg did not affect overall feed intake and growth performance, hence broiler quails digested and absorbed nutrients better favoured by microbiota composition (Mazizi *et al*., 2019). Digestive capacity and diverse microbial population in pigs could improve the surface area for digestion and absorption when fed on fibrous amarula nut cake. It is recommended that amarula nut cake be incorporated up to 200 g/kg inclusion level in pig diets to account for the physicochemical properties of bulkiness that could interfere with feed intake and nutrient digestibility.

No difference was observed in organic matter and acid detergent fibre digestibility in cold-pressed amarula nut cake and undecorticated sunflower cake (Mlambo *et al*., 2011). However, amarula nut cake and sunflower cake had a similar digestibility coefficient of organic matter, approximating 0.73, which is higher than previously reported values (Mdziniso *et al*., 2016). The discrepancies could be ascribed to improvements in oil extraction techniques employed in recent years for processing amarula nut cake and decorticated sunflower cake, further reducing residual fats' effect on digestibility. High residual fat content in energy-rich ingredients interferes with feed intake, nutrient digestion and absorptive capacity (Choe *et al*., 2018). Excess fat surpassing 5 % coats the fibre structure by reducing hydrophilicity of feed particles (Mdziniso *et al*., 2016), thus negatively affecting colonization of resident microbes and digestive capacity of pigs. Mdziniso *et al.* (2016) reported that 29 % ether extract content in amarula nut cake exceeding recommendations but did not lower digestibility as expected, attributed to divalent macrominerals concentrations. The fat content of amarula nut cake could benefit growing pigs as an energy source with a good mineral profile and antioxidant activity enriching digestive efficiency. Therefore, the effect of high fat and fibre components on total dietary fibre digestibility needs attention in growing pigs fed on incremental levels of amarula nut cake.

Presence of polyphenolic compounds in amarula nut cake could interfere with nutrient digestibility, absorption rate, and utilization efficiency. These compounds have adverse effects on protein digestibility, amino acid bioavailability, and protein quality (Gilani *et al*., 2012). Muhammad *et al*. (2011) reported polyphenols concentrations in amarula seed nuts lower than the critical level, which indexes low toxicity for pig consumption. Gilani *et al*. (2012) showed that heat treatment of protein-rich agro-industrial by-products may result in Maillard reaction products, negatively affecting digestibility. Oven heat treatment during oil extraction processing of amarula nut cake could reduce antinutrients-induced by high temperatures. Hence, precise evaluation of defatted amarula nut cake's potential to pig feeding systems requires *in vivo* digestibility trials. On account of this, limitations can be accounted for to reduce the impairment of nutrient digestibility. It is of paramount importance to assess dietary components that could influence the digestibility and utilization of amarula nut cake as a protein ingredient in pigs. Consequently, improving fibre digestibility in growing pigs can increase nutrient digestion.

The fibre and fat fractions of the cake exhibit a dilution effect on its crude protein concentration (Malebane *et al*., 2018). Series of studies showed that nitrogen intake decreased with a reduction in dietary crude protein concentration (Loh *et al*., 2010; Zhang & Kim, 2014; Hlatini *et al*., 2020). The decline in dietary protein content with increasing rapeseed cake levels reduced nitrogen intake in growing pigs (McDonnell *et al*., 2011). The fibre composition varies amongst different oilcakes. Increased proportion of insoluble non-starch polysaccharides binds the nitrogen fraction, reducing nitrogen intake (Liu *et al*., 2019). Patráš *et al*. (2012) reported an increased nitrogen intake in pigs fed fibrous diets containing sugar beet pulp rich in soluble non-starch polysaccharides. Feeding increasing fibrous amarula nut cake levels to growing pigs could increase nitrogen intake due to increased soluble fraction of dry matter (Muya *et al*., 2020). Low concentration of polyphenolic compounds below critical index level forming protein-bound complexes could reduce nitrogen-bound and dietary-protein complexes interfering with intake (Muhammad *et al*., 2011).

Mlambo *et al*. (2011) reported that supplementation of amarula nut cake in grass hay-based diet improved nitrogen retention, with a positive response implying efficiency in gaining protein. The prior study availed that nitrogen supplied facilitates the formation of microbial protein viable for various metabolic functions. The positive nitrogen retention is an indication that livestock fed amarula nut cake acquired protein efficiently (Mlambo *et al*., 2011). The capability of pigs in utilizing the protein source greatly influences the secretion of trypsin and chymotrypsin enzymes, hydrolyzing protein into peptides and free amino acids (Liu *et al*., 2019). Increased protein intake improves intestinal integrity for absorption and retention of nitrogen. Increment of amarula nut cake in diets increased crude protein subjected to digestion (Muya *et al*., 2020), hence could improve absorption and retention of nitrogen in pigs due to higher absorptive capacity. Undigested protein that escapes digestion decreased with increasing amarula nut cake, reducing endogenous protein losses. Growing pigs fed on incremental levels of amarula nut cake could have a small proportion of indigestible protein fraction subjected to microbial fermentation, thus reducing protein fermentation. Hlatini *et al*. (2020) showed that feeding reduced protein levels from 193 to 97 g/kg DM to slow-growing pigs resulted in a linear decrease in nitrogen retention, apparent digestibility, and absorption attributed to decreased dietary protein intake. Hence, protein intake in growing pigs largely determines the extent of absorption and retention of ingested nitrogen.

Increasing acid detergent fibre interferes with efficiency in digestion while high ether extract masks feed particles from being broken down, hindering utilization (Muya *et al*., 2020). Feeding amarula nut cake improved nitrogen retention and utilization as ascribed to longer periods of retaining digesta in the digestive tract, facilitating optimum capture of nitrogen (Mlambo *et al*., 2011). Amarula nut cake contains slowly digested protein cumulating exposure of essential nutrients to increased absorptive surface area for utilization. Hlatini *et al*. (2020) reported an increasing quadratic response in nitrogen utilization as protein concentration decreased in the pig diet. These findings show that growing pigs fed on low-protein level diets utilized nitrogen better with less nitrogen excreted into the environment. Reduction in crude protein concentration with increasing levels of amarula nut cake could improve nitrogen utilization. Inefficient utilization of absorbed nitrogen increases urinary nitrogen excretion and, as such, ammonia volatilization. Consequently, providing surplus protein in pig diets is costly and increases nitrogen-based pollutants through urine and faeces into the environment.

Mlambo *et al*. (2011) reported that the inclusion of amarula nut cake in diets resulted in the lowest faecal nitrogen excretion. Higher inclusion levels (138.4 and 185.9 g/kg) of the cake caused soluble and degradable protein components to be efficiently digested (Muya *et al*., 2020). Consequently, a small proportion escaped digestion to be prone to microbial action initiating microbial protein synthesis. The presence of both soluble and insoluble fractions of bulkiness (Nkosi *et al*., 2019; Muya *et al*., 2020) shifts the nitrogen excretion pattern to less volatile organic nitrogen through faeces than via urine. It is conceivable that high fibre content reduces nitrogen excretion in the form of urea in urine to bacterial protein in faeces (Mlambo *et al.*, 2011). Increased nitrogen excretion via urine in pigs is undesirable as a medium surface for excessive ammonia emissions into the surroundings. Hence, the inclusion of amarula nut cake in pig diets could significantly reduce urinary nitrogen excreta and total nitrogen excretion.

Characterization of amarula nut cake through the perception of nitrogen balance trials could better describe its nutritional characteristics, optimum requirements, and limitations prior to diet formulation. In addition, supplementation of exogenous limiting amino acids, chiefly lysine in amarula nut cake-based diet, could improve nitrogen intake and nitrogen available in pigs (Ball *et al*., 2013). Providing optimal inclusion level of fibrous amarula nut cake in compliance with fast-growing and slow-growing pigs protein allowances and recommendations could contemplate reducing total nitrogen excretion, odorous nitrogenous compounds, and further lowering costs. Thereby, there is a need to assess the fibre digestibility and nitrogen balance response in growing pigs fed dietary amarula nut cake.

2.8 Colon fermentation and digesta physicochemical properties of bulkiness in pigs fed on amarula nut cake

Inclusion of fibrous feedstuffs has been beneficial for gut health, microflora functioning, and promoting the welfare of pigs (Ndou *et al*., 2013). The most common agro-industrial oilcakes used in feeding systems are fibre-rich, varying in physicochemical properties of bulkiness, thus influences hydration properties such as water retention capacity, viscosity, and swelling capacity (Ngoc *et al*., 2012). Consequently, it becomes a challenge to use these oilcakes to their full potential in monogastric nutrition (Acheampong-Boateng *et al*., 2017). The degree of fibre solubility in amarula nut cake significantly affects dietary fibre fermentability and absorptive capacity of pigs. Measuring colon volatile fatty acids and physicochemical characteristics of digesta can better predict the gut capacity of pigs to utilize and ferment dietary fibre components of amarula nut cake.

2.8.1 Dietary fibre fermentation

Dietary fibre is the fraction of nutritional carbohydrates resistant to degradation by endogenous enzymes in the foregut (Knuden, 2001; Zhou *et al*., 2018). These dietary carbohydrates are fermented to a certain degree by microflora in the hindgut owing to the pig's inability to secrete enzymes hydrolyzing fibre fractions. Apart from the carbohydrate sugars and oligosaccharides, the polysaccharides can be broadly divided into starch and non-starch polysaccharides (NSPs) (Lindberg, 2014). The NSPs comprise between 700 and 900 g/kg of the plant cell wall, causing the dietary fibre to vary on the degree of water solubility, consequently influencing digesta flow, rheological gastric content properties, digestion, absorption and gastric emptying (Knudsen, 2001). The hotspot sites for carbohydrate fermentation are mainly the caecum and proximal colon due to the colonic microbiome, with more than 92 % of carbohydrates fermented in these segments of pigs (Agyekum & Nyachoti, 2017).

During dietary fibre fermentation, microbes in the large intestines initiate degradation action to break down polysaccharides into constituent monosaccharides and conserve partial energy for microbial growth. Except for *Bifidobacteria* in the hindgut, a significant population of anaerobic microbes uses the glycogenesis pathway to degrade glucose to pyruvate via glucose-6-phosphate, which is further oxidized to produce short-chain fatty acids (SCFAs) (Jah and Berrocoso, 2016). Pectin and pentose based polysaccharides are metabolized first by the pentose phosphate pathway (Macfarlane & Macfarlane, 2003), starting from the pentose to fructose-6-phosphate and glyceraldehyde-3-phosphate via xylulose-5-phosphate. The primary end-products SCFAs of dietary fibre fermentation, mainly acetic acid, propionic acid, and butyric acid, are produced with carbon dioxide, hydrogen, and methane gases. Other metabolites such as ethanol, lactate, and succinate are also formed by different types of bacteria (Drochner *et al*., 2004). With ethanol exception, these metabolites do not accumulate in a healthy gut because they serve as substrate and electron donors for cross-feeding bacteria and are further converted into SCFAs (Macfarlane & Gibson, 1995).

Short-chain fatty acids (SCFAs) play a crucial role in intestinal metabolic and physiological functions as an energy precursor for maintenance requirements. The resulting microbial fermentation by-products promote mucosal epithelium proliferation by increasing the gut length, mass, and villus height, increasing the intestinal surface area for absorption (Agyekum &

Nyachoti, 2017). The degree of fermentation in the hindgut is primarily influenced by the feed ingredients, fibre source and type, and dietary factors (Bindelle *et al.*, 2008). Faster fermentation rate in the proximal colon is observed in pigs fed diets containing soluble fibre than diets comprised of insoluble fibre fractions (Agyekum & Nyachoti, 2017). Molist *et al*. (2009) reported a pronounced increase in SCFAs concentrations in pigs fed on a diet containing sugar-beet pulp (soluble NSPs), which could be associated with a higher water retention capacity of digesta. The amounts and relative molar proportions of SCFAs vary broadly on diet composition and deviates from the general ratio of acetate: propionate: butyrate (65:20:15), with acetic acid predominating the SCFAs concentrations followed by propionic and butyric acid (Bindelle *et al*., 2008). Type of fibre also contributes to varying acetate: propionate: butyrate ratio with the fermentation of NSPs, pectins, and starch yielding 63:22:8, 80:12:8, and 62:15:23, respectively (Drochner *et al*., 2004).

Topping *et al*. (1996) outlined the beneficial effects of carboxylic acids from carbohydrate fermentation with the total SCFAs lowering the pH level creating an undesirable environment for pH-sensitive microbes. Promotion of acetic acid (CH_3-COOH) increases calcium and magnesium absorption diminishing their loss through faeces (Topping *et al*., 1996). While propionic acid (CH_3-CH_2-COOH) enhances colonic muscular contraction, stimulates electrolyte transport, proliferates the colon's epithelial cells, increases absorptive capacity, and prevents scouring incidences. Butyric acid (CH_3-$(CH_2)_2$-COOH) acts as an oxidative fuel for colonocytes and exhibits a trophic effect on inflamed caeca-colonic mucosa, maintaining mucosal integrity and colonocytes proliferation (Topping *et al*., 1996; Topping & Clifton, 2001). Diets containing higher amounts of insoluble NSPs or the combination of insoluble and soluble NSPs promoted a beneficial shift in microbial colonization with higher butyric acid production and lower counts of *enterobacteria* in the hindgut digesta (Molist *et al*., 2009). Muya *et al*. (2020) show that the inclusion of amarula nut cake in diets increases soluble and insoluble bulkiness fractions. The combination of soluble and insoluble NSPs in amarula nut cake could promote a beneficial shift with increased butyric acid production in growing pigs. Higher fermentative capacity in slow-growing pigs, influenced by their longer and larger caecum-colon (Ndindana *et al*., 2002), could ferment the fibrous fraction of amarula nut cake. It becomes essential to assess SCFAs concentration in the colon digesta of pigs fed fibrous amarula nut cake to understand their total dietary fibre physiological impact on gut health.

2.8.2 Protein fermentation

As digesta in the gastrointestinal tract moves through the colon's distal parts, the carbohydrates become depleted, and saccharolytic bacteria activities are reduced (Macfarlane *et al*., 1992). Such action reduces the concentration of SCFAs, limiting energy availability and causes deamination of amino acids as an alternative energy source for the microflora (Jørgensen *et al*., 199). Dietary proteins that escape digestion in small intestines and some endogenous origin proteins become available to proteolytic bacteria for fermentation. The proteolytic fermentation also yields SCFAs, particularly end products are branched-chain fatty acids (BCFAs). These BCFAs include iso-butyrate (CH_3-$CH(CH_3)$-COOH), valerate (CH_3-$(CH_2)_3$-COOH), and iso-valerate (CH_3-$CH(CH_3)$-CH_2-COOH), mainly formed by the metabolism of branched-chain amino acids such as valine, leucine, and isoleucine (Jah & Berrocoso, 2016). Branched-chain fatty acids constitute about 20 % of approximately 30 % of the total SCFAs resulting from fermented protein (Macfarlane *et al*., 1992). Proteolytic fermentation also produces toxic metabolites such as ammonia, biogenic amines, phenolic compounds, and volatile sulfurous compounds, potentially harmful to gut health.

A small proportion of these harmful substances are excreted directly via faeces, and unfermented protein in the faeces is further fermented in the manure (Jah & Berrocoso, 2016). Feeding amarula nut cake to growing pigs could result to lower BCFAs, with the nut cake containing a large proportion of soluble and degradable dietary protein with a smaller fraction of indigestible protein escaping digestion (Muya *et al*., 2020). Evaluating the occurrence of BCFAs to the total SCFAs production in the colon content could provide an apparent contribution of protein, thereby indigestible protein fraction in the diet containing amarula nut cake can be determined.

2.8.3 Nutritional mitigation strategy to reduce protein fermentation

Supplying excess protein enhances the growth of nitrogen-requiring bacterial population fermenting available protein, which increases ammonia and biogenic amines density in the colon (Macfarlane *et al*., 1992). The yield of these toxic metabolites can impair epithelial integrity, impose enteric disorders incidences, and potentially interfere with the oxidative metabolism of SCFAs in colonocytes (Jha & Berrocoso, 2016). An effective strategy employed in reducing protein fermentation in the pig gut is lowering the crude protein level supplied in the pig diet. Heo *et al*. (2009) reported that feeding a low protein diet supplemented with crystalline valine and isoleucine reduced the incidence of postweaning diarrhoea and indices of protein fermentation. Inclusion of highly fermentable dietary fibre such as hemicellulose and pectin stimulate healthy bacterial growth in the hindgut (Jha & Leterme, 2012), which increases microbial protein in the colon and faecal nitrogen excretion in the form of bacterial biomass. A decreased ammonia concentration in the colon digesta was observed in response to the addition of highly fermentable fibre sources (Jha & Leterme, 2012). Reduction in ammonia concentration in the colon alternatively indicates reduced ammonia volatilization from excreta into the environment. The soluble fraction of fibre in the nut cake can reduce ammonia in the colon digesta and proteolytic fermentation (Muya *et al*., 2020). Feeding pigs on fibrous amarula nut cake could promote the proliferation of beneficial intestinal microbiome and hinder colonization of the harmful bacterial population, thereby congesting their detrimental effects on intestinal health. The nut cake inclusion for pigs should be in acquiescence with recommendations outlined by numerous literature (Barea *et al*., 2007; NRC, 2012; Carter *et al*., 2016; Hlatini *et al*., 2020).

2.8.4 Digesta physicochemical properties of bulkiness in pigs fed on amarula nut cake

The physiological role of dietary fibre dramatically influences digesta physicochemical properties of bulkiness, particularly hydration properties, namely water retention capacity, swelling capacity, and solubility (Wate *et al*., 2014). Water retention capacity (WRC) is defined as the ability of the fibre source to grasp or immobilise water within its matrix, swell and form gels with high water contents, and carried out under atmospheric pressure (Kyriazakis & Emmans, 1995; Kunzek *et al*., 1999). Technically, WRC portrays the measure of water that can be held or taken up by a known volume of the fibre under known conditions (Guillon & Champ, 2000; Elleuch *et al*., 2011). It is often used with water-binding capacity (WBC), which describes the feed's capacity to bind water when exposed to external stresses (Kunzek *et al*., 1999). The WRC and WBC terms are used differently in the literature; however, both measure the extent to which the fibre constituent can swell. Fibre polymers bind water at varying strengths and in various quantities. Water in the digesta can be held by dietary particles or remain unbound as either trapped or free water (Chaplin, 2003; Anguita *et al*., 2006). The fibre matrix's WRC along the gut depends on each particular gastrointestinal tract segment (Canibe & Knudsen, 2002). It is significant to collect colon digesta representative samples when evaluating the physicochemical properties of digesta to relate hindgut fermentability strength. To fully establish the bulkiness property of feed containing amarula cake, WRC should be described together with swelling capacity and viscosity (Elhardallou & Walker, 1993; Takahashi *et al*., 2009). The most common methods for measuring WRC include filtration, centrifugation, and the use of dialysis bags (Elhardallou & Walker, 1993; Kyriazakis & Emmans, 1995).

Swelling capacity (SWC) is the volume occupied by a known weight of fibre as it absorbs water within its matrix under specified conditions (Guillon & Champ, 2002; Borchani *et al*., 2011). Swelling and solubility are closely related as it forms the first stage in the solubilization process of fibre polymers (Canibe & Knudsen, 2002). Briefly, incoming water spreads the macromolecules until they are entirely expanded and dispersed after being solubilized. Fibres with high hydration properties tend to ferment to the highest degree (Canibe & Knudsen, 2002). Consequently, feed with a high swelling capacity is expected to occupy more space in the gut, interfering with voluntary feed intake. In the digesta, solid particles by their volume can change the digesta flow (Guillon & Champ, 2002). The increase in the SWC of the digesta could be associated with increased digesta exposure to microflora activity due to the longer transit time of digesta in the hindgut (Knudsen, 2001). The swelling of soluble NSPs increases the substrate's surface area to microflora rendering effective colonization and degradation (Knudsen, 2001). There is no literature on the hydration properties of amarula nut cake. It becomes necessary to investigate the influence of cake inclusion on the digesta physicochemical characteristics of growing pigs. Such information can correlate with nutrient digestion and absorption during gut transit and fermentability strength of pigs fed amarula nut cake.

2.9 Potential of amarula nut cake in boars and sows

Low plane of nutrition hampers the reproductive efficiency of breeding pigs. Louis *et al.* (1994) show that boars fed on a low protein diet (70 g/kg protein level) compared to high protein (160 g/kg protein level) resulted in reduced semen volume per ejaculate, low libido, and prolongs required time to breed a sow. The protein richness (360 – 470 g/kg) of amarula nut cake can be beneficial in breeding boars and sows (Malebana *et al*., 2018; Nkosi *et al*., 2019). Inclusion of amarula nut cake provides supplementary proteins and divalent macro and micro minerals as key drivers for enhancing and sustaining structural soundness in the breeding stock. Adequate quantities of calcium, phosphorus, iron, copper, magnesium, and potassium in the nut cake can be sufficient to improve spermatozoa characteristics in boars, which largely influences the farrowing rate and litter size of sows. Growing boars have a higher energy requirement for maintenance and tissue accretion. High gross energy (28.5 MJ/kg) of amarula nut cake could serve as a dietary energy source attributed to their oil content. Dietary supplementation of antioxidants and synthetic limiting amino acids, chiefly lysine in the diet containing amarula nut cake, can better improve its nutritive value to fulfill amino acid requirements of breeding pigs. Feeding amarula nut cake to slow-growing pigs can better increase boar herd size for breeding and reduce inbreeding, increasing fecundity, litter sizes, sows' longevity, and better conserve their valued genetic resources. Presumably, amarula nut cake could increase the chances of crossbreeding slow-growing pigs with fast-growing imported pig breeds to exploit their superior genetics into the commercial sectors.

2.10 Summary

The generation of amarula by-products into the environment has increased the interest in utilizing amarula nut cake for pig feeding systems to ameliorate competition for ingredients and lessen pollution to the surroundings. The use of amarula nut cake for feeding pigs is a novel alternative protein ingredient for substituting expensive conventional oilcakes. Amarula nut cake contains appreciable nutrient biomass with substantial digestible protein, bulkiness, and minerals. Soluble non-starch polysaccharides in the nut cake improve fibre digestibility and fermentation, hence maintaining efficiency in nutrient digestion and absorption. Exploiting fibrous amarula nut cake nutritional characteristics on pig growth and production performance entails evaluating the response in nutrient digestibility, nitrogen balance, and characterization of digesta physicochemical properties of bulkiness in the colon of growing pigs fed dietary amarula nut cake. Assessment of the response in digestibility, retention, utilization, and excretion of nitrogen could provide the potential amarula nut cake exhibit in assimilating protein for efficient metabolic and physiological functions and in reducing nitrogen loss into the environment. Further evaluation of physicochemical characteristics of colon digesta of pigs fed on the nut cake could ascertain its influence on intestinal gut health. Its nutritional attributes can be explored to improve the breeding of boars and sows, hence conserving the genetic resources of endangered pig population. Thus, addressing nitrogen balance, fibre digestibility, and digesta physicochemical properties of growing pigs' response to amarula nut cake inclusion can extrapolate an optimum level of efficient protein accretion and compact nitrogenous gas emissions and further alleviate ammonia volatilization.

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CHAPTER THREE: Response in nitrogen balance of slow-growing Windsnyer pigs fed on incremental levels of amarula (*Sclerocarya birrea* subsp. *Caffra*) nut cake

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Abstract

Feeding derived amarula cake to growing pigs can overcome a narrow range of ingredients challenges and improve productivity. The objective of the current study was to determine the response in nitrogen (N) balance in slow-growing pigs fed on incremental levels of amarula nut cake (ANC). Thirty clinically healthy male growing Windsnyer (30.7 kg ± 6.57) (mean ± standard deviation) were individually assigned to separate pens in a completely randomized design, with six pigs per dietary treatment. Iso-energetic experimental diets were formulated to contain 0, 50, 100, 150, and 200 g/kg dry matter (DM) of ANC using the summit and dilution technique. Pigs were given 10 days of dietary adaptation and a collection period of 5 consecutive days after 31 days of feeding. Nitrogen intake increased linearly with incremental levels of ANC ($P < 0.01$). As ANC inclusion increased, the nitrogen (N) absorption, apparent N digestibility and N retention in pigs increased until it reached a maximum, then started to decrease ($P < 0.05$). Nitrogen utilization increased at the rate of 0.63 g for each 1 g increase in ANC ($P < 0.01$). There was a linear decrease ($P < 0.01$) in total nitrogen excretion through urine and faeces with ANC inclusion. Urinary pH levels decreased quadratically in response to graded levels of ANC ($P < 0.01$). The relationship between urinary pH and ANC inclusion was $Y = 0.0115x^2 - 0.3491x + 4.872$ ($P < 0.01$). The nitrogen balance responses were due to ANC inclusion in diets that were balanced for limiting amino acids. It can be concluded that ANC reduces N excretion, potentially minimizing ammonia volatilization, which makes it an alternative protein source for slow-growing pigs.

Keywords: ammonia volatilization, nitrogen absorption, nitrogen intake, nitrogen utilisation, urinary pH level

3.1 Introduction

The unprecedented disease pandemics coupled with changes in climate and the ever-growing human population have exacerbated food insecurity in the world. The susceptibility of staple grains to unreliable weather conditions intensifies competition between humans and livestock for maize and soya beans (Daryanto *et al*., 2016). Changes in the production of staple crops reduce the contribution of livestock in alleviating food insecurity and poverty, particularly in the developing world (Meissner *et al*., 2013). The biofuel and food processing industries have urged the use of by-products as potential livestock feed ingredients, such as amarula nut cake (ANC).

Amarula (*Sclerocarya birrea* subsp. *Caffra*) nut cake is an agro-industrial by-product of oil extraction from amarula fruits (Mdziniso *et al*., 2016). Amarula is prevalent and commercialized in most countries of Southern Africa (Mokgolodi *et al*., 2011), with major cooperatives processing approximately 2 000 tonnes of fruits in the production of value-added commodities (Wynberg *et al*., 2002). Pig production systems could benefit from the utilisation of the cake, which is typically discarded into the environment. Despite the protein richness feature comparable to the commercial soybean oilcake, the presence of antinutrients, fibre and fat content may interfere with protein metabolism vital for pig growth, particularly for fast-growing pigs (Muhammad *et al*., 2011). Utilising dietary fibre in pigs improves the fermentability of fibre, which reduces ammonia emission and further ameliorates greenhouse gas emissions (Mpendulo *et al*., 2018). Appropriate pig strains to use are the slow-growing pigs that can efficiently utilise fibrous diets, for example, the South African Windsnyer pigs.

Slow-growing pigs are sidelined due to biasness in the carcass grading systems (Halimani *et al*., 2010; 2012), despite their high efficiency in utilising agricultural by-products (Kanengoni *et al*., 2015; Ncobela *et al*., 2018; Mkwanazi *et al*., 2019). The use of these slow-growing pigs increases efficiency of farming systems and sustainability of smallholder pig production. Amarula nut cake has the potential to best suit Windsnyer pigs given the postulations to adapt to low planes of nutrition than fast-growing pigs. Its nutritive value in slow-growing pigs can enhance their productivity. The cake has potential beneficial effects of reducing protein fermentation, reducing nitrogen excretion, and repartitioning the nitrogen pathway imposing intense volatilization of ammonia (Jha & Berrocoso, 2016; Mpendulo *et al*., 2018), thus the optimum inclusion of ANC could reduce environmental pollution and, at the same time, enhance performance of slow-growing pigs.

Assessing nitrogen balance has been used for the characterization of nutritional aspects, requirements, and limitations of protein (Canh *et al*., 1997; Hansen *et al*., 2007; Hlatini *et al*., 2017). The physicochemical characteristics of fibrous ANC can influence voluntary feed intake, gut capacity, and protein absorption and utilisation efficiency. Hereafter the extent of fibre digestibility through physicochemical properties of digesta in growing pigs have been outlined in Chapter four. The availability of such knowledge expands the pool of protein ingredients for pig nutritionists. Utilisation of ANC in local pig production could further incentivize and strengthen livelihoods, reduce environmental pollution and greenhouse gas emission. The objective of the study was to determine the response in nitrogen balance of Windsnyer pigs fed on ANC. It was hypothesized that ANC inclusion reduces N excretion from slow-growing pigs.

3.2 Materials and methods

3.2.1 Study site

The study was conducted at the Agricultural Research Council (ARC), Animal Production (AP), (ARC-AP: Irene, Pretoria) situated in the Highveld region of South Africa. The ARC-AP campus is located at 25°55′ south; 28°12′ East, and at an altitude of 1525 m above sea level.

3.2.2 Pigs, experimental design and their management

The study was approved by the Agricultural Research Council Animal Ethics committee (Reference number: APAEC 2019/17). Thirty clinically healthy male growing Windsnyer pigs aged three months with an average initial body weight of 30.7 ± 6.57 kg (mean ± standard deviation) were randomly selected from Agricultural Research Council (ARC), Animal Production (AP) – Irene pig breeding unit. The trial housing facility was thoroughly cleaned and disinfected a week before the trial commenced. Pigs were housed individually in 1.5 x 0.9 m pens in an environmentally controlled house with a temperature ranging between 22 to 25 °C. The pigs were allocated to five experimental diets in a completely randomized design, with six pigs per dietary treatment.

An adaptation to the experimental condition of 10 days was given, and the experiment took 63 days. The feeders were monitored and adjusted twice daily to maintain constant access to fresh feed and minimize any possible wastage and water was freely available through nipple drinkers.

3.2.3 Experimental diets

Batches of ANC were collected in Phalaborwa, Limpopo Province, South Africa. The cake was brought to ARC-AP for diet formulation. Five diets were formulated to contain different levels of defatted ANC: 0 (control), 50, 100, 150, and 200 g/kg DM inclusion of ANC. The diets provided 14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg, and 11.6 g lysine/kg, which meet and exceed the requirements of growing pigs (NRC, 1998). A total mixed ration was formulated without ANC meal and used as the basal or summit diet, and bulky ration with 200 g ANC /kg was formulated and used as the diluent diet (Table 3.1). Five dietary treatments were formulated by diluting a concentrated summit diet (0 g/kg DM of ANC) with a dilution diet (200 g/kg DM of ANC) as described by Gous and Morris (1985). The proportions of the summit to diluent diet were 100, 75, 50, 25 and 0. Table 3.1 shows the ingredient composition of the summit and dilution diets. The sunflower oil cake was used as the source of fibre in the basal diet. The diets were balanced for lysine, methionine and cysteine limiting in ANC such that the differences in nitrogen responses in pigs are due to the inclusion of the nut cake (Malebana *et al*., 2018). Each pig received an allocation of feed twice daily, provided with 1-2 kg in the morning and 10 % adjustments in the afternoon based on their ability to finish.

Table 3.1 Ingredient composition (g/kg DM) of the summit and dilution diets

Ingredient	Control	200 g/kg DM ANC
Maize	545.1	609.8
Sunflower oil cake	150	-
Wheat bran	150	147.9
Sunflower oil	26.4	-
Monocalcium phosphate	8.9	8.3
Feed lime	12.4	19.7
Lysine	17.0	12.3
Amarula nut cake	-	200
Soybean oil cake	88.2	-
Pig supplement	2	2

[#]Mineral and vitamin premix contributed to the diets supplemented (/kg DM of diet) : vitamin A Acetate, 2 000 000 IU; vitamin D_3, 400 000 IU; vitamin E, 1 000 mg; vitamin B_1, 200 mg; vitamin B_{12}, 0.5 mg; vitamin C, 1 000 mg; vitamin B_2, 250 mg; vitamin B_6, 200 mg; vitamin K_3, 200 mg; calcium pantothenate, 500 mg; nicotinic acid, 1 000 mg; folic acid, 50 mg; di-methionine, 2 000 mg; cystine, 300 mg; lysine, 3 000 mg; arginine, 2 000 mg; tryptophan, 1 000 mg. ANC – amarula nut cake.

3.2.4 Physicochemical analyses of the experimental diets and amarula nut cake

The physicochemical composition of the ANC samples and experimental diets were analysed in triplicate at the University of KwaZulu-Natal, Animal and Poultry Science Laboratory, Pietermaritzburg, South Africa. Dry samples were ground through a 1 mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA, USA) for chemical analyses. Dry matter (DM) content was determined by oven drying the samples at 60 ^{0}C for 24 hours. The ash content was determined after incineration of the sample at 550 °C for 4 hours according to method 990.05 (AOAC, 1990). Crude protein (CP) content was calculated using the formula: N × 6.25, nitrogen content was determined following the Dumas Combustion method in a Leco Truspec Nitrogen Analyser, St. Joseph, MI, USA by method 990.3 (AOAC, 1990). Ether extract (EE) was determined using the Soxhlet apparatus according to method 920.39 (AOAC 1990). The gross energy (GE) was determined with bomb calorimetry (MS-1000 modular calorimeter, Energy Instrumentation, Centurion, South Africa).

The neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined following the procedures of Van Soest *et al*. (1991) using ANKOM Fibre Analyser (Ankom, Macedon, NY, USA). Separate samples were used for ADF and amylase-treated neutral detergent fibre (aNDF) analyses and both included residual ash. The swelling capacity of the experimental diets was measured according to Canibe & Bach Knudsen (2002). Water holding capacity of the experimental diets were measured according to Whittemore *et al*. (2003). Amino acids (AAs) were analysed using acid hydrolysis following method 982.30 of AOAC (1990). Table 3.2 and 3.3 shows the physicochemical analyses and amino acid composition of amarula nut cake and experimental diets, respectively.

Table 3.2 Proximate composition and physicochemical properties of amarula nut cake (ANC) and experimental diets

		ANC inclusion level (g/kg DM)				
Component	ANC	0	50	100	150	200
Chemical composition						
Dry matter	963.5	956.8	958.9	954.4	953.8	956.3
Ash	49.8	51.2	50.6	46.9	47.2	47.0
Crude protein	362.2	194.9	176.8	162.6	157.0	140.3
Ether extracts	343.9	44.4	56.0	72.4	78.1	96.8
Gross energy (MJ/kg DM)	25.34	17.65	17.67	17.77	18.09	18.50
Bulkiness properties						
NDF	357.3	305.9	312.3	327.5	341.3	353.0
ADF	245.6	85.8	93.4	99.5	111.2	121.1
ADL	114.3	19.4	25.8	34.7	42.9	48.6
Bulk density (g/ml)	1.66	1.45	1.49	1.55	1.64	1.71
Swelling capacity (ml/g)	3.75	2.89	3.04	3.21	3.39	3.63
WHC (g_{water}/g_{feed} DM)	4.89	3.36	3.63	3.80	4.19	4.43
Calculated nutrients						
Dry matter	-	860.4	-	-	-	896.6
Crude protein	-	180.0	-	-	-	180.0
Ether extracts	-	63.2	-	-	-	87.6
Crude fibre	-	36.8	-	-	-	62.5
DE (MJ/kg DM)	-	13.80	-	-	-	13.80
Calcium	-	8.00	-	-	-	10.0
Phosphorus	-	6.00	-	-	-	6.00
M+C (%)	-	0.62	-	-	-	0.56
Methionine (%)	-	0.30	-	-	-	0.27
Lysine (%)	-	2.0	-	-	-	1.36

ADF – acid detergent fibre (g/kg DM), ADL – acid detergent lignin (g/kg DM); NDF – neutral detergent fibre (g/kg DM); WHC – water holding capacity (g_{water}/g_{feed} DM); M+C – methionine + cysteine.

Table 3.3 Amino acid composition of amarula nut cake (ANC) and experimental diets

		ANC inclusion level (g/kg DM)				
Component (g/100g DM)	ANC	0	50	100	150	200
Essential amino acids						
Histidine	0.61	0.49	0.52	0.50	0.44	0.34
Isoleucine	1.29	0.64	0.62	0.47	0.53	0.51
Leucine	1.23	1.23	1.24	1.20	1.16	1.07
Lysine	0.68	2.23	2.06	1.86	1.51	1.78
Methionine	0.44	0.25	0.26	0.25	0.25	0.22
Phenylalanine	1.28	0.70	0.71	0.68	0.61	0.60
Threonine	0.81	0.62	0.56	0.53	0.49	0.43
Tryptophan	0.44	0.23	0.22	0.22	0.85	0.49
Valine	1.56	0.79	0.74	0.74	0.67	0.65
Non-essential amino acids						
Arginine	5.51	1.34	1.34	1.40	1.37	1.43
Glycine	1.80	0.86	0.77	0.77	0.71	0.67
Ho-Proline	0.09	0.05	0.06	0.07	0.04	0.04
Proline	1.13	0.94	0.90	0.88	0.88	0.83
Tyrosine	0.69	0.50	0.62	0.67	0.51	0.44
Alanine	1.04	0.75	0.73	0.71	0.68	0.63
Aspartic acid	2.74	1.43	1.29	1.19	1.08	0.98
Glutamic acid	8.40	3.04	2.92	2.95	2.93	2.80
Serine	1.65	0.83	0.77	0.75	0.70	0.66

3.2.5 Measurements

3.2.5.1 Pig performance

Pigs were individually weighed at the start of the trial and every week until the end of the trial to determine average daily gain (ADG). The pigs were fed the experimental diets *ad-libitum* in the morning, allowing a 10 % of feed refusal, and free access to water was allowed. Daily feed offered and weekly refusals were recorded. Weights of feed refusals were subtracted from the total weight of the feed allocated to determine feed intake for that week. Weight of the feed consumed each week was divided by seven to determine the average daily feed intake (ADFI). Gain: feed ratio was calculated by dividing ADG/ADFI.

3.2.5.2 Collection and storage of faeces and urine

The total collection of faeces and urine for nitrogen balance was performed after day 31 of feeding for five consecutive days (Hlatini *et al*., 2017). The collection took place every morning at 08h00 to allow 24 hours period from the point of time to the following sample collection. For faecal sample collection, the hand-picking method was practiced for collecting all faecal material from the pen, and a 1 mm sieve was suspended below each pen to capture faeces that fallen out of pen (Ouellet *et al*., 2004). The total daily faecal material collected for each pig was weighed as the combination of faeces on the pen and sieve underneath the pen (record weight as daily faecal output) and placed in plastic bags and frozen at -20 ºC. At the end of the collection period, faecal samples of each pig were mixed thoroughly, weighed, sub-sampled into 250 g, and dried at 60 ºC (fixed at 1% formalin before dried for conservation) pending analyses.

Urine was collected in plastic trays which were placed underneath each pen for a total collection of urine. The pens were suspended with 1mm sieve to improve the flow of urine free from faecal contamination. Each tray containing urine was treated with 2 ml of sulphuric acid (25% H_2SO_4) to lower the pH level post-collection to prevent the volatilisation of nitrogenous compounds (Hlatini *et al*., 2017) and microbial growth. The urine was filtered through a filter mesh fabric to remove any contaminants. The total urine for each pig was weighed, volume recorded as daily urine output, and placed in a clean plastic container. After weighing total urine, 250 ml aliquots were sampled and frozen at -20 ºC pending analyses. The urinary pH level was determined by inserting Crison 52 02 glass pH electrodes.

3.2.5.3 Determination of nitrogen levels in faeces and urine

The DM content of faeces was determined by oven drying 2 g sample from the collected faeces at 103 ºC for 24 hours. Then each dried sample was re-weighed, and the difference recorded as the DM content of the faeces. The concentration of faecal nitrogen (N) and urinary N was determined using the Dumas Combustion method in a Leco Truspec Nitrogen Analyser following method 990.3 of AOAC (1990). Briefly, 0.2 g of the faecal sample was weighed into the crucible, mass recorded and then 1 g of Leco COM-CAT was added to improve the burning profile. The crucibles were then transferred into the appropriate positions of the autoloader for determination of N percentage in the faecal samples. For urine N concentration, 1 g of Leco Com-Aid for liquids was weighed into the crucibles covered with Leco foils, then 0.2 g of urine weighed and mass recorded. Afterward, 1 g of Leco COM-CAT was added, and the crucibles transferred into the appropriate positions of the autoloader for determination of N percentage in the urine samples.

3.2.5.4 Calculations for nitrogen balance

The pre-requisite for estimation of nitrogen balance includes nitrogen intake (NI), faecal nitrogen output (FNO), urinary nitrogen output (UNO), total nitrogen excretion (TNE), absorbed nitrogen (AN), nitrogen retention (NR) and nitrogen utilisation (NU) as described by Otto *et al*. (2003). Daily NI was estimated as the product of feed nitrogen percentage and daily feed intake, determined on a dry matter basis. The FNO and UNO used a similar technique by multiplying the nitrogen concentration in the faeces and urine with daily faecal output and daily urinary output, respectively. The summation of FNO and UNO estimated TNE. The difference between NI and FNO determined AN. The difference between NI and TNE then estimates NR, expressed as a proportion of NI retained to determine NU further. Table 3.4 shows the equations used to determine nitrogen balance parameters.

Table 3.4 Equations for estimating nitrogen balance components

Component	Formula	Units
Nitrogen Intake (NI)	(N feed/100) x daily feed intake	g/day
Faecal Nitrogen Output (FNO)	(N faeces/100) x daily faecal output	g/day
Urinary Nitrogen Output (UNO)	(N urine/100) x daily urine output	g/day
Total Nitrogen Excretion (TNE)	FNO + UNO	g/day
Nitrogen Retention (NR)	NI – TNE	g/day
Absorbed Nitrogen (AN)	NI – FNO	g/day
Apparent Nitrogen Digestibility (ND)	(AN/ NI) x 100	%
Nitrogen Utilization (NU)	NR/ NI x 100	%
The biological value of feed protein (BVFP)	NR/ND x 100	%

Sources: Otto *et al.* (2003); Patráš *et al*. (2009)

3.2.6 Statistical analyses

The general linear model (PROC GLM) procedure of Statistical Analysis System Institute (SAS, 2009) version 9.4 was used for the separation and comparison of least squares means (LSMEANS). A polynomial regression (PROC REG) procedure (SAS, 2009) was used to determine the relationship between ANC inclusion levels and ADFI, ADG, Gain: Feed ratio and nitrogen balance. The regression model was:

$$Y = \beta_0 + \beta_1 D + \beta_2 D^2 + \varepsilon$$

Where: Y is the response variables (ADFI, ADG, Gain: Feed ratio, Dry matter intake, N intake, N absorption, N digestibility, N retention, N utilisation, urinary N, faecal N, urinary N: faecal N ratio and urinary pH level)

β_0 is the intercept

$\beta_1 D$ is the linear component

$\beta_2 D^2$ is the quadratic component

D is the inclusion level of amarula nut cake (ANC)

ε is the residual error.

3.3 Results

3.3.1 Influence of amarula nut cake-based diets on growth performance

The effect of feeding incremental levels of ANC on average daily feed intake (ADFI), average daily gain (ADG), and gain: feed (G: F) ratio is shown in Table 3.5. The ADFI had an increasing quadratic response to incremental levels of ANC ($P < 0.05$). As ANC inclusion level increased, the ADFI increased until it reached 108 g/kg ANC level, and then it decreased afterwards. A positive linear relationship between the inclusion level of ANC and ADG was observed ($P < 0.05$). Addition of ANC also caused a linear increase in G: F ratio ($P < 0.05$).

3.3.2 Relationship between amarula nut cake inclusion and nitrogen balance

Table 3.5 shows the influence of increasing levels of ANC in nitrogen balance. There was an increasing quadratic relationship between dry matter intake (DMI) and ANC inclusion levels ($P < 0.05$). As the levels of ANC increased, the DMI initially increased until it peaked at 106 g/kg inclusion level, and then started to decrease. A positive linear relationship between nitrogen intake (NI) and ANC inclusion was observed ($P < 0.05$). Urinary nitrogen output (UNO) decreased linearly with ANC inclusion ($P < 0.05$). Similarly, faecal nitrogen output (FNO) had a decreasing linear response to ANC inclusion ($P < 0.01$). There was a negative linear relationship between total nitrogen excretion (TNE) and ANC inclusion ($P < 0.01$; Table 3.5).

Although ANC incremental levels had a negative linear relationship with nitrogen excretion through urine and faeces, there was no relationship between ANC inclusion and urinary N: faecal N (UN: FN) ratio ($P > 0.05$). Inclusion of ANC exhibited a quadratic increase in nitrogen absorption (NA) (g) ($P < 0.05$). As ANC inclusion levels increased, NA increased up to 81 g/kg inclusion, and then it decreased onwards. The net protein utilisation (NPU), however, had an increasing linear response to ANC inclusion ($P < 0.01$). There was also a positive linear relationship between the biological value of feed protein (BVFP) and graded levels of ANC ($P < 0.01$).

The relationship between nitrogen retention (NR), apparent nitrogen digestibility (ND), nitrogen utilisation (NU), urinary pH level, and ANC inclusion is shown in Figures 3.1, 3.2, 3.3 and 3.4. Nitrogen retention (NR) (g) increased quadratically with ANC inclusion levels ($P < 0.05$). As the inclusion levels of ANC increased, the retained N initially increased, peaks at 24.4 g/day with ANC inclusion of 94 g/kg, and then started to decrease (Figure 3.1). Similar increasing quadratic response in apparent nitrogen digestibility (ND) (%) with graded levels of ANC was observed ($P < 0.01$). As ANC level increased, apparent (ND) (%) increased until it reached 128 g/kg inclusion and then started to decrease (Figure 3.2). Nitrogen utilisation (NU) (%) increased linearly with ANC inclusion levels (Figure 3.3; $P < 0.01$). A decreasing quadratic relationship between the addition of ANC and urinary pH levels was observed (Figure 3.4; $P < 0.01$). Urinary pH level decreased initially until it reached a minimum at 152 g/kg of ANC inclusion, and after that, it slightly increased.

Table 3.5 The influence of feeding amarula nut cake (ANC) inclusion on growth performance and nitrogen balance of slow-growing Windsnyer pigs

Item	Inclusion level of ANC (g/kg)					SEM	P-value	Regression coefficient	
	0	**50**	**100**	**150**	**200**			**Linear**	**Quadratic**
ADFI (kg/day)	1.04	1.23	1.37	1.18	1.14	0.122	0.3456	NS	-0.002*
ADG (kg/day)	0.22	0.27	0.31	0.34	0.31	0.037	0.2975	0.013*	NS
Gain: Feed ratio	0.21	0.22	0.23	0.29	0.27	0.027	0.2571	0.002*	NS
DM intake (g/day)	961.6	1144.6	1271.2	1085.1	1057.5	113.52	0.3306	NS	-2.31*
N intake (g/day)	32.3	34.8	35.5	29.7	25.6	3.22	0.1638	0.85*	NS
Urinary N (g/day)	5.8	5.0	4.5	3.8	2.3	1.14	0.2597	-0.08*	NS
Faecal N (g/day)	7.4[a]	6.1[b]	5.6[bc]	5.1[cd]	4.5[d]	0.23	<.0001	-0.22***	NS
Total N excretion (g/day)	13.3[a]	11.0[ab]	10.1[ab]	8.9[ab]	6.8[b]	1.21	0.0233	-0.30***	NS
Urinary N: Faecal N	0.78	0.82	0.80	0.74	0.51	0.20	0.7650	NS	NS
N absorption (g/day)	24.8	28.7	29.9	24.5	21.1	3.11	0.2240	NS	-0.06*
NPU (%)	58.9[b]	67.6[ab]	71.0[ab]	69.8[ab]	73.7[a]	3.17	0.0484	1.54**	NS
BVFP (%)	76.6	82.7	84.7	84.3	89.9	3.59	0.1723	0.83**	NS

[ab]Values with different superscripts within a row are different; SEM: standard error of mean; *$*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS: not significant ($P > 0.05$);* ADFI: average daily feed intake; ADG, average daily gain; DM: dry matter; N: nitrogen; NPU; net protein utilisation; BVFP: biological value of feed protein.

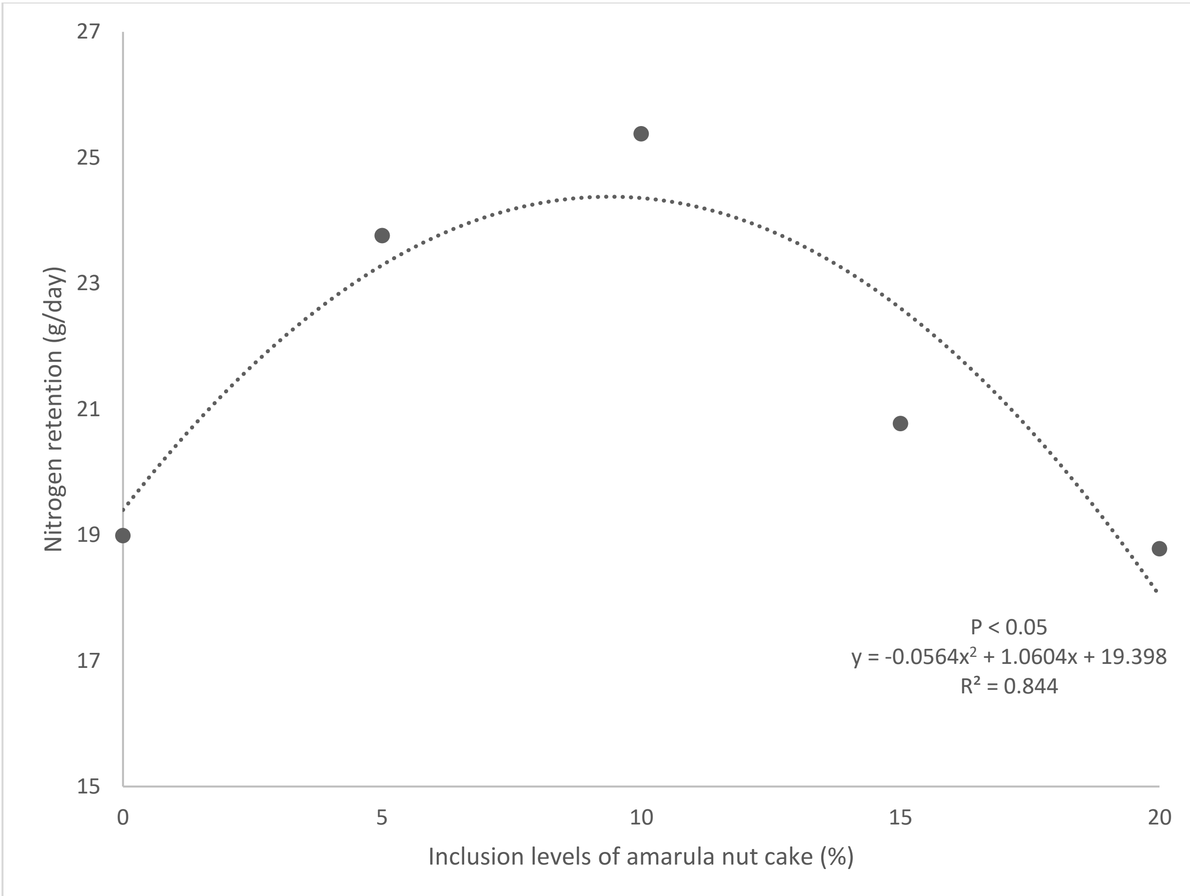

Figure 3. 1 Relationship between nitrogen retention and amarula nut cake inclusion levels

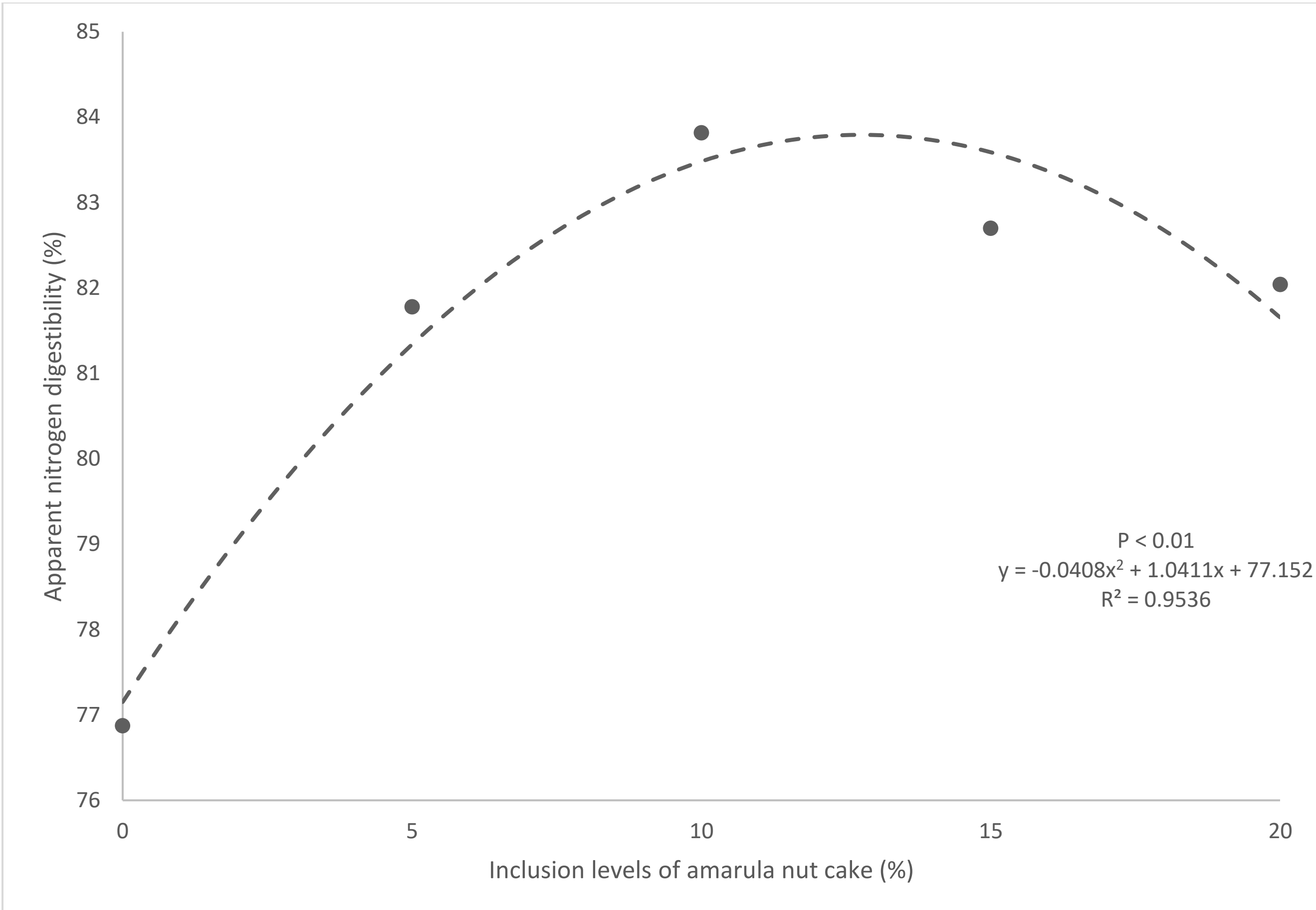

Figure 3. 2 Relationship between apparent nitrogen digestibility and amarula nut cake inclusion levels

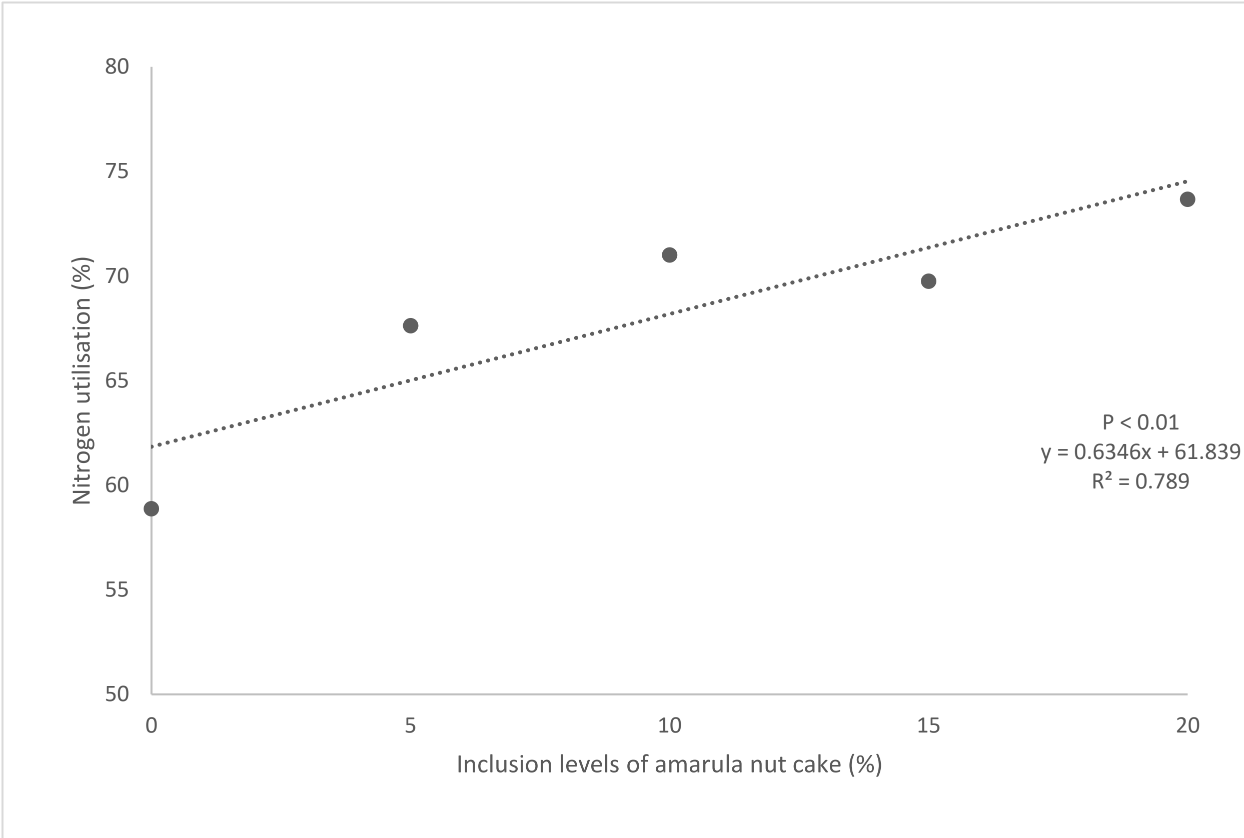

Figure 3. 3 Relationship between nitrogen utilisation and amarula nut cake inclusion levels

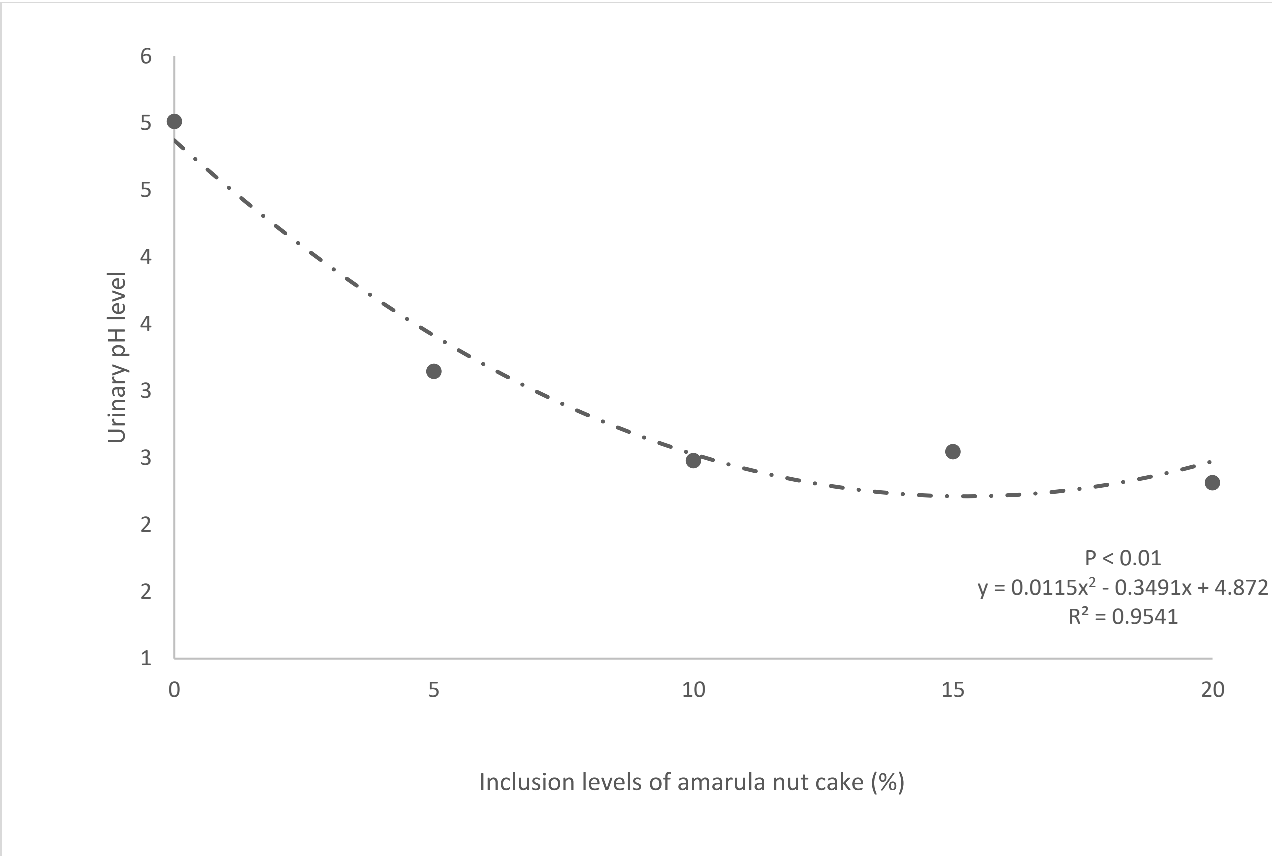

Figure 3. 4 Relationship between urinary pH level and amarula nut cake inclusion levels

3.4 Discussion

To understand the nutrient requirements of slow-growing pigs, a satellite population has been established in research stations around South Africa. Amarula nut cakes are produced in bulk, and their disposal pollutes the environment. The current study was, therefore, designed to assess whether slow-growing Windsnyer pigs can thrive on fibrous and fat-rich ANC. Most oilseed by-products such as baobab and macadamia nut cakes are fibrous- and fat-rich with low-protein content, which is inadequate for sustainable commercial pig production of fast-growing pigs (Boateng *et al*., 2017; Nkosi *et al*., 2019). The use of ANC for feeding pigs has not been explored, however, there is enough evidence that proves that the nut cake has sufficient nutrients that can meet nutrient requirements of pigs (Mdziniso *et al*., 2016). High fibre and fat contents may hinder the utilisation of ANC in fast-growing pigs (Kanengoni *et al*., 2002; Chimonyo *et al*., 2010). However, the use of ANC in Windsnyer pigs could better exploit the dietary protein potential of ANC, overcome ingredient competition with humans, improve productivity and securing Windsnyer pig populations. It is imperative to assess the nitrogen metabolism in slow-growing pigs fed on increasing levels of ANC-based diets and further contemplate its acceptability.

The quadratic increase in ADFI with increasing levels of ANC could be associated with the NDF and ADF proportions of the diets. Mlambo *et al.* (2011) reported that the inclusion of ANC in diets increased the NDF and ADF proportions, which contribute to the bulkiness of the feed. Feed bulk has a significant effect on feed intake of pigs as the physicochemical characteristics of feed bulkiness increase, feed intake of pigs also increases to a certain point until satiety or requirements are met (Lindberg, 2014). It can be assumed that the decrease in feed intake at higher levels of the nut cake beyond 108 g/kg was due to the physiological restriction of the gut capacity of pigs which is correlated to the compact body size of Windsnyer pigs (Ncobela *et al*., 2018). A decrease in ADFI could also be ascribed to EE content with graded levels of ANC (Mogonka *et al*., 2018). Mthiyane and Mhlanga (2017) reported depressed feed intake in broilers offered an ANC-based diet subjected to extensive lipid peroxidation.

The increasing linear response in ADG and Gain: Feed ratio to ANC inclusion could be associated with decreasing ADFI as the cake inclusion increased in the current study. The positive effects on growth suggest that ANC-based diets contain sufficient nutrients that are efficiently utilized by slow-growing pigs. Slow-growing pig genotypes display exceptional dietary preference and selection characteristics when utilising fibrous diets (Kanengoni *et al*., 2015). The observed decrease in ADFI and DM intake at higher inclusion levels of ANC could suggest the potential of these slow-growing pigs in assimilating and converting nutrients into muscle for growth. It also implies that antinutrients that could be present in the cake did not exceed the standard toxic level considered to interfere with protein metabolism vital for pigs to attain their growth potential (Muhammad *et al*., 2011). Mthiyane and Mhlanga (2018) reported depressed growth performance in broilers fed on ANC despite exogenous supplementation of DL-methionine and phytase to encounter deleterious effects of hydrocyanic acid and phytate, respectively.

The observed linear increase in N intake to ANC inclusion levels suggests that the nut cake comprises of larger proportion of nitrogen unbound to fibrous fraction. However, addition of ANC in diets decreased CP concentration due to high fibre and EE content which act as nutrient diluents. The decrease in CP level per unit increase in ANC could also be ascribed to the diet dilution technique as exact proportions of ingredients in diets formulated by the admixture of summit and diluent diets are unknown. The diet dilution method was developed to measure response to increasing intakes and requirements of amino acids (Gous & Morris, 1985). Incorporation of ANC in diets indirectly comply with feeding low crude protein diets as a common practice in reducing N excretion (Galassi *et al*., 2010). McDonnell *et al*. (2011) reported a decreased N intake in growing pigs fed on rapeseed meal. The difference could be due to variability in fibrous components between ingredient sources and possibly reduced N intake could be caused by greater proportion of N enclosed in the fibre (Bindelle *et al*., 2005; Liu *et al*., 2019). The fibre fraction could increase N intake as ANC increased. Patráš *et al*. (2012) reported an increased N intake in pigs fed fibrous diets. The rise in N intake could also be an indication of low concentration of polyphenolic compounds bound to dietary proteins (Muhammad *et al*., 2011).

The observed reduction in N excretion via urine and faeces could be caused by decreased dietary protein levels with incremental levels of ANC (Galassi *et al*., 2010). More N was excreted through faeces than via urine, with an average contribution of 58 % to the total N excretion amongst inclusion levels of ANC. Consequently, urinary N excretion was the major contributor to the marked reduction in total N excretion. Otto *et al.* (2003) reported a decrease in total N excretion as more N was excreted via faeces than urine in pigs fed on low protein diets, which corroborates with the empirical findings. It could also suggest that ANC contains fermentable non-starch polysaccharides (NSPs) fraction reducing N entrapped to fibre (Regnier *et al*., 2013). The undigested fibre is used by intestinal microbiota as an energy source and exploits N from urea and other N bearing sources, thus, less N excreted through urine (Patráš *et al*., 2012). The low protein requirements for slow-growing pigs decreases N loss through urea form in urine (Acciaioli *et al.*, 2011; Kanengoni *et al.*, 2015). Excess protein supply increases N excretion into the environment.

The linear decrease in total N excretion in pigs fed ANC-based diets was expected. It could imply that ANC contains a significant digestible protein fraction, such that the proportion of undigested dietary protein together with endogenous protein binds to form a bacterial protein (Bindelle *et al.*, 2008). High N excretion through faeces indicates that more N is used for microbial protein synthesis, thereby increasing microbial biomass in the hindgut. Excretion of organic N through faeces is less volatile and favourable because of slower degradation than urinary N (Patráš *et al.*, 2012; Jha and Berrocoso, 2016). It is, however, difficult to explain the lack of response of urinary N: faecal N ratio to ANC inclusion as more N was voided through faeces. The inconsistent response of urinary N: faecal N ratio could be attributed to the observed lower values than those reported in literature (Canh *et al.*, 1997; Shirali *et al.*, 2012).

The decreasing quadratic response of urinary pH level to ANC inclusion suggests that increasing ANC decreases urinary pH level which is corroborated by the reduced urinary N output. The degree at which ammonia volatilizes and the extent of odour offensiveness in pig barns depends on urea concentration and urinary pH level. Any change in dietary cation-anion balance affects the acid-base balance of blood pH, considered crucial for normal body function and renal regulation of fluid and electrolyte balance. Non-starch polysaccharide (NSP) content and dietary electrolyte balance (dEB) greatly influences slurry and urinary pH level (Canh *et al.*, 1997). A lower urinary pH level in pigs fed on sugar-beet pulp rich in NSP content and lower dEB level compared to diets containing a higher dEB level was reported (Canh *et al*., 1997). Feeding diets containing ANC could have altered electrolyte balance, increasing the acidity status of urine. The reduction in urine and slurry pH levels is a characteristic of lower ammonia emissions (Portejoiei *et al*., 2004).

Highly fermentable NSP content is expected to change N repartitioning pattern. The altered N pathway to less volatile organic N in faeces could be attributed to the lower urinary pH levels, thus minimizing the volatilisation of ammonia and odour emissions causing air pollution (Patráš *et al*., 2009; Jha and Berrocoso, 2016). It can also be assumed that the urinary pH level could have also been affected by water intake since it was provided *ad libitum* and high protein diets increase water intake (Mroz *et al*., 1995). Jongbloed *et al*. (1997) reported that reducing dietary protein, fat, sodium chloride, or increasing diet bulkiness may partially limit *ad libitum* water consumption. In the prior study, dietary crude protein reduced by 3 % (from 158 to 128 g/kg) did not affect water intake, while urinary total N and blood urea concentration decreased (Jongbloed *et al*., 1997). Mroz *et al*. (1995) showed that water deprivation increased plasma osmolality, which triggers antidiuretic hormone production to retain more water within blood vessels and causes concentrated urine predisposing pigs to renal and respiratory diseases. The observed quadratic response in urinary pH level could also indicate that ANC inclusion did not affect water intake in pigs and the herd remained healthy throughout the trial.

The increase in N absorption as ANC inclusion increased could be explained by the dietary protein content, which was sufficient as N intake increased. Its an indication that the AA composition was proper for protein deposition. Malebana *et al*. (2018) reported the lysine, methionine and cysteine concentrations as the most limiting AAs in the nut cake, which were supplemented in the current study to balance AAs and closely meet protein requirements of growing pigs (NRC, 2012). The differences in AAs of diets varying in inclusion levels of ANC regardless of synthetic AAs supplementation could have been influenced by the diet formulation technique. The study, however, focused on the effect of nut cake inclusion rather than differences in the CP content of diets. Beyond 81 g/kg inclusion level of ANC, the decrease in absorption of consumed N by slow-growing pigs is in line with the findings of (Otto *et al*., 2003). Otto *et al*. (2003) reported a decrease in N absorbed by pigs fed on reduced protein concentration from 150 to 90 g/kg DM. Lowering dietary protein content may interfere with intestinal protein synthesis in the gut cells rendered by limited bioavailability of peptides (Otto *et al*., 2003). As a result, a reduction in protein concentration in pig diets below a certain threshold considered to impair efficiency in gut functioning and absorptive capacity negatively affects N absorption.

The quadratic increase in apparent N digestibility to ANC inclusion suggests the availability of digestible protein biomass that is efficiently digested by pigs (Mlambo *et al*., 2011). Previous literature reported that CP could be reduced by between 3 and 4 % without compromising nutrient digestibility (Jongbloed *et al*., 1997; Jah *et al*., 2013) in agreement with the initial increase in N digestibility. Depressed N digestibility beyond the breakpoint of ANC at 128 g/kg inclusion level could adhere to the protein concentration reduction exceeding 4 %. Furthermore, the presence of anti-nutritional components could be the reason for a decrease in N digestibility at higher inclusion levels of ANC (McDonnell *et al*., 2011). Antongiovanni *et al.* (2007) reported that tannin concentration surpassing the critical level (2.5 g/kg DM of feed) interferes with protein availability and impair digestibility. Reduced N digestibility could also be attributed to NDF and ADF components as levels of ANC increased. Mlambo *et al*. (2011) reported that the fibre content of ANC is characterized by the hard shells' remnants, which encompasses the kernels that are compressed during the decortication process. In the current study, at inclusion levels of 150 and 200 g/kg of ANC pigs tended to select the diet leaving only the remnants of the kernels or shell in the feeding trough. The observed behaviour of indigenous pigs could explain the reduction in N digestibility of pigs fed diets that are above 100 g/kg of ANC.

An increasing quadratic response in N retention expressed as a proportion of N intake could be explained by the indispensable AAs composition profile. Shiver *et al*. (2003) reported improved N retention in growing pigs fed a diet containing 4 % reduced CP content with the addition of lysine, threonine and tryptophan. The improved N retention in pigs fed on ANC below 150 g/kg suggesting that these levels provide adequate amounts of AAs (Nkosi *et al*., 2019). Another possible explanation of the increase in N absorbed could be attributed to the longer retention time of digesta of indigenous pigs caused by their large gastrointestinal tract segments which partially increase due to growth potential (Ngoc *et al*., 2013). Previous literature proves that reducing dietary protein concentration by at least 2 to 3 % with crystalline AA supplementation does not compromise growth performance of growing pigs (Liu *et al*., 1999). Beyond this level, N retention was significantly reduced (Liu *et al*., 1999), which explains the slight reduction in N retention in pigs fed on 150 and 200 g/kg ANC inclusion levels.

The linear increase in N utilisation as ANC inclusion level increased could be explained by the N intake response in the current study. Improvement in N utilisation is an indication of improved efficiency in protein accretion in slow-growing pigs despite a reduction in protein concentration per unit increase in ANC, which complies with their low dietary protein requirements (Kanengoni *et al*., 2015). This is in consistent with the previous report when maximum protein deposition in Iberian-indigenous growing pigs with a body weight between 50 and 100 kg fed diets containing an ideal CP concentration of 95 g/kg DM (Barea *et al*., 2006). According to Chikagwa-Malunga *et al.* (2009), inefficiency in the utilisation of absorbed N results in elevated urinary N losses and may impair growth performance. The improved N utilisation with ANC inclusion levels indicates a significant proportion of protein ingested and retained for general health, growth, and maintenance of slow-growing pigs. Increasing N utilised explains the observed positive linear response on BVFP, a proportion of absorbed protein, suggesting efficiency in utilising N digested for protein synthesis in gut cells.

3.5 Conclusions

Amarula nut cake inclusion caused a linear response to nitrogen intake, utilisation and excretion through urine and faeces, although quadratic response to absorption, retention, and digestibility of nitrogen and urinary pH level was observed. Dietary inclusion of amarula nut cake was associated with improving nitrogen utilisation and reducing volatile nitrogen without compromising the growth performance of slow-growing pigs, which indicates that amino acid composition was proper to meet growth requirements. The current study showed that the nitrogen balance responses may be associated with pig genotype and that inclusion of the cake with supplementation of the most limiting amino acids caused differences in pig responses. Utilisation of the nut cake can alternatively implicate feeding low crude protein diet to growing pigs as a measure of reducing nitrogen excretion and ammonia emissions. Therefore, the cake inclusion reduced urinary nitrogen excretion mitigating the adverse ammonia emissions into the environment, making it a safe protein ingredient for slow-growing pigs. Physical properties of fibrous and ether extract content of the nut cake need to be considered for diet formulation to encounter insoluble and indigestible fractions on the nutrient density of formulated diets. Future research should focus on minimization for greenhouse gas emissions by feeding amarula nut cake and reassessment using isoprotein diets.

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CHAPTER FOUR: Influence of amarula (*Sclerocarya birrea* subsp. *Caffra*) nut cake inclusion on fibre digestibility and physicochemical characteristics of colon digesta in slow-growing Windsnyer pigs

Abstract

The objective of the study was to assess the relationship between amarula nut cake (ANC) inclusion and fibre digestibility and physicochemical characteristics of colon digesta in Windsnyer pigs. Thirty growing pigs were individually assigned to separate pens in a completely randomized design, with six pigs per dietary treatment. Iso-energetic experimental diets were formulated to contain 0, 50, 100, 150, and 200 g/kg dry matter (DM) of ANC using the summit and dilution technique. All formulated diets were mixed with chromic oxide and fed to pigs (32.3 kg ± 6.07) after six weeks and were given three days of adaptation. After the apparent total tract digestibility (ATTD) study, digesta liquor from the colon segment was collected for bulkiness physicochemical characteristics. There was a linear increase in ATTD of hemicellulose (Hemi) and neutral detergent fibre (αNDF) with ANC inclusion ($P < 0.01$). The ATTD of dry matter (DM), acid detergent fibre (ADF) and acid detergent lignin (ADL) in pigs initially increased until it reached a peak and then decreased with ANC inclusion ($P < 0.01$). There was a quadratic increase between digesta dry matter (DM) content and ANC inclusion levels ($P < 0.01$). The digesta pH level decreased quadratically with ANC inclusion ($Y = 0.0027x^2 – 0.0997x + 6.4423$ ($P < 0.01$)). The quadratic equation $Y = -0.0017x^2 + 0.0867x + 3.0929$ and $Y = 0.017x^2 + 0.0389x + 2.9637$ described the response of swelling capacity (SWC) ($P < 0.01$) and water retention capacity (WRC) ($P < 0.05$) to ANC inclusion levels, respectively. It can be concluded that slow-growing pigs better digest dietary fibre components and improves nutrient utilization when fed dietary amarula nut cake.

Keywords: by-products, digesta properties, digestion, dry matter, dietary fibre, swine

4.1 Introduction

Dietary fibre in pig diets increases fibre fermentation and is considered as an important determinant of nutrient utilization and absorption depending on the physicochemical properties (Zhang *et al*., 2013; Mpendulo *et al*., 2018). Amarula nut cake (ANC) exhibits the potential of reducing ammonia, proteolytic fermentation and volatile organic compound emissions. It could also facilitate fibre digestion and volatile fatty acid production in pigs, increasing nutrient utilization for optimum growth (Mlambo *et al*., 2011). Several studies have focused on assessing physicochemical properties of digesta in the stomach and small intestines to predict feed intake and gut capacity in pigs fed fibrous diets (Ndou *et al*.., 2013; Wate *et al*., 2014). It can be speculated that feeding fibrous ANC to growing pigs influences feed intake and limits gut volume at higher inclusion levels (Mthiyane and Mhlanga, 2018). Assessing the effect of ANC on physicochemical properties of colon digesta could estimate fibre fermentability capacity and thus the extent of nutrient digestion and absorption in pigs. The use of slow-growing pigs, such as Windsnyer pigs, can better digest and ferment fibrous ANC.

Windsnyer pigs are ignored for numerous reasons as reported by Halimani *et al*. (2012). Utilizing their admirable qualities could conserve their superior genetic resources, increase the sustainability and efficiency of farming systems. Pig producers are channelled to monitor and sustain efficient pork production compounded with little greenhouse gas emissions to the environment. Slow-growing pigs could serve a dual-purpose of ameliorating compact nitrogenous-containing gas contributions to the surroundings and reduce waste disposal. These slow-growing pigs have higher fermentative capacity influenced by their longer and larger caecum-colon (Ndindana *et al*., 2002). Increased fibre can intensify cell proliferation rate and crypt depth in the large intestines of swine depending on physicochemical characteristics (Montagne *et al*., 2003). There is a gap in understanding the total dietary fibre digestibility, physicochemical properties of colon digesta and degree of fermentability response in slow-growing Windsnyer pigs fed on graded levels of ANC.

To fully utilize and accept the nutritional attributes of the cake, the assertion of evaluating colonal digesta physicochemical properties and digestibility of fibre should be drawn. Such information could benefit pig diet formulators to opt for various available energy and protein ingredients. Besides using locally available feed resources to reduce production costs, animal nutritionists should consider diet formulations with compact ammonia emission implications to the environment. The objective of the current study was to assess the relationship between ANC inclusion and fibre digestibility and physicochemical characteristics of digesta in Windsnyer pigs. It was hypothesized that dietary ANC increases fibre digestibility and physicochemical properties of bulkiness in colon digesta of slow-growing pigs.

4.2 Materials and methods

4.2.1 Study site

The study site has been described in detail in section 3.2.1

4.2.2 Pigs, experimental design and management

Details on pigs, experimental design and management are described in section 3.2.2

4.2.3 Experimental diets

Details on experimental diets are also described in section 3.2.3.

4.2.4 Physicochemical analyses of the experimental diets and amarula nut cake

Physicochemical analyses of the experimental diets and amarula nut cake have been described in section 4.2.5. The mineral composition of amarula nut cake and diets was determined from ground samples ashed at 550 °C overnight and dissolved in a 1 M HCL (Abdou *et al*., 2011). The samples were then analysed using the Varian 720 Inductively Coupled Plasma Emission Spectrometer (ICP- OES, Frankfurt, Germany) with atomic absorption. Further, chromium oxide (Cr_2O_3) concentration in faeces and representative diets was also determined using the Inductively Coupled Plasma Emission Spectrometer. The mineral composition of the experimental diets and amarula nut cake are given in Table 4.1.

Table 4.1 Mineral composition of amarula nut cake (ANC) and experimental diets

		Amarula nut cake inclusion level (g/kg DM)				
Component	ANC	0	50	100	150	200
Calcium (g/kg)	1.8	7.1	7.9	8.3	8.3	8.2
Magnesium (g/kg)	5.7	2.4	2.2	2.1	2.1	2.0
Phosphorus (g/kg)	9.4	6.3	5.5	5.5	5.5	5.1
Potassium (g/kg)	8.3	7.90	6.6	6.2	5.8	5.1
Sodium (mg/kg)	345.7	243.2	255.4	239.8	233.8	227.3
Iron (mg/kg)	95.5	161.5	169.8	149.5	143.3	135.5
Copper (mg/kg)	27.9	73.3	58.4	58.2	60.2	59.8
Manganese (mg/kg)	10.2	84.9	73.8	70.2	84.6	63.0
Zinc (mg/kg)	60.2	48.5	44.8	45.0	42.2	38.7
Cobalt (mg/kg)	0.14	0.30	0.26	0.28	0.29	0.23
Molybdenum (mg/kg)	0.31	0.45	0.35	0.31	0.26	0.21

4.2.5 Measurements

4.2.5.1 Apparent total tract digestibility of fibre

After six weeks of feeding, faecal collection was done from pigs weighing about 32.3 ± 6.07 kg at the commencement of the digestibility trial. A 3 g chromic oxide/kg was added to the daily ration of the pigs and was used as an indigestible marker (Brandy *et al.*, 2017). Representative chromium feed samples from each diet were taken each time diets were mixed and stored at room temperature pending analyses. A 3-day adaptation to chromic oxide was given, followed by a 5-day faecal collection using grab sampling methods (Brandy *et al.*, 2017). Faecal samples were collected once per day between 08h00 to 13h00 and immediately chilled at -20 C for further analysis. At the end of the collection period, faecal samples of each pig were thawed overnight, pooled and dried at 60 ºC for 24 hours, then mixed thoroughly and representative samples were taken for laboratory analyses. The digestibility of dry matter (DMD), neutral detergent fibre (NDFD), acid detergent fibre (ADFD), acid detergent lignin (ADLD) and hemicellulose (HemiD) were measured. The total tract digestibility (ATTD) of fibres were calculated using the formula:

ATTD = 100 – (100*(% Indicator feed/ % Indicator faeces) *(% Nutrient faeces/ % Nutrient feed))

Where ATTD is the total tract digestibility, indicator feed is the percentage of Cr_2O_3 in the feed, indicator faeces is the percentage of Cr_2O_3 in the faeces, nutrient faeces is the percentage of nutrient in the faeces, and nutrient feed is the percentage of nutrient in the feed.

4.2.5.2 Digesta physicochemical characteristics of colon digesta

Pigs weighing 34 kg ± 6.25 kg were slaughtered after fasting for 24 hours. They were provided with fresh-water *ad libitum.* The pigs were taken to the abattoir situated less than 1 km from the pens at around 08h00. Pig processing followed routine abattoir procedures, including an antemortem inspection and rest for the pigs before slaughter. The pigs were stunned with an electrical stunner set at 220 V and 1.8 A with a current flow for 6 s and exsanguinated within 10 s of stunning. Dehairing and evisceration followed, and the gastrointestinal tracts were set aside for sample collection. 15 to 20 g digesta samples were obtained from the colon within 1 h after slaughter. A 10 cm section of the proximal colon 50 cm from the ileo-caecal junction was ligated, incised, and its total contents were collected to determine physicochemical characteristics of digesta. The contents were put in 50 ml sealed plastic bottles and frozen immediately at -20 ºC within 1 h of collection pending analysis.

After digesta collection in plastic bottles, pH was determined by inserting Crison 52 02 glass pH electrode. Dry matter content analyses of the digesta samples were determined by first freeze-drying and then dried at 103 ºC for 24 hours. Digesta swelling capacity (SWC) was carried out on freeze-dried materials, while water retention capacity (WRC) was performed on wet materials.

The swelling capacity (SWC) of digesta samples was measured according to the modified bed volume technique described by Canibe & Bach Knudsen (2002). Briefly, 2 g of digesta samples were weighed into 15 ml measuring plastic tubes, a solution of 9 g/l NaCl containing 0.2 g/l NaN_3 was added to a final volume of 10 ml. The samples were then incubated at 39 °C in a water shaking bath overnight. After 16 hours, the water shaker bath was stopped, and the samples were left in the water for 1 hour before being removed to measure the volume occupied by the fibre and digesta. The results were expressed as ml of swollen sample per gram of dry residue.

Water retention capacity (WRC) of fresh digesta samples was determined by centrifugation following the method described by Anguita *et al.* (2007). Briefly, the digesta samples in plastic bottles were thawed. Then 4.5 to 5 g was measured into previously weighed plastic centrifuge tubes, which were centrifuged at 2 500 x g for 25 min, and then the supernatant was discarded. The centrifuge tubes were then inverted and left to drain for 25 min to remove water altogether and then weighed. The centrifuged samples were then dried at 103 °C for 16 hours. The weight of the water retained by the sample was calculated from the difference between fresh and dried samples. The WRC was then determined by dividing the weight of the water retained by the weight of the dried sample, which was expressed in g water / g of dry material.

4.2.6 Statistical analyses

A polynomial regression (PROC REG) procedure (SAS, 2009) was used to determine the relationship between apparent total tract digestibility and physicochemical characteristics of bulkiness with inclusion levels of amarula nut cake. The model used was:

$$Y = \beta_0 + \beta_1 D + \beta_2 D^2 + \varepsilon$$

Where:

Y is the response variables (fibre digestibility and physicochemical characteristics)

B_0 is the intercept

B_1D is the linear regression component

B_2D^2 is the quadratic regression component

D is the inclusion level of amarula nut cake

ε is the residual error

4.3 Results

4.3.1 Relationship between apparent total tract digestibility (ATTD) of fibre and levels of amarula nut cake

The ATTD of dietary fibre in slow-growing pigs fed on incremental levels of amarula nut cake (ANC) is shown in Table 4.2. There was an increasing quadratic relationship between ATTD of DM and ANC inclusion ($P < 0.01$). As ANC levels increased, DMD initially increased until it peaked and then started to decrease. There was a linear increase in ATTD of hemicellulose (Hemi) of pigs against rising levels of ANC ($P < 0.01$). Similarly, the ATTD of NDF increased linearly in response to ANC inclusion ($P < 0.01$). Apparent digestibility of ADF had an increasing quadratic relationship with the inclusion of ANC ($P < 0.01$). As ANC inclusion increased, ADFD increased until it plateaus and then decreased. A similar increasing quadratic response in ATTD of acid detergent lignin (ADL) to incremental levels of ANC was observed ($P < 0.01$).

Table 4.2 Influence of incremental levels of amarula nut cake on apparent total tract digestibility of fibre in slow-growing Windsnyer pigs

Nutrient	Treatment (g/kg dry matter)					SEM	Regression Coefficients	
	Control	50	100	150	200		Linear	Quadratic
DM	86.9	89.9	90.7	89	87.1	1.36	NS	-0.04**
NDF	63.3	66.4	70.9	76.9	80.9	2.25	0.72***	NS
ADF	51.2	59.2	64.1	70.1	68.6	1.04	1.93***	-0.05***
ADL	48.1	55.1	61.3	67.5	65.4	1.76	1.99***	-0.05**
Hemi	66.2	69.4	73.9	80.2	85.3	3.06	0.66***	NS

SEM = standard error of means, Level of significance (*P < 0.05; **P < 0.01; ***= P <0.001), NS = not significant; control = no amarula nut meal, ANC = amarula nut cake, ADF – acid detergent fibre, ADL – acid detergent lignin; DM – dry matter; Hemi = hemicellulose; NDF – neutral detergent fibre.

4.3.2 Influence of amarula nut cake on physicochemical characteristics of digesta

The relationship between DM content, pH level, SWC and WRC of proximal colon digesta and amarula nut cake (ANC) inclusion is shown in Figures 4.1, 4.2 and 4.3. There was an increasing quadratic response in DM (%) with increasing ANC levels (Figure 4.1). As ANC inclusion increased, the digesta DM (%) initially increased until it reached a peak and then started to decrease ($P < 0.01$). The digesta pH level decreased quadratically with ANC inclusion levels (Figure 4.2). As ANC inclusion increased from 50 to 150 g/kg DM, the pH decreased then increased from 150 to 200 g/kg DM ($P < 0.01$). There was an increasing quadratic relationship between the SWC (ml/g) and ANC inclusion (Figure 4.3). As ANC levels increased, the digesta SWC initially increased until it reached a plateau ($Y = -0.0017x^2 + 0.0867x + 3.0929$; $P < 0.01$). A decreasing quadratic response in WRC (g water/ g DM) to ANC inclusion was observed (Figure 4.3; $P < 0.05$). The quadratic equation $Y = 0.0017x^2 + 0.0389x + 2.9637$ describe the relationship between WRC and ANC inclusion.

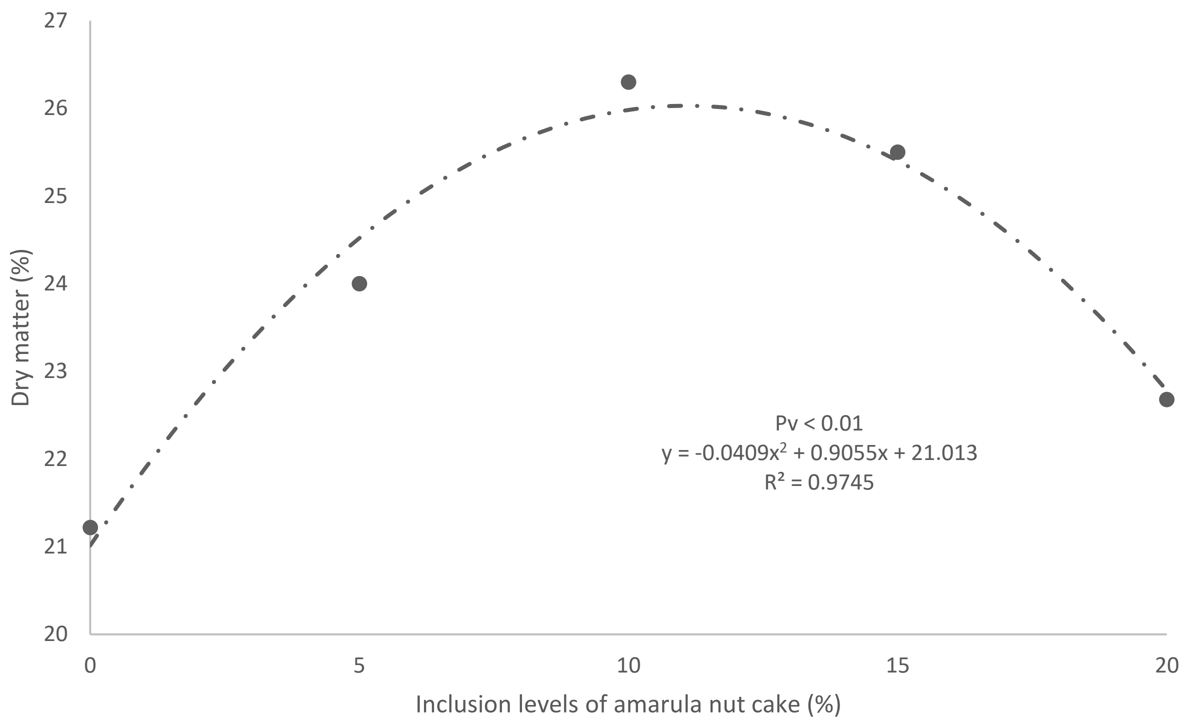


Figure 4.1 Relationship between inclusion levels of amarula nut cake and digesta dry matter content

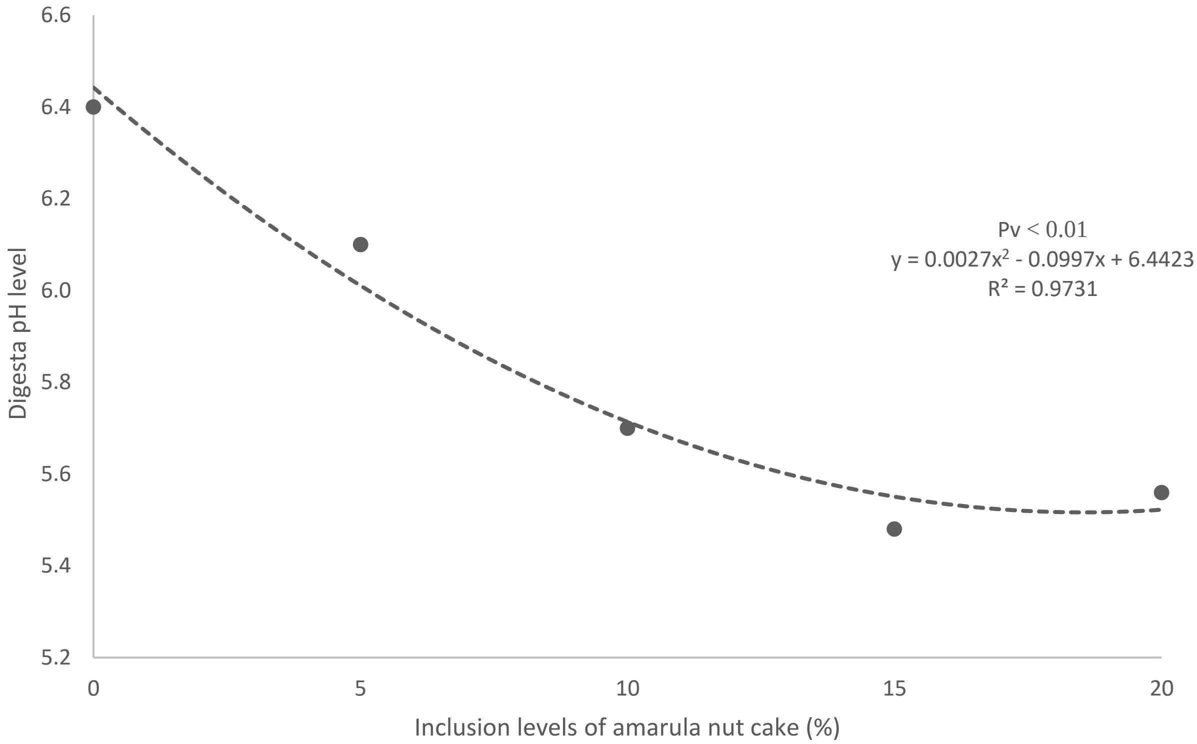
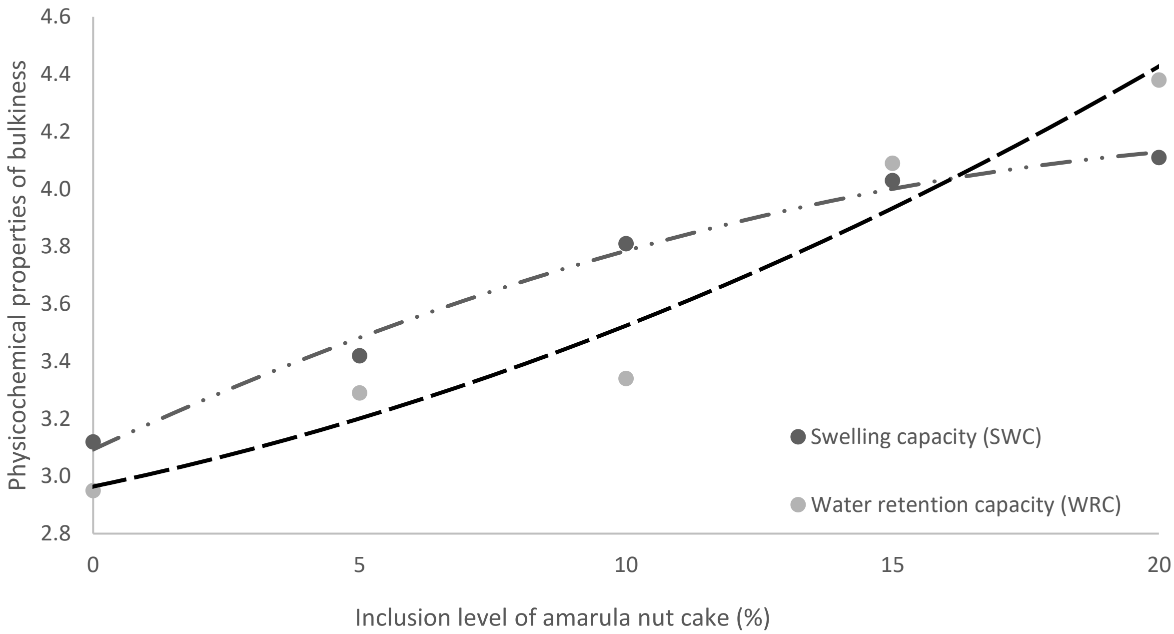


Figure 4.2 Relationship between digesta pH level and increasing levels of amarula nut cake



SWC (ml/g) = $-0.0017x^2 + 0.0867x + 3.0929$ $R^2 = 0.9908$;   Pv < 0.01

WRC (g water/g DM) = $0.0017x^2 + 0.0389x + 2.9637$ $R^2 = 0.9519$;   Pv < 0.05

Figure 4.3 Relationship between inclusion levels of amarula nut cake against water retention capacity and swelling capacity

4.4 Discussion

Variability in the response of fibre digestibility and colon digesta physicochemical characteristics were influenced by a broad spectrum of chemical and physical properties of experimental diets formulated to contain varying levels of ANC. The physiological functions and fermentability of fibre are predicted accurately by its physical properties more pronounced on the large intestines (Ndou *et al.*, 2013). The physicochemical properties of digesta in the stomach and small intestines predict only feed intake (Wate *et al*., 2014) while fibre fermentation occurs in the hindgut, thus, foregut segments were excluded from the analysis. The current study, therefore, focused on colonic digesta to estimate the extent of fibre fermentation in pigs fed on fibrous ANC. The colon is the leading intestinal site that drives fibre fermentation and bacterial colonization beneficial for pig health (Jha & Berrocoso, 2016). To measure nutrient digestion, absorption and fibre fermentability capacity in pigs fed dietary ANC, slow-growing Windsnyer-type are appropriate pig strain efficient in utilizing fibrous diets (Ncobela *et al*., 2018). The current study aimed to evaluate the influence of ANC inclusion on fibre digestibility and physicochemical properties of bulkiness in colon digesta of slow-growing pigs. Inclusion of ANC in pig diets can exhibit health benefits for the intestinal environment, microflora functioning and promoting welfare (Ndou *et al*., 2013).

The quadratic increase in ATTD of DM to ANC inclusion can be explained by the increasing ADF and NDF with dietary ANC addition. In the study of Ndindana *et al*. (2002), a quadratic decrease in ATTD of DM was observed in slow-growing pigs fed increasing levels of maize cobs. Differences in fibre properties of the ingredients could explain the discrepancies between the two studies. The initial increase in DM digestibility is an indication of improved DM intake ascribed to effective fibre degradation by microbial action in the intestinal tract (Nkosi *et al*., 2019). Muya *et al*. (2020) reported that soluble proportions of DM increased with increasing ANC levels in diets. The decrease in DM digestibility at higher ANC levels could be attributed to the digesta bulkiness effect on feed intake, consequently interfering with digestion and absorption capacity. The observed ATTD of DM also aligns with the quadratic response in DM content of colon digesta as ANC inclusion increased, which portrays the relation between fibre digestibility and digesta properties.

The increasing linear response in ATTD of hemicellulose (Hemi) and NDF indicates that pigs adapted well to diets containing ANC and digested fibre components efficiently. Series of studies support these findings following the assertion that slow-growing pigs better digest and utilize fibrous diets than their counterparts (Kanengoni *et al*., 2002; Ndindana *et al*., 2002). The empirical findings agree with Nkosi *et al*. (2019), who reported increased NDF digestibility in growing pigs fed avocado oil cake. Kanengoni *et al*. (2015a) also reported increased hemicellulose and NDF digestibility in Windsnyer pigs fed on diets containing graded levels of fibrous maize cobs. It can also be attributed to the active intestinal microbial community in indigenous-type pigs, enhancing their ability to digest high fibre diets (Kanengoni *et al*. 2015b). Another possible explanation could be ascribed to the improvement in the oil-extraction processing technique employed in the current study, which resulted in 67 units lower ether extract (EE) than the value reported by Malebana *et al*. (2018). Reduction in residual oil hampered its physical covering effect on fibre, which increases dietary fibre digestibility. In addition, a good mineral profile of ANC could have reverted the interactive effect between divalent minerals and hemicellulose components, forming complexes impairing absorption (Barszcz *et al*., 2019). Improvement in fibre digestibility also indicates increased nutrient digestion, absorption, and fibre fermentability strength in the hindgut. Thus, increased hemicellulose and NDF digestibility imply that fewer nutrient effluents were excreted into the environment.

An increasing quadratic response in ATTD of ADF and ADL could be explained by increasing ADF concentration in diets containing ANC (Mlambo *et al*., 2011). Nkosi *et al*. (2019) reported a quadratic relationship between ADF digestibility and avocado oilcake inclusion. Diets containing a high level of ADF content constitute an indigestible fibre fraction depressing the degradability rate (Muya *et al*., 2020). Cellulose is less digestible than hemicellulose in swine due to the acid hydrolytic effect on carboxylic groups of uronic acids in hemicelluloses (Barszcz *et al*., 2019). Indigestible cellulosic cell walls are insoluble and characterized as poorly digested and fermented in pigs (Knudsen, 2001), which explains the reduction in fibre digestibility beyond ANC inclusion level of 150 g/kg DM. Consequently, this could have increased endogenous losses resulting in more rapid transit and hinder dietary protein and other nutrients absorption (Ndindana *et al*., 2002). The latter caused a larger fraction of insoluble fibre escaping digestion and directed to microbial attack by colonic flora, increasing substrate for hindgut fermentation.

The observed initial increase in colon digesta DM (%) as ANC inclusion increased to 100 g/kg DM could be explained by a larger proportion of soluble non-starch polysaccharides (NSPs). Muya *et al*. (2020) reported that soluble fractions of DM and protein increased with ANC inclusion indicating high content of easily degradable nutrients. There is the proximity between DM concentration in the digesta and the nutrient content assimilated through the gastrointestinal tract. An increase in digesta DM content could indicate a significant disappearance of DM and soluble dietary fibre before the proximal colon. Jaworski & Stein (2017) concur with these findings reporting the remarkable disappearance of soluble dietary fibre in the caecum and DM degradation in the colon of pigs fed on soybean hulls. The decrease in DM concentration of digesta at higher inclusion levels of ANC could be explained by increasing ADF fraction per unit increase in ANC (Mlambo *et al*., 2011). Increasing dietary ANC levels reduces DM degradation rate and effectiveness attributed to higher ADF and EE (Muya *et al*., 2020). Higher ADF interferes with nutrient digestion (Lindberg, 2014), and EE masks fibre components limiting their attack by the microbial community. The insoluble DM fraction of ANC at higher inclusion levels could have diluted nutrient density and reduced DM intake and digestibility.

The decreasing quadratic response in colon digesta pH as ANC inclusion increased indicates a beneficial effect on pig health. It can be explained by increasing NDF per ANC addition comprising fermentable hemicellulose proportion, which stimulates the hindgut microbiome (Jha & Leterme, 2012). Low colonic pH acidifies the intestinal environment suppressing acid intolerant pathogenic bacteria populace such as *Enterobacteria* and production of toxic metabolites (Bird *et al.*, 2006). The reduction in colonic pH as the ANC inclusion level increased can also promote a beneficial shift in colonic microflora, increasing colonization resistance. There were no incidences of scouring in pigs fed on diets containing ANC, which indicates an insignificant population of opportunistic microorganisms. Molist *et al*. (2009) reported the lowest counts of *Enterobacteria* in the colon of pigs fed a diet containing a combination of wheat bran (insoluble NSPs) and sugar beet pulp (soluble NSPs). Amarula nut cake encompasses both soluble and insoluble dietary fibre fractions (Muya *et al*., 2019), which could favour a synergistic effect on colonic microflora and reduce protein fermentation associated with toxic metabolites. These metabolites could impair epithelial integrity, impose colonic disorders emergencies and interfere with oxidative metabolism of short-chain fatty acids (SCFAs) in colonocytes (Jha & Berrocoso, 2016). The concentration of SCFAs produced and the production rate, absorption and utilisation, including the buffering capacity of digesta contents, affect digesta pH along the gastrointestinal tract (Wate *et al*., 2014). As a result, pigs fed on ANC were healthy and had a lower risk of developing colon cancer or diseases indexed in the luminal colon.

The increasing quadratic response on swelling capacity (SWC) at a decreasing rate as ANC inclusion increased could be explained by the high proportion of soluble dietary fibre available in the nut cake. Similar findings were observed in growing pigs fed on graded maize cobs that were rich in soluble fibres (Wate *et al*. 2014), indicating that SWC increased at higher rates in the foregut than the hindgut segments. The swelling of fibre is closely related to the solubility forming the first stage of the solubilization process (Canibe & Knudsen, 2002). Incoming water into the fibre polymers spreads macromolecules until they are entirely expanded and dispersed after being solubilized (Knudsen, 2011). Knudsen (2011) also reported that swelling of soluble NSPs increases the substrate's surface area to microbial action for significant degradation and colonization. High dietary fibre is expected to occupy more space in the gut and upsurge digesta bulkiness, influencing transit time and flow rate of digesta content (Guillon & Champ, 2002). Consequently, increased SWC with ANC inclusion could be associated with increased digesta exposure to microflora activity for effective degradation due to the longer transit time of digesta in the hindgut of slow-growing pigs (Knudsen, 2001; Ndindana *et al*., 2002).

The quadratic decrease in WRC of proximal colon digesta at an increasing rate with ANC inclusion was expected. A plausible explanation could be ascribed to the increase in NSPs content predominantly soluble than insoluble fractions per unit addition of ANC. Wate *et al*. (2014) reported a linear increase in digesta WRC of the proximal colon. Increased WRC in the proximal sites of the hindgut in growing pigs fed sugar beet pulp and wheat bran was attributed to a higher amount of NSPs in the diet (Anguita *et al*., 2007). The effect of increasing NDF and bulkiness properties in the diet with ANC inclusion could have increased the ability of fibre to retain water (Mlambo *et al*., 2011). Dietary fibre matrix can hold water by either trapping or bounding water in the digesta (Anguita *et al*., 2006). Water in digesta can also remain unbound, which increases the concentration of water content in the gastrointestinal tract. Molist *et al*. (2009) reported that the colon contains high water content, facilitating colonic mucosa function for reabsorption of water and some macro-minerals. Inclusion of insoluble fibre can diminish unbound water in the colonic digesta and reduce transit time (Molist *et al*., 2005; 2009). The highly lignified remains of hard shells' remnants in the nut cake could, possibly, account for an insoluble fraction. The insoluble fibre could have influenced particle size, viscosity, flow rate and retention time of digesta, which were not measured in the current study. Such confounding aspects interlink with digesta bulkiness and increase hydration properties response, reflecting high fibre volume available for fermentation.

4.5 Conclusions

Dietary amarula nut cake inclusion improved apparent digestibility of neutral detergent fibre, hemicellulose, acid detergent fibre and acid detergent lignin in Windsnyer pigs. Inclusion of amarula nut cake caused a quadratic response in dry matter content, pH level, swelling and water retention capacity in the colon digesta. Increasing colonal digesta bulkiness is an indicator of increased fibre fermentation, hence physicochemical properties of colon digesta should be accounted for the prediction of fermentability strength. Therefore, the inclusion of nut cake improved nutrient digestion and dietary fibre fermentation, reducing toxic metabolites production associated with colonic mucosa diseases.

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CHAPTER FIVE: General discussion, Conclusions and Recommendations

5.1 General discussion

The study aimed to explore the use of amarula nut cake (ANC) in pig feeding systems to substitute costly dietary protein sources. Its fibrous and high ether extract (EE) content leaves room for discussion regarding their utilisation efficiency by slow-growing pigs compared to imported genotypes. The main hypothesis tested was that increasing inclusion levels of ANC influences nitrogen balance, fibre digestibility and physicochemical characteristics of digesta in Windsnyer pigs. Slow-growing Windsnyer pigs digest fibrous diets efficiently than their counterparts, which was reflected in their ability to utilize the nut cake. The successful use of ANC in pig diets promotes the dual-purpose of using valuable by-products with minimal environmental pollution.

Growth performance and nitrogen (N) balance of Windsnyer pigs fed on incremental levels of ANC were measured in Chapter 3. It was hypothesized that ANC reduces N excretion from slow-growing pigs. The average daily feed intake (ADFI) increased quadratically with ANC inclusion, which could be ascribed to feed bulk regulating feed intake of slow-growing pigs. Increasing acid detergent fibre (ADF) and neutral detergent fibre (NDF) per unit addition of the cake in pig diets interferes with intake due to satiety and physiological restriction of the gut capacity of Windsnyer pigs. The average daily gain (ADG) and gain: feed ratio increased linearly with graded ANC levels. The observed positive linear relationship between ADG and Gain: Feed and ANC inclusion could be associated with decreasing ADFI at higher inclusion levels of ANC and suggest that the cake contains sufficient nutrients efficiently utilized by Windsnyer pigs. The increasing response in ADG and Gain: Feed ratio can be further explained by the observed N intake.

Increasing levels of ANC caused a linear increase in N intake of pigs. The linear relationship between N intake and ANC inclusion could indicate a low concentration of polyphenolic compounds bound to dietary protein. Further, decreasing crude protein (CP) with the addition of ANC complied with low protein requirements for slow-growing pigs. Increasing N intake response explains the negative linear relationship between ANC inclusion and N excretion through faeces and urine. More N was excreted through faeces than via urine, suggesting a larger proportion of fermentable non-starch polysaccharides (NSPs), making ANC repartition N excretion to less volatile organic N in faeces. This was also in line with decreasing quadratic response in urinary pH level, indicating reduced ammonia emissions. A quadratic increase in N digestibility, absorption and retention could be attributed to a larger fraction of digestible protein biomass and soluble NSPs, improving assimilation of ingested N supported by a linear increase in N utilisation. Inclusion of the nut cake, therefore, enhanced net protein utilisation without compromising pig growth. As a result, the hypothesis that ANC reduces N excretion is accepted as more N was excreted through faeces than via urine, which alternatively reduces ammonia volatilization.

In Chapter 4, the hypothesis tested was that dietary ANC increases fibre digestibility and physicochemical properties of bulkiness in colon digesta of slow-growing pigs. Incorporation of ANC produced a positive linear response in apparent total tract digestibility (ATTD) of hemicellulose and neutral detergent fibre (NDF). The increasing response could indicate that a considerable fraction of digestible and degradable fibre was subjected to the active microbial community in slow-growing pigs, enhancing digestion. An increasing quadratic relationship between ANC inclusion and ATTD of acid detergent fibre (ADF) and acid detergent lignin (ADL) indicates that indigestible fibre fraction is insoluble and characterized as poorly digested in pigs. Higher inclusion levels of ANC could increase endogenous losses, resulting in more rapid digesta transit and impeding divalent nutrients absorption. This could be in relation to the decrease in apparent N digestibility, absorption and retention at higher inclusion levels of ANC. In this respect, larger quantities of undigested insoluble dietary fibre escaping digestion are fermented by colonized microbiota in the colon.

Increasing ANC inclusion caused a quadratic increase in dry matter (DM) content and could be related to soluble DM and protein fraction in the nut cake. The digesta DM content interlink with the decreasing feed intake at higher inclusion levels of ANC due to elevated ADF concentration per unit increase of the cake. The insoluble DM fractions tend to dilute nutrient density and interfere with DM intake and digestibility at higher inclusion levels of ANC. The quadratic relationship between colon digesta pH and ANC inclusion assures its safety for consumption suppressing acid intolerant pathogenic bacteria and toxic metabolites production. The quadratic relationships between ANC and digesta swelling capacity (SWC) and water retention capacity (WRC) at different rates was ascribed to the nut cake's physical properties. Increasing WRC and SWC expand the digesta bulkiness, which reduces DM and feed intake at higher inclusion levels of ANC due to gut fill. These increasing quadratic responses estimates increased fibre fermented and reduced proteolytic fermentation. Consequently, the hypothesis that ANC increases fibre digestibility and physicochemical properties of colon digesta is accepted as increasing digesta bulkiness influenced nitrogen metabolism to an optimum level until physical properties restrict efficiency in digestion and absorption.

5.2 Conclusions

Amarula nut cake inclusion resulted in a positive linear relationship in nitrogen utilisation and a decreasing linear response in total nitrogen excretion. Increasing inclusion levels of ANC improved fibre digestibility and increased hydration properties of digesta, hence physicochemical properties need to be accounted for when predicting fibre fermentability. Further, incorporating the cake implicates feeding low-protein diets to slow-growing pigs, making it a potential dietary protein source reducing nitrogen loss and ammonia emissions into the environment without depressing growth. Therefore, dietary amarula nut cake can increase ingredients' availability, improve the productivity of slow-growing pigs, and preserve their endangered genetics.

5.3 Recommendations

Proper processing of the nut cake can reduce the residual oil effect on nutrient digestion and utilisation. The inclusion of ANC in pig diets should not exceed 100 g/kg DM when feeding slow-growing pigs. Extrapolation from nitrogen balance, fibre digestibility and colonal digesta physicochemical characteristics relationships with ANC inclusion exhibits this optimum inclusion level.

Further research should dwell on the following aspects

1. Physical properties of the nut cake need to be considered prior to diet formulation as the significant influencer on digesta characteristics changes and further account for the risk of confounding effects.

2. Assessing ammonia volatilization in growing pigs fed incremental levels of ANC to measure its extent in reducing ammonia emission.
3. Determining physicochemical properties of digesta on different intestinal segments in pigs fed increasing levels of ANC.
4. Assessing the relationship between incremental levels of ANC against digesta passage rate and digestibility in growing pigs.
5. Assessing volatile fatty acids production to understand the fermentability strength in slow-growing pigs fed dietary ANC.

APPENDIX 1: Ethical Approval Letter

ARC • LNR

Excellence in Research and Development

Date: 9th of July, 2019

Dear Dr R Thomas,

Re: **"The impact of different inclusion levels of Marula (Sclerocarya birrea subsp. Caffra) Nut Cake on nutritional and anti-nutritional factors, nutrient digestibility, growth performance and carcass traits of South African Windsnyer and Large White x Landrace crossbred pigs "**

Your application for the ethical evaluation of the project entitled "**The impact of different inclusion levels of Marula (Sclerocarya birrea subsp. Caffra) Nut Cake on nutritional and anti-nutritional factors, nutrient digestibility, growth performance and carcass traits of South African Windsnyer and Large White x Landrace crossbred pigs**" has been finalized and approved; its reference number is **APAEC [2019/17]**.

I would like to inform you that the project was evaluated and found to be ethically acceptable.

Please note that should any more amendments or changes be made to the protocol, you are obliged to submit an amended application to the Animal Ethics Committee. A hard-copy of this application letter of approval must be available at the site office where animals are kept, including a copy of the protocol, ethical application, all SOP's and data monitoring sheets.

Regards,

[redacted]

Dr. Klaas-Jan Leeuw

Chairperson: ARC-API Animal Ethics Committee

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